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IARC MONOGRAPHS

# BIOLOGICAL AGENTS

VOLUME 100 B

A REVIEW OF HUMAN CARCINOGENS

IARC MONOGRAPHS  
ON THE EVALUATION  
OF CARCINOGENIC RISKS  
TO HUMANS

International Agency for Research on Cancer





# BIOLOGICAL AGENTS

VOLUME 100 B  
A REVIEW OF HUMAN CARCINOGENS

This publication represents the views and expert  
opinions of an IARC Working Group on the  
Evaluation of Carcinogenic Risks to Humans,  
which met in Lyon, 24 February-3 March 2009

LYON, FRANCE - 2012

IARC MONOGRAPHS  
ON THE EVALUATION  
OF CARCINOGENIC RISKS  
TO HUMANS

## IARC MONOGRAPHS

In 1969, the International Agency for Research on Cancer (IARC) initiated a programme on the evaluation of the carcinogenic risk of chemicals to humans involving the production of critically evaluated monographs on individual chemicals. The programme was subsequently expanded to include evaluations of carcinogenic risks associated with exposures to complex mixtures, lifestyle factors and biological and physical agents, as well as those in specific occupations. The objective of the programme is to elaborate and publish in the form of monographs critical reviews of data on carcinogenicity for agents to which humans are known to be exposed and on specific exposure situations; to evaluate these data in terms of human risk with the help of international working groups of experts in chemical carcinogenesis and related fields; and to indicate where additional research efforts are needed. The lists of IARC evaluations are regularly updated and are available on the Internet at <http://monographs.iarc.fr/>.

This programme has been supported since 1982 by Cooperative Agreement U01 CA33193 with the United States National Cancer Institute, Department of Health and Human Services. Additional support has been provided since 1986 by the Health, Safety and Hygiene at Work Unit of the European Commission Directorate-General for Employment, Social Affairs and Equal Opportunities, and since 1992 by the United States National Institute of Environmental Health Sciences, Department of Health and Human Services. The contents of this volume are solely the responsibility of the Working Group and do not necessarily represent the official views of the U.S. National Cancer Institute, the U.S. National Institute of Environmental Health Sciences, the U.S. Department of Health and Human Services, or the European Commission Directorate-General for Employment, Social Affairs and Equal Opportunities.

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Lorenzo Tomatis (1929-2007)  
Founder of the *IARC Monographs* Programme

Lorenzo Tomatis, MD, with other colleagues knowledgeable in primary prevention and environmental carcinogenesis, perceived in the 1960s the growing need to objectively evaluate carcinogenic risks by international groups of experts in chemical carcinogenesis. His vision and determination to provide a reliable source of knowledge and information on environmental and occupational causes of cancer led to his creating the *IARC Monographs* Programme for evaluating cancer risks to humans from exposures to chemicals. The first meeting, held in Geneva in December 1971, resulted in Volume 1 of the *IARC Monographs* on the Evaluation of Carcinogenic Risk of Chemicals to Man [1972], a series known affectionately since as the “orange books”. As a champion of chemical carcinogenesis bioassays, Tomatis defined and promoted the applicability and utility of experimental animal findings for identifying carcinogens and for preventing cancers in humans, especially in workers and children, and to eliminate inequalities in judging cancer risks between industrialized and developing countries. Tomatis’ foresight, guidance, leadership, and staunch belief in primary prevention continued to influence the *IARC Monographs* as they expanded to encompass personal habits, as well as physical and biological agents. Lorenzo Tomatis had a distinguished career at the Agency, arriving in 1967 and heading the Unit of Chemical Carcinogenesis, before being Director from 1982 to 1993.

Volume 100 of the *IARC Monographs* Series is respectfully dedicated to him.

(photo: Roland Dray)



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## NOTE TO THE READER

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The term ‘carcinogenic risk’ in the *IARC Monographs* series is taken to mean that an agent is capable of causing cancer. The *Monographs* evaluate cancer hazards, despite the historical presence of the word ‘risks’ in the title.

Inclusion of an agent in the *Monographs* does not imply that it is a carcinogen, only that the published data have been examined. Equally, the fact that an agent has not yet been evaluated in a *Monograph* does not mean that it is not carcinogenic. Similarly, identification of cancer sites with *sufficient evidence* or *limited evidence* in humans should not be viewed as precluding the possibility that an agent may cause cancer at other sites.

The evaluations of carcinogenic risk are made by international working groups of independent scientists and are qualitative in nature. No recommendation is given for regulation or legislation.

Anyone who is aware of published data that may alter the evaluation of the carcinogenic risk of an agent to humans is encouraged to make this information available to the Section of IARC Monographs, International Agency for Research on Cancer, 150 cours Albert Thomas, 69372 Lyon Cedex 08, France, in order that the agent may be considered for re-evaluation by a future Working Group.

Although every effort is made to prepare the *Monographs* as accurately as possible, mistakes may occur. Readers are requested to communicate any errors to the Section of IARC Monographs, so that corrections can be reported in future volumes.



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<sup>5</sup> Dr Mahieux served briefly as a consultant to MP Biomedicals (which makes tests for Epstein-Barr virus and Helicobacter pylori), ending in 2007.

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# PREAMBLE

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The Preamble to the *IARC Monographs* describes the objective and scope of the programme, the scientific principles and procedures used in developing a *Monograph*, the types of evidence considered and the scientific criteria that guide the evaluations. The Preamble should be consulted when reading a *Monograph* or list of evaluations.

## A. GENERAL PRINCIPLES AND PROCEDURES

### 1. Background

Soon after IARC was established in 1965, it received frequent requests for advice on the carcinogenic risk of chemicals, including requests for lists of known and suspected human carcinogens. It was clear that it would not be a simple task to summarize adequately the complexity of the information that was available, and IARC began to consider means of obtaining international expert opinion on this topic. In 1970, the IARC Advisory Committee on Environmental Carcinogenesis recommended ‘...that a compendium on carcinogenic chemicals be prepared by experts. The biological activity and evaluation of practical importance to public health should be referenced and documented.’ The IARC Governing Council adopted a resolution concerning the role of IARC in providing government authorities with expert, independent, scientific opinion on environmental carcinogenesis. As one means to that end, the Governing Council recommended that IARC should prepare monographs on the evaluation of carcinogenic

risk of chemicals to man, which became the initial title of the series.

In the succeeding years, the scope of the programme broadened as *Monographs* were developed for groups of related chemicals, complex mixtures, occupational exposures, physical and biological agents and lifestyle factors. In 1988, the phrase ‘of chemicals’ was dropped from the title, which assumed its present form, *IARC Monographs on the Evaluation of Carcinogenic Risks to Humans*.

Through the *Monographs* programme, IARC seeks to identify the causes of human cancer. This is the first step in cancer prevention, which is needed as much today as when IARC was established. The global burden of cancer is high and continues to increase: the annual number of new cases was estimated at 10.1 million in 2000 and is expected to reach 15 million by 2020 ([Stewart & Kleihues, 2003](#)). With current trends in demographics and exposure, the cancer burden has been shifting from high-resource countries to low- and medium-resource countries. As a result of *Monographs* evaluations, national health agencies have been able, on scientific grounds, to take measures to reduce human exposure to carcinogens in the workplace and in the environment.

The criteria established in 1971 to evaluate carcinogenic risks to humans were adopted by the Working Groups whose deliberations resulted in the first 16 volumes of the *Monographs* series. Those criteria were subsequently updated by further ad hoc Advisory Groups ([IARC, 1977, 1978, 1979, 1982, 1983, 1987, 1988, 1991; Vainio et al., 1992; IARC, 2005, 2006](#)).

The Preamble is primarily a statement of scientific principles, rather than a specification of working procedures. The procedures through which a Working Group implements these principles are not specified in detail. They usually involve operations that have been established as being effective during previous *Monograph* meetings but remain, predominantly, the prerogative of each individual Working Group.

## 2. Objective and scope

The objective of the programme is to prepare, with the help of international Working Groups of experts, and to publish in the form of *Monographs*, critical reviews and evaluations of evidence on the carcinogenicity of a wide range of human exposures. The *Monographs* represent the first step in carcinogen risk assessment, which involves examination of all relevant information to assess the strength of the available evidence that an agent could alter the age-specific incidence of cancer in humans. The *Monographs* may also indicate where additional research efforts are needed, specifically when data immediately relevant to an evaluation are not available.

In this Preamble, the term ‘agent’ refers to any entity or circumstance that is subject to evaluation in a *Monograph*. As the scope of the programme has broadened, categories of agents now include specific chemicals, groups of related chemicals, complex mixtures, occupational or environmental exposures, cultural or behavioural practices, biological organisms and physical agents. This list of categories may expand as

causation of, and susceptibility to, malignant disease become more fully understood.

A cancer ‘hazard’ is an agent that is capable of causing cancer under some circumstances, while a cancer ‘risk’ is an estimate of the carcinogenic effects expected from exposure to a cancer hazard. The *Monographs* are an exercise in evaluating cancer hazards, despite the historical presence of the word ‘risks’ in the title. The distinction between hazard and risk is important, and the *Monographs* identify cancer hazards even when risks are very low at current exposure levels, because new uses or unforeseen exposures could engender risks that are significantly higher.

In the *Monographs*, an agent is termed ‘carcinogenic’ if it is capable of increasing the incidence of malignant neoplasms, reducing their latency, or increasing their severity or multiplicity. The induction of benign neoplasms may in some circumstances (see Part B, Section 3a) contribute to the judgement that the agent is carcinogenic. The terms ‘neoplasm’ and ‘tumour’ are used interchangeably.

The Preamble continues the previous usage of the phrase ‘strength of evidence’ as a matter of historical continuity, although it should be understood that *Monographs* evaluations consider studies that support a finding of a cancer hazard as well as studies that do not.

Some epidemiological and experimental studies indicate that different agents may act at different stages in the carcinogenic process, and several different mechanisms may be involved. The aim of the *Monographs* has been, from their inception, to evaluate evidence of carcinogenicity at any stage in the carcinogenesis process, independently of the underlying mechanisms. Information on mechanisms may, however, be used in making the overall evaluation ([IARC, 1991; Vainio et al., 1992; IARC, 2005, 2006](#); see also Part B, Sections 4 and 6). As mechanisms of carcinogenesis are elucidated, IARC convenes international scientific conferences to determine whether a broad-based consensus has emerged

on how specific mechanistic data can be used in an evaluation of human carcinogenicity. The results of such conferences are reported in IARC Scientific Publications, which, as long as they still reflect the current state of scientific knowledge, may guide subsequent Working Groups.

Although the *Monographs* have emphasized hazard identification, important issues may also involve dose-response assessment. In many cases, the same epidemiological and experimental studies used to evaluate a cancer hazard can also be used to estimate a dose-response relationship. A *Monograph* may undertake to estimate dose-response relationships within the range of the available epidemiological data, or it may compare the dose-response information from experimental and epidemiological studies. In some cases, a subsequent publication may be prepared by a separate Working Group with expertise in quantitative dose-response assessment.

The *Monographs* are used by national and international authorities to make risk assessments, formulate decisions concerning preventive measures, provide effective cancer control programmes and decide among alternative options for public health decisions. The evaluations of IARC Working Groups are scientific, qualitative judgements on the evidence for or against carcinogenicity provided by the available data. These evaluations represent only one part of the body of information on which public health decisions may be based. Public health options vary from one situation to another and from country to country and relate to many factors, including different socioeconomic and national priorities. Therefore, no recommendation is given with regard to regulation or legislation, which are the responsibility of individual governments or other international organizations.

### 3. Selection of agents for review

Agents are selected for review on the basis of two main criteria: (a) there is evidence of human

exposure and (b) there is some evidence or suspicion of carcinogenicity. Mixed exposures may occur in occupational and environmental settings and as a result of individual and cultural habits (such as tobacco smoking and dietary practices). Chemical analogues and compounds with biological or physical characteristics similar to those of suspected carcinogens may also be considered, even in the absence of data on a possible carcinogenic effect in humans or experimental animals.

The scientific literature is surveyed for published data relevant to an assessment of carcinogenicity. Ad hoc Advisory Groups convened by IARC in 1984, 1989, 1991, 1993, 1998 and 2003 made recommendations as to which agents should be evaluated in the *Monographs* series. Recent recommendations are available on the *Monographs* programme web site (<http://monographs.iarc.fr>). IARC may schedule other agents for review as it becomes aware of new scientific information or as national health agencies identify an urgent public health need related to cancer.

As significant new data become available on an agent for which a *Monograph* exists, a re-evaluation may be made at a subsequent meeting, and a new *Monograph* published. In some cases it may be appropriate to review only the data published since a prior evaluation. This can be useful for updating a database, reviewing new data to resolve a previously open question or identifying new tumour sites associated with a carcinogenic agent. Major changes in an evaluation (e.g. a new classification in Group 1 or a determination that a mechanism does not operate in humans, see Part B, Section 6) are more appropriately addressed by a full review.

### 4. Data for the *Monographs*

Each *Monograph* reviews all pertinent epidemiological studies and cancer bioassays in experimental animals. Those judged inadequate

or irrelevant to the evaluation may be cited but not summarized. If a group of similar studies is not reviewed, the reasons are indicated.

Mechanistic and other relevant data are also reviewed. A *Monograph* does not necessarily cite all the mechanistic literature concerning the agent being evaluated (see Part B, Section 4). Only those data considered by the Working Group to be relevant to making the evaluation are included.

With regard to epidemiological studies, cancer bioassays, and mechanistic and other relevant data, only reports that have been published or accepted for publication in the openly available scientific literature are reviewed. The same publication requirement applies to studies originating from IARC, including meta-analyses or pooled analyses commissioned by IARC in advance of a meeting (see Part B, Section 2c). Data from government agency reports that are publicly available are also considered. Exceptionally, doctoral theses and other material that are in their final form and publicly available may be reviewed.

Exposure data and other information on an agent under consideration are also reviewed. In the sections on chemical and physical properties, on analysis, on production and use and on occurrence, published and unpublished sources of information may be considered.

Inclusion of a study does not imply acceptance of the adequacy of the study design or of the analysis and interpretation of the results, and limitations are clearly outlined in square brackets at the end of each study description (see Part B). The reasons for not giving further consideration to an individual study also are indicated in the square brackets.

## 5. Meeting participants

Five categories of participant can be present at *Monograph* meetings.

### (a) *The Working Group*

The Working Group is responsible for the critical reviews and evaluations that are developed during the meeting. The tasks of Working Group Members are: (i) to ascertain that all appropriate data have been collected; (ii) to select the data relevant for the evaluation on the basis of scientific merit; (iii) to prepare accurate summaries of the data to enable the reader to follow the reasoning of the Working Group; (iv) to evaluate the results of epidemiological and experimental studies on cancer; (v) to evaluate data relevant to the understanding of mechanisms of carcinogenesis; and (vi) to make an overall evaluation of the carcinogenicity of the exposure to humans. Working Group Members generally have published significant research related to the carcinogenicity of the agents being reviewed, and IARC uses literature searches to identify most experts. Working Group Members are selected on the basis of (a) knowledge and experience and (b) absence of real or apparent conflicts of interests. Consideration is also given to demographic diversity and balance of scientific findings and views.

### (b) *Invited Specialists*

Invited Specialists are experts who also have critical knowledge and experience but have a real or apparent conflict of interests. These experts are invited when necessary to assist in the Working Group by contributing their unique knowledge and experience during subgroup and plenary discussions. They may also contribute text on non-influential issues in the section on exposure, such as a general description of data on production and use (see Part B, Section 1). Invited Specialists do not serve as meeting chair or subgroup chair, draft text that pertains to the description or interpretation of cancer data, or participate in the evaluations.

(c) *Representatives of national and international health agencies*

Representatives of national and international health agencies often attend meetings because their agencies sponsor the programme or are interested in the subject of a meeting. Representatives do not serve as meeting chair or subgroup chair, draft any part of a *Monograph*, or participate in the evaluations.

(d) *Observers with relevant scientific credentials*

Observers with relevant scientific credentials may be admitted to a meeting by IARC in limited numbers. Attention will be given to achieving a balance of Observers from constituencies with differing perspectives. They are invited to observe the meeting and should not attempt to influence it. Observers do not serve as meeting chair or subgroup chair, draft any part of a *Monograph*, or participate in the evaluations. At the meeting, the meeting chair and subgroup chairs may grant Observers an opportunity to speak, generally after they have observed a discussion. Observers agree to respect the Guidelines for Observers at *IARC Monographs* meetings (available at <http://monographs.iarc.fr>).

(e) *The IARC Secretariat*

The IARC Secretariat consists of scientists who are designated by IARC and who have relevant expertise. They serve as rapporteurs and participate in all discussions. When requested by the meeting chair or subgroup chair, they may also draft text or prepare tables and analyses.

Before an invitation is extended, each potential participant, including the IARC Secretariat, completes the WHO Declaration of Interests to report financial interests, employment and consulting, and individual and institutional research support related to the subject of the meeting. IARC assesses these interests to determine

whether there is a conflict that warrants some limitation on participation. The declarations are updated and reviewed again at the opening of the meeting. Interests related to the subject of the meeting are disclosed to the meeting participants and in the published volume ([Cogliano et al., 2004](#)).

The names and principal affiliations of participants are available on the *Monographs* programme web site (<http://monographs.iarc.fr>) approximately two months before each meeting. It is not acceptable for Observers or third parties to contact other participants before a meeting or to lobby them at any time. Meeting participants are asked to report all such contacts to IARC ([Cogliano et al., 2005](#)).

All participants are listed, with their principal affiliations, at the beginning of each volume. Each participant who is a Member of a Working Group serves as an individual scientist and not as a representative of any organization, government or industry.

## 6. Working procedures

A separate Working Group is responsible for developing each volume of *Monographs*. A volume contains one or more *Monographs*, which can cover either a single agent or several related agents. Approximately one year in advance of the meeting of a Working Group, the agents to be reviewed are announced on the *Monographs* programme web site (<http://monographs.iarc.fr>) and participants are selected by IARC staff in consultation with other experts. Subsequently, relevant biological and epidemiological data are collected by IARC from recognized sources of information on carcinogenesis, including data storage and retrieval systems such as PubMed. Meeting participants who are asked to prepare preliminary working papers for specific sections are expected to supplement the IARC literature searches with their own searches.

For most chemicals and some complex mixtures, the major collection of data and the preparation of working papers for the sections on chemical and physical properties, on analysis, on production and use, and on occurrence are carried out under a separate contract funded by the US National Cancer Institute. Industrial associations, labour unions and other knowledgeable organizations may be asked to provide input to the sections on production and use, although this involvement is not required as a general rule. Information on production and trade is obtained from governmental, trade and market research publications and, in some cases, by direct contact with industries. Separate production data on some agents may not be available for a variety of reasons (e.g. not collected or made public in all producing countries, production is small). Information on uses may be obtained from published sources but is often complemented by direct contact with manufacturers. Efforts are made to supplement this information with data from other national and international sources.

Six months before the meeting, the material obtained is sent to meeting participants to prepare preliminary working papers. The working papers are compiled by IARC staff and sent, before the meeting, to Working Group Members and Invited Specialists for review.

The Working Group meets at IARC for seven to eight days to discuss and finalize the texts and to formulate the evaluations. The objectives of the meeting are peer review and consensus. During the first few days, four subgroups (covering exposure data, cancer in humans, cancer in experimental animals, and mechanistic and other relevant data) review the working papers, develop a joint subgroup draft and write summaries. Care is taken to ensure that each study summary is written or reviewed by someone not associated with the study being considered. During the last few days, the Working Group meets in plenary session to review the subgroup drafts and develop the evaluations. As a result,

the entire volume is the joint product of the Working Group, and there are no individually authored sections.

IARC Working Groups strive to achieve a consensus evaluation. Consensus reflects broad agreement among Working Group Members, but not necessarily unanimity. The chair may elect to poll Working Group Members to determine the diversity of scientific opinion on issues where consensus is not readily apparent.

After the meeting, the master copy is verified by consulting the original literature, edited and prepared for publication. The aim is to publish the volume within six months of the Working Group meeting. A summary of the outcome is available on the *Monographs* programme web site soon after the meeting.

## B. SCIENTIFIC REVIEW AND EVALUATION

The available studies are summarized by the Working Group, with particular regard to the qualitative aspects discussed below. In general, numerical findings are indicated as they appear in the original report; units are converted when necessary for easier comparison. The Working Group may conduct additional analyses of the published data and use them in their assessment of the evidence; the results of such supplementary analyses are given in square brackets. When an important aspect of a study that directly impinges on its interpretation should be brought to the attention of the reader, a Working Group comment is given in square brackets.

The scope of the *IARC Monographs* programme has expanded beyond chemicals to include complex mixtures, occupational exposures, physical and biological agents, lifestyle factors and other potentially carcinogenic exposures. Over time, the structure of a *Monograph* has evolved to include the following sections:

## Exposure data

Studies of cancer in humans

Studies of cancer in experimental animals

Mechanistic and other relevant data

Summary

Evaluation and rationale

In addition, a section of General Remarks at the front of the volume discusses the reasons the agents were scheduled for evaluation and some key issues the Working Group encountered during the meeting.

This part of the Preamble discusses the types of evidence considered and summarized in each section of a *Monograph*, followed by the scientific criteria that guide the evaluations.

## 1. Exposure data

Each *Monograph* includes general information on the agent: this information may vary substantially between agents and must be adapted accordingly. Also included is information on production and use (when appropriate), methods of analysis and detection, occurrence, and sources and routes of human occupational and environmental exposures. Depending on the agent, regulations and guidelines for use may be presented.

### (a) General information on the agent

For chemical agents, sections on chemical and physical data are included: the Chemical Abstracts Service Registry Number, the latest primary name and the IUPAC systematic name are recorded; other synonyms are given, but the list is not necessarily comprehensive. Information on chemical and physical properties that are relevant to identification, occurrence and biological activity is included. A description of technical products of chemicals includes trade names, relevant specifications and available information on composition and impurities. Some of the trade names given may be those of mixtures in

which the agent being evaluated is only one of the ingredients.

For biological agents, taxonomy, structure and biology are described, and the degree of variability is indicated. Mode of replication, life cycle, target cells, persistence, latency, host response and clinical disease other than cancer are also presented.

For physical agents that are forms of radiation, energy and range of the radiation are included. For foreign bodies, fibres and respirable particles, size range and relative dimensions are indicated.

For agents such as mixtures, drugs or lifestyle factors, a description of the agent, including its composition, is given.

Whenever appropriate, other information, such as historical perspectives or the description of an industry or habit, may be included.

### (b) Analysis and detection

An overview of methods of analysis and detection of the agent is presented, including their sensitivity, specificity and reproducibility. Methods widely used for regulatory purposes are emphasized. Methods for monitoring human exposure are also given. No critical evaluation or recommendation of any method is meant or implied.

### (c) Production and use

The dates of first synthesis and of first commercial production of a chemical, mixture or other agent are provided when available; for agents that do not occur naturally, this information may allow a reasonable estimate to be made of the date before which no human exposure to the agent could have occurred. The dates of first reported occurrence of an exposure are also provided when available. In addition, methods of synthesis used in past and present commercial production and different methods of production,

which may give rise to different impurities, are described.

The countries where companies report production of the agent, and the number of companies in each country, are identified. Available data on production, international trade and uses are obtained for representative regions. It should not, however, be inferred that those areas or nations are necessarily the sole or major sources or users of the agent. Some identified uses may not be current or major applications, and the coverage is not necessarily comprehensive. In the case of drugs, mention of their therapeutic uses does not necessarily represent current practice nor does it imply judgement as to their therapeutic efficacy.

#### (d) Occurrence and exposure

Information on the occurrence of an agent in the environment is obtained from data derived from the monitoring and surveillance of levels in occupational environments, air, water, soil, plants, foods and animal and human tissues. When available, data on the generation, persistence and bioaccumulation of the agent are also included. Such data may be available from national databases.

Data that indicate the extent of past and present human exposure, the sources of exposure, the people most likely to be exposed and the factors that contribute to the exposure are reported. Information is presented on the range of human exposure, including occupational and environmental exposures. This includes relevant findings from both developed and developing countries. Some of these data are not distributed widely and may be available from government reports and other sources. In the case of mixtures, industries, occupations or processes, information is given about all agents known to be present. For processes, industries and occupations, a historical description is also given, noting variations in chemical composition, physical properties and levels of occupational exposure with date and

place. For biological agents, the epidemiology of infection is described.

#### (e) Regulations and guidelines

Statements concerning regulations and guidelines (e.g. occupational exposure limits, maximal levels permitted in foods and water, pesticide registrations) are included, but they may not reflect the most recent situation, since such limits are continuously reviewed and modified. The absence of information on regulatory status for a country should not be taken to imply that that country does not have regulations with regard to the exposure. For biological agents, legislation and control, including vaccination and therapy, are described.

## 2. Studies of cancer in humans

This section includes all pertinent epidemiological studies (see Part A, Section 4). Studies of biomarkers are included when they are relevant to an evaluation of carcinogenicity to humans.

#### (a) Types of study considered

Several types of epidemiological study contribute to the assessment of carcinogenicity in humans — cohort studies, case-control studies, correlation (or ecological) studies and intervention studies. Rarely, results from randomized trials may be available. Case reports and case series of cancer in humans may also be reviewed.

Cohort and case-control studies relate individual exposures under study to the occurrence of cancer in individuals and provide an estimate of effect (such as relative risk) as the main measure of association. Intervention studies may provide strong evidence for making causal inferences, as exemplified by cessation of smoking and the subsequent decrease in risk for lung cancer.

In correlation studies, the units of investigation are usually whole populations (e.g. in

particular geographical areas or at particular times), and cancer frequency is related to a summary measure of the exposure of the population to the agent under study. In correlation studies, individual exposure is not documented, which renders this kind of study more prone to confounding. In some circumstances, however, correlation studies may be more informative than analytical study designs (see, for example, the *Monograph* on arsenic in drinking-water; [IARC, 2004](#)).

In some instances, case reports and case series have provided important information about the carcinogenicity of an agent. These types of study generally arise from a suspicion, based on clinical experience, that the concurrence of two events — that is, a particular exposure and occurrence of a cancer — has happened rather more frequently than would be expected by chance. Case reports and case series usually lack complete ascertainment of cases in any population, definition or enumeration of the population at risk and estimation of the expected number of cases in the absence of exposure.

The uncertainties that surround the interpretation of case reports, case series and correlation studies make them inadequate, except in rare instances, to form the sole basis for inferring a causal relationship. When taken together with case-control and cohort studies, however, these types of study may add materially to the judgement that a causal relationship exists.

Epidemiological studies of benign neoplasms, presumed preneoplastic lesions and other end-points thought to be relevant to cancer are also reviewed. They may, in some instances, strengthen inferences drawn from studies of cancer itself.

### (b) *Quality of studies considered*

It is necessary to take into account the possible roles of bias, confounding and chance in the interpretation of epidemiological studies.

Bias is the effect of factors in study design or execution that lead erroneously to a stronger or weaker association than in fact exists between an agent and disease. Confounding is a form of bias that occurs when the relationship with disease is made to appear stronger or weaker than it truly is as a result of an association between the apparent causal factor and another factor that is associated with either an increase or decrease in the incidence of the disease. The role of chance is related to biological variability and the influence of sample size on the precision of estimates of effect.

In evaluating the extent to which these factors have been minimized in an individual study, consideration is given to several aspects of design and analysis as described in the report of the study. For example, when suspicion of carcinogenicity arises largely from a single small study, careful consideration is given when interpreting subsequent studies that included these data in an enlarged population. Most of these considerations apply equally to case-control, cohort and correlation studies. Lack of clarity of any of these aspects in the reporting of a study can decrease its credibility and the weight given to it in the final evaluation of the exposure.

First, the study population, disease (or diseases) and exposure should have been well defined by the authors. Cases of disease in the study population should have been identified in a way that was independent of the exposure of interest, and exposure should have been assessed in a way that was not related to disease status.

Second, the authors should have taken into account — in the study design and analysis — other variables that can influence the risk of disease and may have been related to the exposure of interest. Potential confounding by such variables should have been dealt with either in the design of the study, such as by matching, or in the analysis, by statistical adjustment. In cohort studies, comparisons with local rates of disease may or may not be more appropriate than those with national rates. Internal comparisons of

frequency of disease among individuals at different levels of exposure are also desirable in cohort studies, since they minimize the potential for confounding related to the difference in risk factors between an external reference group and the study population.

Third, the authors should have reported the basic data on which the conclusions are founded, even if sophisticated statistical analyses were employed. At the very least, they should have given the numbers of exposed and unexposed cases and controls in a case–control study and the numbers of cases observed and expected in a cohort study. Further tabulations by time since exposure began and other temporal factors are also important. In a cohort study, data on all cancer sites and all causes of death should have been given, to reveal the possibility of reporting bias. In a case–control study, the effects of investigated factors other than the exposure of interest should have been reported.

Finally, the statistical methods used to obtain estimates of relative risk, absolute rates of cancer, confidence intervals and significance tests, and to adjust for confounding should have been clearly stated by the authors. These methods have been reviewed for case–control studies ([Breslow & Day, 1980](#)) and for cohort studies ([Breslow & Day, 1987](#)).

#### (c) *Meta-analyses and pooled analyses*

Independent epidemiological studies of the same agent may lead to results that are difficult to interpret. Combined analyses of data from multiple studies are a means of resolving this ambiguity, and well conducted analyses can be considered. There are two types of combined analysis. The first involves combining summary statistics such as relative risks from individual studies (meta-analysis) and the second involves a pooled analysis of the raw data from the individual studies (pooled analysis) ([Greenland, 1998](#)).

The advantages of combined analyses are increased precision due to increased sample size and the opportunity to explore potential confounders, interactions and modifying effects that may explain heterogeneity among studies in more detail. A disadvantage of combined analyses is the possible lack of compatibility of data from various studies due to differences in subject recruitment, procedures of data collection, methods of measurement and effects of unmeasured co-variates that may differ among studies. Despite these limitations, well conducted combined analyses may provide a firmer basis than individual studies for drawing conclusions about the potential carcinogenicity of agents.

IARC may commission a meta-analysis or pooled analysis that is pertinent to a particular *Monograph* (see Part A, Section 4). Additionally, as a means of gaining insight from the results of multiple individual studies, ad hoc calculations that combine data from different studies may be conducted by the Working Group during the course of a *Monograph* meeting. The results of such original calculations, which would be specified in the text by presentation in square brackets, might involve updates of previously conducted analyses that incorporate the results of more recent studies or de-novo analyses. Irrespective of the source of data for the meta-analyses and pooled analyses, it is important that the same criteria for data quality be applied as those that would be applied to individual studies and to ensure also that sources of heterogeneity between studies be taken into account.

#### (d) *Temporal effects*

Detailed analyses of both relative and absolute risks in relation to temporal variables, such as age at first exposure, time since first exposure, duration of exposure, cumulative exposure, peak exposure (when appropriate) and time since cessation of exposure, are reviewed and summarized when available. Analyses of temporal

relationships may be useful in making causal inferences. In addition, such analyses may suggest whether a carcinogen acts early or late in the process of carcinogenesis, although, at best, they allow only indirect inferences about mechanisms of carcinogenesis.

(e) *Use of biomarkers in epidemiological studies*

Biomarkers indicate molecular, cellular or other biological changes and are increasingly used in epidemiological studies for various purposes ([IARC, 1991](#); [Vainio et al., 1992](#); [Toniolo et al., 1997](#); [Vineis et al., 1999](#); [Buffler et al., 2004](#)). These may include evidence of exposure, of early effects, of cellular, tissue or organism responses, of individual susceptibility or host responses, and inference of a mechanism (see Part B, Section 4b). This is a rapidly evolving field that encompasses developments in genomics, epigenomics and other emerging technologies.

Molecular epidemiological data that identify associations between genetic polymorphisms and interindividual differences in susceptibility to the agent(s) being evaluated may contribute to the identification of carcinogenic hazards to humans. If the polymorphism has been demonstrated experimentally to modify the functional activity of the gene product in a manner that is consistent with increased susceptibility, these data may be useful in making causal inferences. Similarly, molecular epidemiological studies that measure cell functions, enzymes or metabolites that are thought to be the basis of susceptibility may provide evidence that reinforces biological plausibility. It should be noted, however, that when data on genetic susceptibility originate from multiple comparisons that arise from subgroup analyses, this can generate false-positive results and inconsistencies across studies, and such data therefore require careful evaluation. If the known phenotype of a genetic polymorphism can explain the carcinogenic mechanism

of the agent being evaluated, data on this phenotype may be useful in making causal inferences.

(f) *Criteria for causality*

After the quality of individual epidemiological studies of cancer has been summarized and assessed, a judgement is made concerning the strength of evidence that the agent in question is carcinogenic to humans. In making its judgement, the Working Group considers several criteria for causality ([Hill, 1965](#)). A strong association (e.g. a large relative risk) is more likely to indicate causality than a weak association, although it is recognized that estimates of effect of small magnitude do not imply lack of causality and may be important if the disease or exposure is common. Associations that are replicated in several studies of the same design or that use different epidemiological approaches or under different circumstances of exposure are more likely to represent a causal relationship than isolated observations from single studies. If there are inconsistent results among investigations, possible reasons are sought (such as differences in exposure), and results of studies that are judged to be of high quality are given more weight than those of studies that are judged to be methodologically less sound.

If the risk increases with the exposure, this is considered to be a strong indication of causality, although the absence of a graded response is not necessarily evidence against a causal relationship. The demonstration of a decline in risk after cessation of or reduction in exposure in individuals or in whole populations also supports a causal interpretation of the findings.

Several scenarios may increase confidence in a causal relationship. On the one hand, an agent may be specific in causing tumours at one site or of one morphological type. On the other, carcinogenicity may be evident through the causation of multiple tumour types. Temporality, precision of estimates of effect, biological plausibility and

coherence of the overall database are considered. Data on biomarkers may be employed in an assessment of the biological plausibility of epidemiological observations.

Although rarely available, results from randomized trials that show different rates of cancer among exposed and unexposed individuals provide particularly strong evidence for causality.

When several epidemiological studies show little or no indication of an association between an exposure and cancer, a judgement may be made that, in the aggregate, they show evidence of lack of carcinogenicity. Such a judgement requires first that the studies meet, to a sufficient degree, the standards of design and analysis described above. Specifically, the possibility that bias, confounding or misclassification of exposure or outcome could explain the observed results should be considered and excluded with reasonable certainty. In addition, all studies that are judged to be methodologically sound should (a) be consistent with an estimate of effect of unity for any observed level of exposure, (b) when considered together, provide a pooled estimate of relative risk that is at or near to unity, and (c) have a narrow confidence interval, due to sufficient population size. Moreover, no individual study nor the pooled results of all the studies should show any consistent tendency that the relative risk of cancer increases with increasing level of exposure. It is important to note that evidence of lack of carcinogenicity obtained from several epidemiological studies can apply only to the type(s) of cancer studied, to the dose levels reported, and to the intervals between first exposure and disease onset observed in these studies. Experience with human cancer indicates that the period from first exposure to the development of clinical cancer is sometimes longer than 20 years; latent periods substantially shorter than 30 years cannot provide evidence for lack of carcinogenicity.

### 3. Studies of cancer in experimental animals

All known human carcinogens that have been studied adequately for carcinogenicity in experimental animals have produced positive results in one or more animal species ([Wilbourn et al., 1986](#); [Tomatis et al., 1989](#)). For several agents (e.g. aflatoxins, diethylstilbestrol, solar radiation, vinyl chloride), carcinogenicity in experimental animals was established or highly suspected before epidemiological studies confirmed their carcinogenicity in humans ([Vainio et al., 1995](#)). Although this association cannot establish that all agents that cause cancer in experimental animals also cause cancer in humans, it is biologically plausible that agents for which there is *sufficient evidence of carcinogenicity* in experimental animals (see Part B, Section 6b) also present a carcinogenic hazard to humans. Accordingly, in the absence of additional scientific information, these agents are considered to pose a carcinogenic hazard to humans. Examples of additional scientific information are data that demonstrate that a given agent causes cancer in animals through a species-specific mechanism that does not operate in humans or data that demonstrate that the mechanism in experimental animals also operates in humans (see Part B, Section 6).

Consideration is given to all available long-term studies of cancer in experimental animals with the agent under review (see Part A, Section 4). In all experimental settings, the nature and extent of impurities or contaminants present in the agent being evaluated are given when available. Animal species, strain (including genetic background where applicable), sex, numbers per group, age at start of treatment, route of exposure, dose levels, duration of exposure, survival and information on tumours (incidence, latency, severity or multiplicity of neoplasms or preneoplastic lesions) are reported. Those studies in experimental animals that are judged to be irrelevant to the evaluation or judged to be inadequate

(e.g. too short a duration, too few animals, poor survival; see below) may be omitted. Guidelines for conducting long-term carcinogenicity experiments have been published (e.g. [OECD, 2002](#)).

Other studies considered may include: experiments in which the agent was administered in the presence of factors that modify carcinogenic effects (e.g. initiation–promotion studies, co-carcinogenicity studies and studies in genetically modified animals); studies in which the end-point was not cancer but a defined pre-cancerous lesion; experiments on the carcinogenicity of known metabolites and derivatives; and studies of cancer in non-laboratory animals (e.g. livestock and companion animals) exposed to the agent.

For studies of mixtures, consideration is given to the possibility that changes in the physicochemical properties of the individual substances may occur during collection, storage, extraction, concentration and delivery. Another consideration is that chemical and toxicological interactions of components in a mixture may alter dose–response relationships. The relevance to human exposure of the test mixture administered in the animal experiment is also assessed. This may involve consideration of the following aspects of the mixture tested: (i) physical and chemical characteristics, (ii) identified constituents that may indicate the presence of a class of substances and (iii) the results of genetic toxicity and related tests.

The relevance of results obtained with an agent that is analogous (e.g. similar in structure or of a similar virus genus) to that being evaluated is also considered. Such results may provide biological and mechanistic information that is relevant to the understanding of the process of carcinogenesis in humans and may strengthen the biological plausibility that the agent being evaluated is carcinogenic to humans (see Part B, Section 2f).

### (a) Qualitative aspects

An assessment of carcinogenicity involves several considerations of qualitative importance, including (i) the experimental conditions under which the test was performed, including route, schedule and duration of exposure, species, strain (including genetic background where applicable), sex, age and duration of follow-up; (ii) the consistency of the results, for example, across species and target organ(s); (iii) the spectrum of neoplastic response, from preneoplastic lesions and benign tumours to malignant neoplasms; and (iv) the possible role of modifying factors.

Considerations of importance in the interpretation and evaluation of a particular study include: (i) how clearly the agent was defined and, in the case of mixtures, how adequately the sample characterization was reported; (ii) whether the dose was monitored adequately, particularly in inhalation experiments; (iii) whether the doses, duration of treatment and route of exposure were appropriate; (iv) whether the survival of treated animals was similar to that of controls; (v) whether there were adequate numbers of animals per group; (vi) whether both male and female animals were used; (vii) whether animals were allocated randomly to groups; (viii) whether the duration of observation was adequate; and (ix) whether the data were reported and analysed adequately.

When benign tumours (a) occur together with and originate from the same cell type as malignant tumours in an organ or tissue in a particular study and (b) appear to represent a stage in the progression to malignancy, they are usually combined in the assessment of tumour incidence ([Huff et al., 1989](#)). The occurrence of lesions presumed to be preneoplastic may in certain instances aid in assessing the biological plausibility of any neoplastic response observed. If an agent induces only benign neoplasms that appear to be end-points that do not readily undergo

transition to malignancy, the agent should nevertheless be suspected of being carcinogenic and requires further investigation.

### (b) Quantitative aspects

The probability that tumours will occur may depend on the species, sex, strain, genetic background and age of the animal, and on the dose, route, timing and duration of the exposure. Evidence of an increased incidence of neoplasms with increasing levels of exposure strengthens the inference of a causal association between the exposure and the development of neoplasms.

The form of the dose–response relationship can vary widely, depending on the particular agent under study and the target organ. Mechanisms such as induction of DNA damage or inhibition of repair, altered cell division and cell death rates and changes in intercellular communication are important determinants of dose–response relationships for some carcinogens. Since many chemicals require metabolic activation before being converted to their reactive intermediates, both metabolic and toxicokinetic aspects are important in determining the dose–response pattern. Saturation of steps such as absorption, activation, inactivation and elimination may produce nonlinearity in the dose–response relationship ([Hoel et al., 1983](#); [Gart et al., 1986](#)), as could saturation of processes such as DNA repair. The dose–response relationship can also be affected by differences in survival among the treatment groups.

### (c) Statistical analyses

Factors considered include the adequacy of the information given for each treatment group: (i) number of animals studied and number examined histologically, (ii) number of animals with a given tumour type and (iii) length of survival. The statistical methods used should be clearly stated and should be the generally accepted techniques refined for this purpose ([Peto et al., 1980](#);

[Gart et al., 1986](#); [Portier & Bailer, 1989](#); [Bieler & Williams, 1993](#)). The choice of the most appropriate statistical method requires consideration of whether or not there are differences in survival among the treatment groups; for example, reduced survival because of non-tumour-related mortality can preclude the occurrence of tumours later in life. When detailed information on survival is not available, comparisons of the proportions of tumour-bearing animals among the effective number of animals (alive at the time the first tumour was discovered) can be useful when significant differences in survival occur before tumours appear. The lethality of the tumour also requires consideration: for rapidly fatal tumours, the time of death provides an indication of the time of tumour onset and can be assessed using life-table methods; non-fatal or incidental tumours that do not affect survival can be assessed using methods such as the Mantel-Haenzel test for changes in tumour prevalence. Because tumour lethality is often difficult to determine, methods such as the Poly-K test that do not require such information can also be used. When results are available on the number and size of tumours seen in experimental animals (e.g. papillomas on mouse skin, liver tumours observed through nuclear magnetic resonance tomography), other more complicated statistical procedures may be needed ([Sherman et al., 1994](#); [Dunson et al., 2003](#)).

Formal statistical methods have been developed to incorporate historical control data into the analysis of data from a given experiment. These methods assign an appropriate weight to historical and concurrent controls on the basis of the extent of between-study and within-study variability: less weight is given to historical controls when they show a high degree of variability, and greater weight when they show little variability. It is generally not appropriate to discount a tumour response that is significantly increased compared with concurrent controls by arguing that it falls within the range of historical controls,

particularly when historical controls show high between-study variability and are, thus, of little relevance to the current experiment. In analysing results for uncommon tumours, however, the analysis may be improved by considering historical control data, particularly when between-study variability is low. Historical controls should be selected to resemble the concurrent controls as closely as possible with respect to species, gender and strain, as well as other factors such as basal diet and general laboratory environment, which may affect tumour-response rates in control animals ([Haseman et al., 1984](#); [Fung et al., 1996](#); [Greim et al., 2003](#)).

Although meta-analyses and combined analyses are conducted less frequently for animal experiments than for epidemiological studies due to differences in animal strains, they can be useful aids in interpreting animal data when the experimental protocols are sufficiently similar.

#### 4. Mechanistic and other relevant data

Mechanistic and other relevant data may provide evidence of carcinogenicity and also help in assessing the relevance and importance of findings of cancer in animals and in humans. The nature of the mechanistic and other relevant data depends on the biological activity of the agent being considered. The Working Group considers representative studies to give a concise description of the relevant data and issues that they consider to be important; thus, not every available study is cited. Relevant topics may include toxicokinetics, mechanisms of carcinogenesis, susceptible individuals, populations and life-stages, other relevant data and other adverse effects. When data on biomarkers are informative about the mechanisms of carcinogenesis, they are included in this section.

These topics are not mutually exclusive; thus, the same studies may be discussed in more than

one subsection. For example, a mutation in a gene that codes for an enzyme that metabolizes the agent under study could be discussed in the subsections on toxicokinetics, mechanisms and individual susceptibility if it also exists as an inherited polymorphism.

##### (a) Toxicokinetic data

Toxicokinetics refers to the absorption, distribution, metabolism and elimination of agents in humans, experimental animals and, where relevant, cellular systems. Examples of kinetic factors that may affect dose-response relationships include uptake, deposition, biopersistence and half-life in tissues, protein binding, metabolic activation and detoxification. Studies that indicate the metabolic fate of the agent in humans and in experimental animals are summarized briefly, and comparisons of data from humans and animals are made when possible. Comparative information on the relationship between exposure and the dose that reaches the target site may be important for the extrapolation of hazards between species and in clarifying the role of in-vitro findings.

##### (b) Data on mechanisms of carcinogenesis

To provide focus, the Working Group attempts to identify the possible mechanisms by which the agent may increase the risk of cancer. For each possible mechanism, a representative selection of key data from humans and experimental systems is summarized. Attention is given to gaps in the data and to data that suggests that more than one mechanism may be operating. The relevance of the mechanism to humans is discussed, in particular, when mechanistic data are derived from experimental model systems. Changes in the affected organs, tissues or cells can be divided into three non-exclusive levels as described below.

### (i) Changes in physiology

Physiological changes refer to exposure-related modifications to the physiology and/or response of cells, tissues and organs. Examples of potentially adverse physiological changes include mitogenesis, compensatory cell division, escape from apoptosis and/or senescence, presence of inflammation, hyperplasia, metaplasia and/or preneoplasia, angiogenesis, alterations in cellular adhesion, changes in steroid hormones and changes in immune surveillance.

### (ii) Functional changes at the cellular level

Functional changes refer to exposure-related alterations in the signalling pathways used by cells to manage critical processes that are related to increased risk for cancer. Examples of functional changes include modified activities of enzymes involved in the metabolism of xenobiotics, alterations in the expression of key genes that regulate DNA repair, alterations in cyclin-dependent kinases that govern cell cycle progression, changes in the patterns of post-translational modifications of proteins, changes in regulatory factors that alter apoptotic rates, changes in the secretion of factors related to the stimulation of DNA replication and transcription and changes in gap-junction-mediated intercellular communication.

### (iii) Changes at the molecular level

Molecular changes refer to exposure-related changes in key cellular structures at the molecular level, including, in particular, genotoxicity. Examples of molecular changes include formation of DNA adducts and DNA strand breaks, mutations in genes, chromosomal aberrations, aneuploidy and changes in DNA methylation patterns. Greater emphasis is given to irreversible effects.

The use of mechanistic data in the identification of a carcinogenic hazard is specific to the mechanism being addressed and is not readily

described for every possible level and mechanism discussed above.

Genotoxicity data are discussed here to illustrate the key issues involved in the evaluation of mechanistic data.

Tests for genetic and related effects are described in view of the relevance of gene mutation and chromosomal aberration/aneuploidy to carcinogenesis ([Vainio et al., 1992](#); [McGregor et al., 1999](#)). The adequacy of the reporting of sample characterization is considered and, when necessary, commented upon; with regard to complex mixtures, such comments are similar to those described for animal carcinogenicity tests. The available data are interpreted critically according to the end-points detected, which may include DNA damage, gene mutation, sister chromatid exchange, micronucleus formation, chromosomal aberrations and aneuploidy. The concentrations employed are given, and mention is made of whether the use of an exogenous metabolic system *in vitro* affected the test result. These data are listed in tabular form by phylogenetic classification.

Positive results in tests using prokaryotes, lower eukaryotes, insects, plants and cultured mammalian cells suggest that genetic and related effects could occur in mammals. Results from such tests may also give information on the types of genetic effect produced and on the involvement of metabolic activation. Some endpoints described are clearly genetic in nature (e.g. gene mutations), while others are associated with genetic effects (e.g. unscheduled DNA synthesis). In-vitro tests for tumour promotion, cell transformation and gap-junction intercellular communication may be sensitive to changes that are not necessarily the result of genetic alterations but that may have specific relevance to the process of carcinogenesis. Critical appraisals of these tests have been published ([Montesano et al., 1986](#); [McGregor et al., 1999](#)).

Genetic or other activity manifest in humans and experimental mammals is regarded to be of

greater relevance than that in other organisms. The demonstration that an agent can induce gene and chromosomal mutations in mammals *in vivo* indicates that it may have carcinogenic activity. Negative results in tests for mutagenicity in selected tissues from animals treated *in vivo* provide less weight, partly because they do not exclude the possibility of an effect in tissues other than those examined. Moreover, negative results in short-term tests with genetic end-points cannot be considered to provide evidence that rules out the carcinogenicity of agents that act through other mechanisms (e.g. receptor-mediated effects, cellular toxicity with regenerative cell division, peroxisome proliferation) ([Vainio et al., 1992](#)). Factors that may give misleading results in short-term tests have been discussed in detail elsewhere ([Montesano et al., 1986](#); [McGregor et al., 1999](#)).

When there is evidence that an agent acts by a specific mechanism that does not involve genotoxicity (e.g. hormonal dysregulation, immune suppression, and formation of calculi and other deposits that cause chronic irritation), that evidence is presented and reviewed critically in the context of rigorous criteria for the operation of that mechanism in carcinogenesis (e.g. [Capen et al., 1999](#)).

For biological agents such as viruses, bacteria and parasites, other data relevant to carcinogenicity may include descriptions of the pathology of infection, integration and expression of viruses, and genetic alterations seen in human tumours. Other observations that might comprise cellular and tissue responses to infection, immune response and the presence of tumour markers are also considered.

For physical agents that are forms of radiation, other data relevant to carcinogenicity may include descriptions of damaging effects at the physiological, cellular and molecular level, as for chemical agents, and descriptions of how these effects occur. ‘Physical agents’ may also be considered to comprise foreign bodies, such as

surgical implants of various kinds, and poorly soluble fibres, dusts and particles of various sizes, the pathogenic effects of which are a result of their physical presence in tissues or body cavities. Other relevant data for such materials may include characterization of cellular, tissue and physiological reactions to these materials and descriptions of pathological conditions other than neoplasia with which they may be associated.

#### (c) *Other data relevant to mechanisms*

A description is provided of any structure–activity relationships that may be relevant to an evaluation of the carcinogenicity of an agent, the toxicological implications of the physical and chemical properties, and any other data relevant to the evaluation that are not included elsewhere.

High-output data, such as those derived from gene expression microarrays, and high-throughput data, such as those that result from testing hundreds of agents for a single end-point, pose a unique problem for the use of mechanistic data in the evaluation of a carcinogenic hazard. In the case of high-output data, there is the possibility to overinterpret changes in individual end-points (e.g. changes in expression in one gene) without considering the consistency of that finding in the broader context of the other end-points (e.g. other genes with linked transcriptional control). High-output data can be used in assessing mechanisms, but all end-points measured in a single experiment need to be considered in the proper context. For high-throughput data, where the number of observations far exceeds the number of end-points measured, their utility for identifying common mechanisms across multiple agents is enhanced. These data can be used to identify mechanisms that not only seem plausible, but also have a consistent pattern of carcinogenic response across entire classes of related compounds.

#### (d) Susceptibility data

Individuals, populations and life-stages may have greater or lesser susceptibility to an agent, based on toxicokinetics, mechanisms of carcinogenesis and other factors. Examples of host and genetic factors that affect individual susceptibility include sex, genetic polymorphisms of genes involved in the metabolism of the agent under evaluation, differences in metabolic capacity due to life-stage or the presence of disease, differences in DNA repair capacity, competition for or alteration of metabolic capacity by medications or other chemical exposures, pre-existing hormonal imbalance that is exacerbated by a chemical exposure, a suppressed immune system, periods of higher-than-usual tissue growth or regeneration and genetic polymorphisms that lead to differences in behaviour (e.g. addiction). Such data can substantially increase the strength of the evidence from epidemiological data and enhance the linkage of in-vivo and in-vitro laboratory studies to humans.

#### (e) Data on other adverse effects

Data on acute, subchronic and chronic adverse effects relevant to the cancer evaluation are summarized. Adverse effects that confirm distribution and biological effects at the sites of tumour development, or alterations in physiology that could lead to tumour development, are emphasized. Effects on reproduction, embryonic and fetal survival and development are summarized briefly. The adequacy of epidemiological studies of reproductive outcome and genetic and related effects in humans is judged by the same criteria as those applied to epidemiological studies of cancer, but fewer details are given.

## 5. Summary

This section is a summary of data presented in the preceding sections. Summaries can be

found on the *Monographs* programme web site (<http://monographs.iarc.fr>).

#### (a) Exposure data

Data are summarized, as appropriate, on the basis of elements such as production, use, occurrence and exposure levels in the workplace and environment and measurements in human tissues and body fluids. Quantitative data and time trends are given to compare exposures in different occupations and environmental settings. Exposure to biological agents is described in terms of transmission, prevalence and persistence of infection.

#### (b) Cancer in humans

Results of epidemiological studies pertinent to an assessment of human carcinogenicity are summarized. When relevant, case reports and correlation studies are also summarized. The target organ(s) or tissue(s) in which an increase in cancer was observed is identified. Dose-response and other quantitative data may be summarized when available.

#### (c) Cancer in experimental animals

Data relevant to an evaluation of carcinogenicity in animals are summarized. For each animal species, study design and route of administration, it is stated whether an increased incidence, reduced latency, or increased severity or multiplicity of neoplasms or preneoplastic lesions were observed, and the tumour sites are indicated. If the agent produced tumours after prenatal exposure or in single-dose experiments, this is also mentioned. Negative findings, inverse relationships, dose-response and other quantitative data are also summarized.

#### (d) Mechanistic and other relevant data

Data relevant to the toxicokinetics (absorption, distribution, metabolism, elimination) and

the possible mechanism(s) of carcinogenesis (e.g. genetic toxicity, epigenetic effects) are summarized. In addition, information on susceptible individuals, populations and life-stages is summarized. This section also reports on other toxic effects, including reproductive and developmental effects, as well as additional relevant data that are considered to be important.

## 6. Evaluation and rationale

Evaluations of the strength of the evidence for carcinogenicity arising from human and experimental animal data are made, using standard terms. The strength of the mechanistic evidence is also characterized.

It is recognized that the criteria for these evaluations, described below, cannot encompass all of the factors that may be relevant to an evaluation of carcinogenicity. In considering all of the relevant scientific data, the Working Group may assign the agent to a higher or lower category than a strict interpretation of these criteria would indicate.

These categories refer only to the strength of the evidence that an exposure is carcinogenic and not to the extent of its carcinogenic activity (potency). A classification may change as new information becomes available.

An evaluation of the degree of evidence is limited to the materials tested, as defined physically, chemically or biologically. When the agents evaluated are considered by the Working Group to be sufficiently closely related, they may be grouped together for the purpose of a single evaluation of the degree of evidence.

### (a) Carcinogenicity in humans

The evidence relevant to carcinogenicity from studies in humans is classified into one of the following categories:

**Sufficient evidence of carcinogenicity:** The Working Group considers that a causal

relationship has been established between exposure to the agent and human cancer. That is, a positive relationship has been observed between the exposure and cancer in studies in which chance, bias and confounding could be ruled out with reasonable confidence. A statement that there is *sufficient evidence* is followed by a separate sentence that identifies the target organ(s) or tissue(s) where an increased risk of cancer was observed in humans. Identification of a specific target organ or tissue does not preclude the possibility that the agent may cause cancer at other sites.

**Limited evidence of carcinogenicity:** A positive association has been observed between exposure to the agent and cancer for which a causal interpretation is considered by the Working Group to be credible, but chance, bias or confounding could not be ruled out with reasonable confidence.

**Inadequate evidence of carcinogenicity:** The available studies are of insufficient quality, consistency or statistical power to permit a conclusion regarding the presence or absence of a causal association between exposure and cancer, or no data on cancer in humans are available.

**Evidence suggesting lack of carcinogenicity:** There are several adequate studies covering the full range of levels of exposure that humans are known to encounter, which are mutually consistent in not showing a positive association between exposure to the agent and any studied cancer at any observed level of exposure. The results from these studies alone or combined should have narrow confidence intervals with an upper limit close to the null value (e.g. a relative risk of 1.0). Bias and confounding should be ruled out with reasonable confidence, and the studies should have an adequate length of follow-up. A conclusion of *evidence suggesting lack of carcinogenicity* is inevitably limited to the cancer sites, conditions and levels of exposure, and length of observation covered by the available studies. In

addition, the possibility of a very small risk at the levels of exposure studied can never be excluded.

In some instances, the above categories may be used to classify the degree of evidence related to carcinogenicity in specific organs or tissues.

When the available epidemiological studies pertain to a mixture, process, occupation or industry, the Working Group seeks to identify the specific agent considered most likely to be responsible for any excess risk. The evaluation is focused as narrowly as the available data on exposure and other aspects permit.

**(b) Carcinogenicity in experimental animals**

Carcinogenicity in experimental animals can be evaluated using conventional bioassays, bioassays that employ genetically modified animals, and other in-vivo bioassays that focus on one or more of the critical stages of carcinogenesis. In the absence of data from conventional long-term bioassays or from assays with neoplasia as the end-point, consistently positive results in several models that address several stages in the multi-stage process of carcinogenesis should be considered in evaluating the degree of evidence of carcinogenicity in experimental animals.

The evidence relevant to carcinogenicity in experimental animals is classified into one of the following categories:

**Sufficient evidence of carcinogenicity:** The Working Group considers that a causal relationship has been established between the agent and an increased incidence of malignant neoplasms or of an appropriate combination of benign and malignant neoplasms in (a) two or more species of animals or (b) two or more independent studies in one species carried out at different times or in different laboratories or under different protocols. An increased incidence of tumours in both sexes of a single species in a well conducted study, ideally conducted under Good Laboratory Practices, can also provide *sufficient evidence*.

A single study in one species and sex might be considered to provide *sufficient evidence of carcinogenicity* when malignant neoplasms occur to an unusual degree with regard to incidence, site, type of tumour or age at onset, or when there are strong findings of tumours at multiple sites.

**Limited evidence of carcinogenicity:**

The data suggest a carcinogenic effect but are limited for making a definitive evaluation because, e.g. (a) the evidence of carcinogenicity is restricted to a single experiment; (b) there are unresolved questions regarding the adequacy of the design, conduct or interpretation of the studies; (c) the agent increases the incidence only of benign neoplasms or lesions of uncertain neoplastic potential; or (d) the evidence of carcinogenicity is restricted to studies that demonstrate only promoting activity in a narrow range of tissues or organs.

**Inadequate evidence of carcinogenicity:**

The studies cannot be interpreted as showing either the presence or absence of a carcinogenic effect because of major qualitative or quantitative limitations, or no data on cancer in experimental animals are available.

**Evidence suggesting lack of carcinogenicity:**

Adequate studies involving at least two species are available which show that, within the limits of the tests used, the agent is not carcinogenic. A conclusion of *evidence suggesting lack of carcinogenicity* is inevitably limited to the species, tumour sites, age at exposure, and conditions and levels of exposure studied.

**(c) Mechanistic and other relevant data**

Mechanistic and other evidence judged to be relevant to an evaluation of carcinogenicity and of sufficient importance to affect the overall evaluation is highlighted. This may include data on preneoplastic lesions, tumour pathology, genetic and related effects, structure–activity relationships, metabolism and toxicokinetics,

physicochemical parameters and analogous biological agents.

The strength of the evidence that any carcinogenic effect observed is due to a particular mechanism is evaluated, using terms such as ‘weak’, ‘moderate’ or ‘strong’. The Working Group then assesses whether that particular mechanism is likely to be operative in humans. The strongest indications that a particular mechanism operates in humans derive from data on humans or biological specimens obtained from exposed humans. The data may be considered to be especially relevant if they show that the agent in question has caused changes in exposed humans that are on the causal pathway to carcinogenesis. Such data may, however, never become available, because it is at least conceivable that certain compounds may be kept from human use solely on the basis of evidence of their toxicity and/or carcinogenicity in experimental systems.

The conclusion that a mechanism operates in experimental animals is strengthened by findings of consistent results in different experimental systems, by the demonstration of biological plausibility and by coherence of the overall database. Strong support can be obtained from studies that challenge the hypothesized mechanism experimentally, by demonstrating that the suppression of key mechanistic processes leads to the suppression of tumour development. The Working Group considers whether multiple mechanisms might contribute to tumour development, whether different mechanisms might operate in different dose ranges, whether separate mechanisms might operate in humans and experimental animals and whether a unique mechanism might operate in a susceptible group. The possible contribution of alternative mechanisms must be considered before concluding that tumours observed in experimental animals are not relevant to humans. An uneven level of experimental support for different mechanisms may reflect that disproportionate resources

have been focused on investigating a favoured mechanism.

For complex exposures, including occupational and industrial exposures, the chemical composition and the potential contribution of carcinogens known to be present are considered by the Working Group in its overall evaluation of human carcinogenicity. The Working Group also determines the extent to which the materials tested in experimental systems are related to those to which humans are exposed.

#### (d) Overall evaluation

Finally, the body of evidence is considered as a whole, to reach an overall evaluation of the carcinogenicity of the agent to humans.

An evaluation may be made for a group of agents that have been evaluated by the Working Group. In addition, when supporting data indicate that other related agents, for which there is no direct evidence of their capacity to induce cancer in humans or in animals, may also be carcinogenic, a statement describing the rationale for this conclusion is added to the evaluation narrative; an additional evaluation may be made for this broader group of agents if the strength of the evidence warrants it.

The agent is described according to the wording of one of the following categories, and the designated group is given. The categorization of an agent is a matter of scientific judgement that reflects the strength of the evidence derived from studies in humans and in experimental animals and from mechanistic and other relevant data.

#### **Group 1: The agent is carcinogenic to humans.**

This category is used when there is *sufficient evidence of carcinogenicity* in humans. Exceptionally, an agent may be placed in this category when evidence of carcinogenicity in humans is less than *sufficient* but there is *sufficient evidence of carcinogenicity* in experimental

animals and strong evidence in exposed humans that the agent acts through a relevant mechanism of carcinogenicity.

### **Group 2.**

This category includes agents for which, at one extreme, the degree of evidence of carcinogenicity in humans is almost *sufficient*, as well as those for which, at the other extreme, there are no human data but for which there is evidence of carcinogenicity in experimental animals. Agents are assigned to either Group 2A (*probably carcinogenic to humans*) or Group 2B (*possibly carcinogenic to humans*) on the basis of epidemiological and experimental evidence of carcinogenicity and mechanistic and other relevant data. The terms *probably carcinogenic* and *possibly carcinogenic* have no quantitative significance and are used simply as descriptors of different levels of evidence of human carcinogenicity, with *probably carcinogenic* signifying a higher level of evidence than *possibly carcinogenic*.

### **Group 2A: The agent is probably carcinogenic to humans.**

This category is used when there is *limited evidence of carcinogenicity* in humans and *sufficient evidence of carcinogenicity* in experimental animals. In some cases, an agent may be classified in this category when there is *inadequate evidence of carcinogenicity* in humans and *sufficient evidence of carcinogenicity* in experimental animals and strong evidence that the carcinogenesis is mediated by a mechanism that also operates in humans. Exceptionally, an agent may be classified in this category solely on the basis of *limited evidence of carcinogenicity* in humans. An agent may be assigned to this category if it clearly belongs, based on mechanistic considerations, to a class of agents for which one or more members have been classified in Group 1 or Group 2A.

### **Group 2B: The agent is possibly carcinogenic to humans.**

This category is used for agents for which there is *limited evidence of carcinogenicity* in humans and less than *sufficient evidence of carcinogenicity* in experimental animals. It may also be used when there is *inadequate evidence of carcinogenicity* in humans but there is *sufficient evidence of carcinogenicity* in experimental animals. In some instances, an agent for which there is *inadequate evidence of carcinogenicity* in humans and less than *sufficient evidence of carcinogenicity* in experimental animals together with supporting evidence from mechanistic and other relevant data may be placed in this group. An agent may be classified in this category solely on the basis of strong evidence from mechanistic and other relevant data.

### **Group 3: The agent is not classifiable as to its carcinogenicity to humans.**

This category is used most commonly for agents for which the evidence of carcinogenicity is *inadequate* in humans and *inadequate* or *limited* in experimental animals.

Exceptionally, agents for which the evidence of carcinogenicity is *inadequate* in humans but *sufficient* in experimental animals may be placed in this category when there is strong evidence that the mechanism of carcinogenicity in experimental animals does not operate in humans.

Agents that do not fall into any other group are also placed in this category.

An evaluation in Group 3 is not a determination of non-carcinogenicity or overall safety. It often means that further research is needed, especially when exposures are widespread or the cancer data are consistent with differing interpretations.

### **Group 4: The agent is probably not carcinogenic to humans.**

This category is used for agents for which there is *evidence suggesting lack of carcinogenicity*

in humans and in experimental animals. In some instances, agents for which there is *inadequate evidence of carcinogenicity* in humans but *evidence suggesting lack of carcinogenicity* in experimental animals, consistently and strongly supported by a broad range of mechanistic and other relevant data, may be classified in this group.

#### (e) Rationale

The reasoning that the Working Group used to reach its evaluation is presented and discussed. This section integrates the major findings from studies of cancer in humans, studies of cancer in experimental animals, and mechanistic and other relevant data. It includes concise statements of the principal line(s) of argument that emerged, the conclusions of the Working Group on the strength of the evidence for each group of studies, citations to indicate which studies were pivotal to these conclusions, and an explanation of the reasoning of the Working Group in weighing data and making evaluations. When there are significant differences of scientific interpretation among Working Group Members, a brief summary of the alternative interpretations is provided, together with their scientific rationale and an indication of the relative degree of support for each alternative.

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## GENERAL REMARKS

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Part B of Volume 100 of the *IARC Monographs on the Evaluation of Carcinogenic Risks to Humans* considers 11 biological agents that were first classified in Volumes 1–99: seven viruses, three worms, and one bacterium.

Most of these agents were evaluated more than 15 years ago, and were classified by IARC as *carcinogenic to humans (Group 1)* with two exceptions: Kaposi sarcoma-associated herpesvirus (KSHV), and the worm *Clonorchis sinensis*, both previously classified as *probably carcinogenic to humans (Group 2A)*. KSHV was last assessed ten years ago and, in view of the extensive scientific literature published since, needed to be re-evaluated. *Clonorchis sinensis* was also included because its biology and epidemiology of infection are very similar to those of *Opisthorchis viverrini*, another worm reviewed in this volume.

### Volume 100 – General Information

About half of the agents classified in Group 1 were last reviewed more than 20 years ago, before mechanistic studies became prominent in evaluations of carcinogenicity. In addition, more recent epidemiological studies and animal cancer bioassays have demonstrated that many cancer hazards reported in earlier studies were later observed in other organs or through different exposure scenarios. Much can be learned by updating the assessments of agents that are known to cause cancer in humans. Accordingly, IARC has selected *A Review of Human Carcinogens* to be the topic for Volume 100. It is hoped that this volume, by compiling the knowledge accumulated through several decades of cancer research, will stimulate cancer prevention activities worldwide, and will be a valued resource for future research to identify other agents suspected of causing cancer in humans.

Volume 100 was developed by six separate Working Groups:

**Pharmaceuticals**

**Biological agents**

**Arsenic, metals, fibres, and dusts**

**Radiation**

**Personal habits and indoor combustions**

**Chemical agents and related occupations**

Because the scope of Volume 100 is so broad, its *Monographs* are focused on key information. Each *Monograph* presents a description of a carcinogenic agent and how people are exposed, critical

overviews of the epidemiological studies and animal cancer bioassays, and a concise review of the toxicokinetic properties of the agent, plausible mechanisms of carcinogenesis, and potentially susceptible populations, and life-stages. Details of the design and results of individual epidemiological studies and animal cancer bioassays are summarized in tables. Short tables that highlight key results appear in the printed version of Volume 100, and more extensive tables that include all studies appear on the website of the *IARC Monographs* programme (<http://monographs.iarc.fr>). For a few well-established associations (for example, tobacco smoke and human lung cancer), it was impractical to include all studies, even in the website tables. In those instances, the rationale for inclusion or exclusion of sets of studies is given.

Each section of Volume 100 was reviewed by a subgroup of the Working Group with appropriate subject expertise; then all sections of each *Monograph* were discussed together in a plenary session of the full Working Group. As a result, the evaluation statements and other conclusions reflect the views of the Working Group as a whole.

Volume 100 compiles information on tumour sites and mechanisms of carcinogenesis. This information will be used in two scientific publications that may be considered as annexes to this volume. One publication, *Tumour Site Concordance between Humans and Experimental Animals*, will analyse the correspondence of tumour sites among humans and different animal species. It will discuss the predictive value of different animal tumours for cancer in humans, and perhaps identify human tumour sites for which there are no good animal models. Another publication, *Mechanisms Involved in Human Carcinogenesis*, will describe mechanisms known to or likely to cause cancer in humans. Joint consideration of multiple agents that act through similar mechanisms should facilitate the development of a more comprehensive discussion of these mechanisms. Because susceptibility often has its basis in a mechanism, this could also facilitate a more confident and precise description of populations that may be susceptible to agents acting through each mechanism. This publication will also suggest biomarkers that could render future research more informative. In this way, IARC hopes that Volume 100 will serve to improve the design of future cancer studies.

## Specific remarks about the review of biological agents in this volume

### 1. Historical aspects\*

Current knowledge linking infection with some biological agents and carcinogenesis in humans is the result of a long and laborious but exciting succession of scientific discoveries, which started more than a century ago. These important historical steps have been thoroughly retraced by Dr Harald Zur Hausen in his recent book “Infections Causing Human Cancer” (2006) from which this paragraph has extracted some highlights.

At the end of the 19<sup>th</sup> century, the first suspicions arose that infectious agents (initially parasites, liver flukes and *Schistosoma* infections) could cause specific human cancers: in 1900 Askanazy reported a link between infection by a liver fluke and liver cancer in former East Prussia ([Askanazy, 1900](#)). Five years later, another report described a case of chronic infection with bilharzia (*schistosomiasis*) and

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\* Part of this text has been adapted from [zur Hausen \(2006\)](#): Infections Causing Human Cancers, 2006. Copyright Wiley-VCH Verlag GmbH & Co. KGaA (reproduced with permission).

bladder cancer ([Goebel, 1905](#)). It took more than 50 additional years before further evidence emerged of an involvement of infectious agents in human cancers.

In 1958, a British surgeon, Dennis Burkitt, described a specific childhood lymphoma in equatorial Africa occurring only in specific geographic regions: it was subsequently called *Burkitt lymphoma*. Because these regions coincided with areas of holoendemic malaria, Burkitt speculated that this tumour should have an infectious etiology, most likely vectored by an arthropod, possibly by a mosquito ([Burkitt, 1962](#)). In a small fraction of cultured Burkitt lymphoma cells Epstein and colleagues (1964) noticed herpesvirus-like particles: a new member of the Herpesvirus family was discovered and later named Epstein-Barr virus (EBV).

The development of immunofluorescence allowed the detection of highly elevated antibody titres against EBV viral antigens in patients with Burkitt lymphoma ([Henle & Henle, 1966](#), [Henle et al., 1969](#)), and subsequently also in a second human cancer, nasopharyngeal carcinoma ([Old et al., 1966](#)). EBV was also identified as the causative agent of infectious mononucleosis ([Henle et al., 1968](#)). Besides, the Henles' laboratory first demonstrated that latent EBV infection was widespread in all human populations.

The first hints of an oncogenic potential of EBV originated from the establishment of lymphoblastoid lines of cord-blood origin when co-cultivated with lethally X-irradiated Burkitt lymphoma cells in culture ([Henle et al., 1967](#)). This was further underlined by the discovery of persisting EBV DNA in "virus-negative" Burkitt lymphoma cells ([zur Hausen & Schulte-Holthausen, 1970](#)), as well as in primary biopsies from Burkitt lymphomas and nasopharyngeal cancer ([zur Hausen et al., 1970](#); [Wolf et al., 1973](#)). The induction of lymphoproliferative disease after inoculation of EBV into cottontop marmosets ([Shope et al., 1973](#)) or owl monkeys ([Epstein et al., 1973](#)) added to the evidence in this early phase.

In the 1970s, more infectious agents were considered as potential human carcinogens. Three independent findings contributed to this development: the discovery of a role of hepatitis B virus (HBV) in liver cancer, the identification of a retrovirus in a rare form of human leukaemia, and the characterization of novel types of papillomaviruses causing the second most frequent cancer in women, i.e. cancer of the cervix.

The frequent coincidence between infectious hepatitis and hepatocellular carcinoma (HCC) was noted early in Africa in the 1950s by British and French pathologists (reviewed in [Szmuness, 1978](#)). While investigating a potential role of human genetic polymorphisms in relation to inherited susceptibility to disease, [Blumberg et al. \(1965\)](#) described a "new" antigen in the blood of an Australian aborigine (the "Au" antigen), which was shortly thereafter recognized to be the surface antigen of HBV, termed today HBsAg ([Blumberg et al., 1967](#); [Blumberg, 1997](#)). In this case, basic research initially remote from studies on infectious agents led to the discovery of HBV, to diagnostic methods for viral detection efficiently used for donor blood testing, and finally to a vaccine. Several epidemiological studies followed this discovery and stressed a role of chronic infection with HBV in HCC development. A key contribution was presented in 1981 by Beasley et al. with a prospective study in Taiwan (China) showing that HBV carriers had a more than 200-fold elevated risk for developing HCC than virus-negative individuals. Vaccination studies today underline the importance of this viral infection for hepatocellular carcinoma. Indeed, the world's first universal HBV vaccination program launched in Taiwan (China) in July 1984 has resulted in a reduction of the original prevalence of HBV infection to approximately one-tenth, and a clear prevention for HCC by the vaccine was recently reported in childhood and early adulthood ([Chang, 2011](#)).

The discovery of the first human retrovirus, human T-cell lymphotropic retrovirus (HTLV-1) and its association with human cancer originated from rather independent studies in Japan and in the USA. In 1977, a new type of leukaemia called adult-T-cell leukaemia/lymphoma (ATLL) was described by Takatsuki and colleagues in the coastal regions of southern and western Japan ([Takatsuki et al., 1977](#); [Uchiyama et al., 1977](#)). The first isolation of HTLV-1 from a cutaneous T-cell lymphoma was reported in the USA ([Poiesz et al., 1980](#)). One year later, Japanese researchers identified the same virus from an ATLL cell line ([Miyoshi et al., 1981](#)), and could link this infection to this specific leukaemia ([Hinuma et al., 1981](#)).

Shortly later, when the acquired immunodeficiency syndrome (AIDS) epidemic just started in the USA, the human immunodeficiency virus type 1 (HIV-1) was discovered as a new T-cell lymphotropic human retrovirus, first isolated from a patient with lymphadenopathy ([Barré-Sinoussi et al., 1983](#)). The following year, the virus was firmly associated with AIDS ([Gallo et al., 1984](#)).

The initial link between human papillomavirus and cancer became clear as a result of research on a rare hereditary condition, *epidermodysplasia verruciformis*, described in 1922 by Lewandowsky and Lutz in Basel, and characterized by an extensive verrucosis. At sun-exposed sites of the body of these patients, some of the papillomatous lesions of the skin converted into squamous cell carcinomas. In [Lutz \(1946\)](#) and later [Jablonska & Milewski \(1957\)](#) proved the viral etiology of these warts in auto-inoculation experiments. Then, in the late 1970s, novel types of papillomaviruses, most frequently HPV 5, were isolated within *epidermodysplasia verruciformis* lesions and biopsies of squamous cell carcinomas of those patients. ([Orth et al., 1978, 1979](#)).

Another track of papillomavirus research resulted in the identification of specific HPV types as causative agents for cancer of the cervix, other anogenital cancers, and a subset of oropharyngeal carcinomas. By the end of the 1960s and during the 1970s, serological studies suggested a role of herpes simplex virus type 2 (HSV 2) in cervical cancer ([Rawls et al., 1968](#), [Naib et al., 1969](#)). However, a possible causal role of papillomavirus infections for cervical cancer was only postulated when several anecdotal reports had shown malignant conversion of genital warts (*condylomata acuminata*). Attempts to characterize the viral DNA in genital warts started ([zur Hausen et al., 1974, 1975](#), [zur Hausen, 1976, 1977](#)) leading initially to the discovery of the heterogeneity of the papillomavirus family ([Gissmann & zur Hausen, 1976](#); [Orth et al., 1977](#); [Gissmann et al., 1977](#)), which today comprises 120 fully sequenced genotypes ([Bernard et al., 2010](#)). The use of hybridization experiments, performed under conditions of reduced stringency with the DNA of the already isolated HPV types 6 and 11 as probes permitted the subsequent cloning of HPV 16 ([Dürst et al., 1983](#)) and of HPV 18 ([Boshart et al., 1984](#)), the two papillomavirus types most frequently found in cervical cancer.

The identification of three viral families with representative types clearly causing widespread human cancers gradually resulted in the acceptance of infectious agents as important human carcinogens. The subsequent identification of additional infections linked to other cancers further strengthened the role of infectious agents in human carcinogenesis. The hepatitis C virus (HCV) was identified in 1989 ([Choo et al., 1989](#)), and first reports on its relationship to a subset of HCC appeared in the same year ([Bargiggia et al., 1989](#); [Simonetti et al., 1989](#)). There had been, however, some earlier reports, linking non-A, non-B hepatitis infections to liver cancer ([Kiyosawa et al., 1982](#), [Resnick et al., 1983](#) and others). Human herpesvirus type 8 (HHV-8) now called Kaposi sarcoma herpes virus (KSHV) was discovered in 1994 ([Chang et al., 1994](#)) as the most likely responsible agent for Kaposi's sarcoma. In the early 1990s *Helicobacter pylori*, as a bacterial infection, was added to the list of potential human carcinogens ([Forman et al., 1991](#); [Nomura et al., 1991](#); [Parsonnet et al., 1991](#);

and [Wotherspoon et al., 1993](#)), and several additional human papillomavirus genotypes were added to the list of oncogenic viruses.

Merkel cell polyomavirus (MCPyV), a novel member of the polyomavirus family has been recently identified ([Feng et al., 2008](#)). The available evidence seems to support an important role for this infection in the development of Merkel-cell carcinomas ([Shuda et al., 2008](#)).

Today, more than one hundred years after early attempts to link infections to human cancer, infections are recognized as a major factor in human carcinogenesis.

## 2. Global contribution of infections to human cancers

In 2002, it was estimated that 17.8% of cancers (1.9 million cases) were caused by viral (12.1%), bacterial (5.6%), and helminth (0.1%) infection ([Parkin, 2006](#)). Of these, the majority occur in developing countries (1.5 million cases), reflecting the higher prevalence of the major oncogenic infections in these areas. If the relevant infections could be controlled, it is conservatively estimated that there would be about 26% fewer cancers in developing countries, and approximately 8% fewer in developed countries. Since then, several new links between infectious agents and specific cancers have been established ([Bouvard et al., 2009](#); this volume), further increasing the burden of infection-related cancers.

A better understanding of the role of infectious agents in the etiology of cancer is an essential element in public health policy, because such cancers are theoretically preventable by vaccination or early treatment of infection. Furthermore, cancer-causing infections often cause substantial morbidity and mortality from non-malignant conditions (e.g. HBV). Therefore, an additional benefit of any public health plan to reduce the burden of cancers caused by infections would involve a reduction in the incidence of other diseases as well.

Temporal changes in the incidence of infection-associated cancers are difficult to predict. Although there is evidence that the prevalence of infection with, for example, *Helicobacter pylori* and HBV is declining as a result of efficient antibiotic treatments and vaccination respectively, other important oncogenic infections remain uncontrolled. For instance, it is unclear how changes in the prevalence of infection with HIV, or indeed how the roll-out of effective antiretroviral treatments for this virus, will impact on the associated cancer burden. Furthermore, vaccination against HPV is still too expensive for many developing countries.

## 3. Mechanistic considerations

### 3.1 *Specific tropism of the infectious agents leads to very specific cancers*

The major distinctive feature of the biological agents as compared to other carcinogenic agents evaluated in the IARC monographs is that they are biological entities that have evolved to preferentially target specific host species, specific organs or cell types within those species, and often – in the case of viruses – even cell types with a specific differentiation status. As a result, cancers associated with viral infections are often very specific cancers, e.g. “adult T-cell leukaemia/lymphoma” associated with HTLV-1 and “extranodal NK/T-cell lymphoma (nasal type)” and “nasopharyngeal carcinoma,” both associated with EBV. Cell type-specific tropism also applies to bacteria such as *Helicobacter pylori*, associated with “non-cardia gastric carcinoma,” whereas worms such as liver

flukes or *Schistosoma haematobium*, associated with cholangiocarcinoma and urinary bladder cancer, respectively, only show organ-specific tropism.

### 3.2 Mechanisms of carcinogenesis

From the overall data summarized in this volume, three major mechanisms of carcinogenesis have been recognized for biological agents that could be defined either as direct, indirect (acting via chronic inflammation), or indirect (acting via immune-suppression). To fully exploit their carcinogenic potential, these agents have developed different strategies to evade the immune system. Finally, the data emphasize the potential role of cofactors, which are, however, largely understudied.

#### 3.2.1 Direct carcinogens

Infectious agents can be direct carcinogens, and the ones known today are all viruses that fulfill the following criteria:

The viral genome or part of it can usually be detected in each cancer cell.

The virus can immortalize after the growth of target cells *in vitro*.

It expresses several oncogenes that interact with cellular proteins and have multifunctional properties leading to disruption of the cell-cycle checkpoints, inhibition of apoptosis and cell immortalization.

Four viral agents have been described as direct carcinogens: several types of the human papillomavirus family, the human T-cell lymphotropic virus type 1 (HTLV-1) and the two herpesviruses: Epstein-Barr virus (EBV) and Kaposi sarcoma-associated herpes virus (KSHV).

#### 3.2.2 Indirect carcinogens that act via chronic inflammation

Infectious agents can be indirect carcinogens by causing chronic inflammation. Chronic infection followed by chronic inflammation will lead to the production of chemokines, cytokines, prostaglandins secreted by infected cells and/or inflammatory cells. This also leads to the production of reactive oxygen species – which have direct mutagenic effects – to the deregulation of the immune system, and to the promotion of angiogenesis, which is essential for tumour neovascularization and tumour survival.

Six infectious agents have been shown to be carcinogenic primarily by inducing chronic inflammation. Those are the two hepatitis viruses, HBV and HCV, the bacterium *Helicobacter pylori* and, the three worms *Schistosoma haematobium*, *Opisthorchis viverrini*, and *Clonorchis sinensis*.

#### 3.2.3 Indirect carcinogens that act via immune-suppression

Infectious agents can act as indirect carcinogens by causing immune-suppression. This has been shown for HIV-1 infection which strongly increases the incidence of many different human cancers. Strikingly, the majority of cancers associated with HIV-1 infection have another known infectious etiology, and HIV-1 infection increases their incidence rate considerably. Among these cancers, those associated with the herpesviruses KSHV and EBV are the most strongly enhanced by immune suppression. The same cancers are also increased by iatrogenic immune-suppression as shown by their increased incidence in transplant recipients, which lends additional support to the notion that HIV-1 acts as a carcinogen mainly through immune-suppression. These considerations may suggest that

other cancers whose incidence is increased by immune suppression (e.g. non-melanoma skin cancer) may also have an infectious etiology.

### 3.2.4 Contribution of additional factors

It is estimated that about 5% of HTLV-1 carriers when infected before the age of 20 years will potentially develop adult-T-cell leukaemia/lymphoma ([Cleghorn et al., 1995](#)). Likewise, only a small proportion of carriers of the high-risk human papillomavirus type 16 in the *cervix uteri* will develop cervical cancer. The fact that infection with these carcinogenic viruses does not always lead to cancer is a common feature for all the infectious agents that were studied strongly suggests the involvement of cofactors in the carcinogenic process. Carcinogenesis would result from the interaction of multiple risk factors including those related to the infectious agent itself (e.g. variants or subtypes), host-related factors (e.g. gene polymorphisms and immune status) and environmental cofactors (e.g. chemicals, ionizing radiation, immunosuppressive drugs, or another infection that may lead to reactivation of latent oncogenic viruses such as EBV or KSHV). The contribution of several of these additional factors to the development of infection-associated cancers is likely to be substantial, but has not yet been elucidated in detail.

## 4. Other remarks

### 4.1 The absence of a Section 3 “Cancer in Experimental Animals” in the Monographs on viruses

The Working Group decided not to include in this Volume a separate section on “Cancer in experimental animals” in the *Monographs* on viruses, but rather to include description of such studies under Section 4 “Other Relevant Data” for the following reasons:

- The use of animals as surrogate hosts for the study of a human tumour virus is often problematic since species-specificity limits the feasibility of this approach for most of these viruses. HTLV-1 is one exception: this virus can infect several different animal species (rabbits, rats and monkeys) but does induce adult T-cell leukaemia/lymphoma in monkeys only. For some human tumour viruses (e.g. KSHV), the use of humanized SCID mice, in which the human target cell for the virus is placed into a mouse host context, can provide a platform for *in-vivo* infection. However, apart from EBV, which causes lymphoproliferative diseases in New World monkeys and humanized SCID mice, the use of surrogate hosts has not proven very useful for assessing the carcinogenicity of human viruses in humans.
- Cancer models for human tumour viruses that make use of animal viruses are very scarce. In fact, although many viruses that infect non-human primate species are related to the human tumour viruses, the incidence of cancer is low in these species – as it is in humans – which makes cancer studies costly and difficult. Moreover, animal tumour virus models in non-primate species often do not accurately reflect the mechanism of the disease caused by the cognate human tumour virus. For instance, woodchuck hepatitis virus induces HCC that is histopathologically very similar to that caused by HBV in humans, but it does so through a different mechanism.
- Transgenic mouse models provide powerful means for performing mechanistic studies to investigate the role of individual viral genes in cancer. Indeed, for many of the human tumour viruses described in this volume, transgenic mouse studies provide critical mechanistic

evidence. However, such transgenic mouse models do not represent models for understanding the cancer etiology in the context of natural viral infections, and are therefore more appropriately discussed in Section 4.

#### 4.2 *Difficulties in assessing causality for some cancers*

The Working Group acknowledged the difficulties of assessing causality, on the basis of epidemiological data, for certain rare cancer types in which presence of a specific infection was already been incorporated into the diagnostic criteria (e.g. HTLV-1 in relation to ATLL; KSHV and primary effusion lymphoma). In these instances, there was generally, but not always, convincing evidence for a causal relationship. Further epidemiological research on these cancers, often hampered by the rarity of the tumour, would require that currently accepted diagnostic criteria be reassessed.

#### 4.3 *Classification of weakly carcinogenic human papillomavirus types*

Previous IARC Monographs summarized the considerable evidence showing that virtually all cases of cervical cancer are caused by persistent infections with a restricted set of human papillomaviruses. However, the carcinogenic potential of HPV types is very heterogeneous. Most are not carcinogenic. Some HPV types, like HPV 16 and HPV 18, are clear and powerful carcinogens. It is the categorization of the most weakly carcinogenic HPV types that is most challenging. The distinctions are important for screening tests and vaccine development. Carcinogenic types are targeted in HPV-screening assays to maximize sensitivity while others are excluded to preserve specificity. The types that are most carcinogenic are included in multivalent HPV vaccines to prevent the largest feasible fraction of cervical and other cancers. Thus, the Working Group made great efforts to consider the carcinogenic potential of each individual HPV type, with a strong emphasis on evolutionary relationships and detection within cases of invasive cancers ([Schiffman et al., 2009](#)).

A summary of the findings of this volume appears in *The Lancet Oncology* ([Bouvard et al., 2009](#)).

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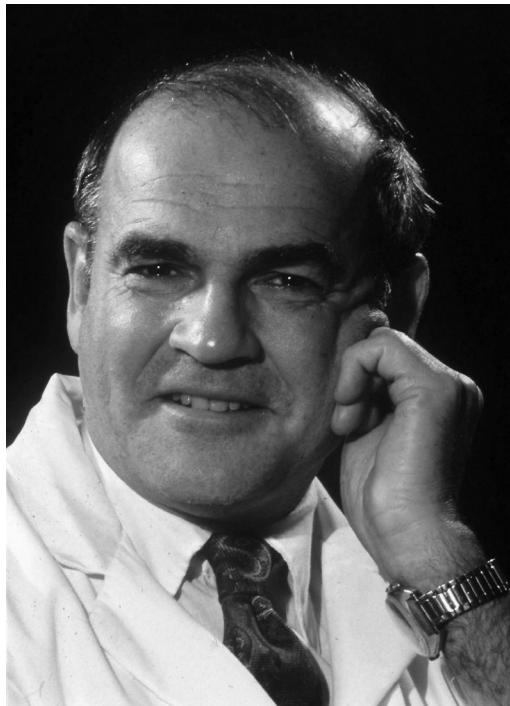
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### **Baruch Blumberg (1925-2011)**

Dr. Baruch Blumberg received the Nobel Prize in Physiology or Medicine in 1976 for his discovery of the hepatitis B virus, one of the agents discussed in this Volume.

Dr. Blumberg died on 5 April 2011.

We wish to commemorate his achievements in the development of an HBV vaccine and the promotion of blood testing, which allowed millions of lives to be saved worldwide.

Photo © The Nobel Foundation



# EPSTEIN-BARR VIRUS

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The Epstein-Barr virus was considered by a previous IARC Working Group in 1997 ([IARC, 1997](#)). Since that time, new data have become available, these have been incorporated in the *Monograph*, and taken into consideration in the present evaluation.

## 1. Exposure Data

### 1.1 Taxonomy, structure, and biology

#### 1.1.1 Taxonomy

The Epstein-Barrvirus (EBV), the first isolated human tumour virus, was identified in 1964 by Epstein's group in a cell line derived from Burkitt lymphoma ([Epstein et al., 1964](#)). EBV is a human herpesvirus, classified within the gammaherpesviruses subfamily, and is the prototype of the *Lymphocryptovirus* genus. In keeping with the systematic nomenclature adopted for all human herpesviruses, the formal designation of EBV is human herpesvirus 4 (HHV-4).

Two major EBV types have been detected in humans: EBV-1 and EBV-2 (also known as types A and B). EBV-1 and EBV-2 differ in the sequence of the genes that code for the EBV nuclear antigens (EBNA-2, EBNA-3A/3, EBNA-3B/4, and EBNA-3C/6) ([Sample et al., 1990](#)). EBV-2 immortalizes B cells less efficiently than EBV-1 *in vitro*, and the viability of EBV-2-infected lymphoblastoid cell lines is less than that of EBV-1-infected lines ([Rickinson et al., 1987](#)). The differences in the immortalizing efficiency of the EBV subtypes may relate to a divergence in the EBNA-2 sequences ([Cohen et al., 1989](#)).

In addition to type-specific polymorphism, significant DNA-sequence heterogeneity has been found when comparing selected regions of the EBV genome isolated in certain geographic areas or even from the same area. These polymorphisms define different viral strains within both types ([Aitken et al., 1994](#)).

#### 1.1.2 Structure of the virion

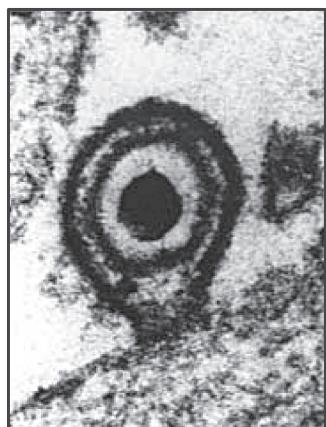
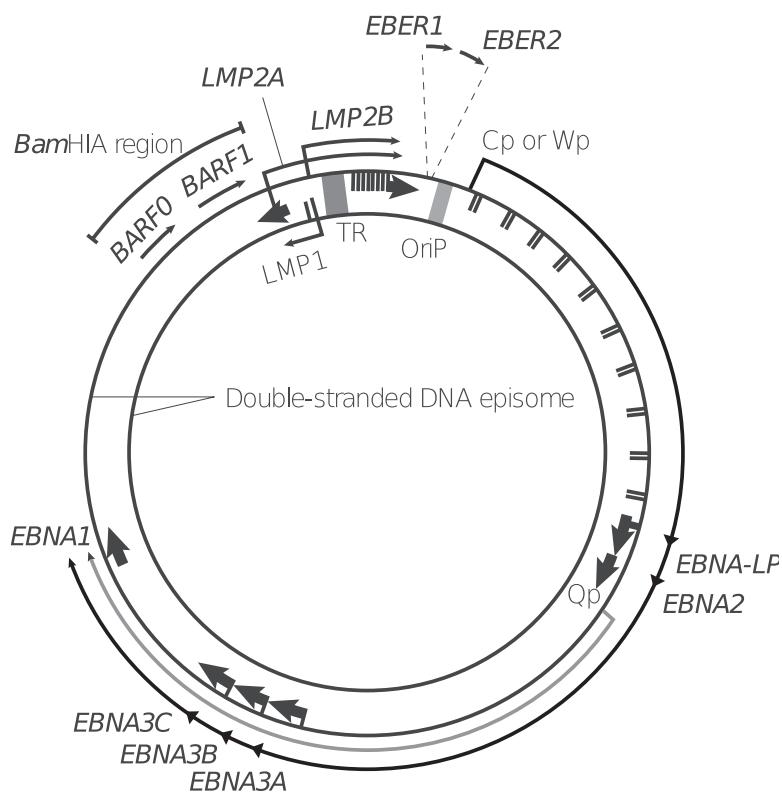
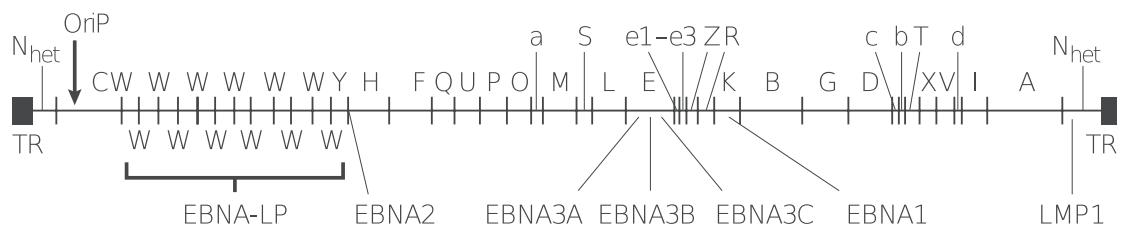
Like other herpesviruses, EBV is a DNA virus with a toroid-shaped protein core that is wrapped with DNA, a nucleocapsid with 162 capsomers, a protein tegument between the nucleocapsid and the envelope, and an outer envelope with external virus-encoded glycoprotein spikes ([Liebowitz & Kieff, 1993](#)).

#### 1.1.3 Structure of the viral genome

The EBV genome is a linear, double-stranded, ~172-kb DNA molecule that encodes > 85 genes (Fig. 1.1).

The nomenclature for EBV open-reading frames (ORFs) is based on the *Bam*HI-restriction fragment in which they are found. For example, the *BARF1* ORF is found in the *Bam*HI A fragment, and extends rightwards (Fig. 1.1).

The many EBV ORFs are divided into latent and lytic genes (further divided into immediate

**Figure 1.1 The Epstein-Barr virus genome****a EBV electron micrograph****b EBV genome: latent genes****c Open reading frames for the EBV latent proteins**

a Electron micrograph of the Epstein-Barr virus (EBV) virion

b Diagram showing the location and transcription of the EBV latent genes on the double-stranded viral DNA episome

c Location of open reading frames of the EBV latent proteins on the Bam H1 restriction-endonuclease map of the prototype B95.8 genome  
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**Table 1.1 Examples of identified EBV gene products and their open reading frames**

Open reading frame	Protein	Main proposed function	
		Common name	Alternative nomenclature
<b>Latent genes</b>			
<i>BKRF1</i>	EBNA-1 <sup>a</sup>		Plasmid maintenance, DNA replication transcriptional regulation
<i>BYRF1</i>	EBNA-2 <sup>a</sup>		<i>trans</i> -activation
<i>BERF1</i>	EBNA-3A <sup>a</sup>	EBNA-3	Transcriptional regulation
<i>BERF2</i>	EBNA-3B <sup>a</sup>	EBNA-4	Unknown
<i>BERF3/4</i>	EBNA-3C <sup>a</sup>	EBNA-6	Transcriptional regulation
<i>BWRF1</i>	EBNA-LP <sup>a</sup>	EBNA-5	<i>trans</i> -activation
<i>BNLF1</i>	LMP-1 <sup>a</sup>		B-cell survival, anti-apoptosis
<i>BNRF1</i>	LMP-2A <sup>a</sup> /2B	TP1/2	Maintenance of latency
<i>BARF0</i>			Not shown to be translated, unknown function
<i>EBER1/2</i>			Non-translated, regulation of innate immunity
<b>Lytic genes</b>			
Immediate early genes			
<i>BZLF1</i>	ZEBRA		<i>trans</i> -activation, initiation of lytic cycle
<i>BRLF1</i>			<i>trans</i> -activation, initiation of lytic cycle
<i>BI'LF4</i>			<i>trans</i> -activation, initiation of lytic cycle
Early genes			
<i>BMRF1</i>			<i>trans</i> -activation
<i>BALF2</i>			DNA binding
<i>BALF5</i>			DNA polymerase
<i>BORF2</i>			Ribonucleotide reductase subunit
<i>BARF1</i>			Ribonucleotide reductase subunit
<i>BXLF1</i>			Thymidine kinase
<i>BGLF5</i>			Alkaline exonuclease
<i>BSLF1</i>			Primase
<i>BBLF4</i>			Helicase
<i>BKRF3</i>			Uracil DNA glycosylase
Late genes			
<i>BLLF1</i>	gp350/220		Major envelope glycoprotein
<i>BXLF2</i>	gp85 (gH)		Virus-host envelope fusion
<i>BKRF2</i>	gp25 (gL)		Virus-host envelope fusion
<i>BZLF2</i>	gp42		Virus-host envelope fusion, binds MHC class II
<i>BALF4</i>	gp110 (gB)		Unknown
<i>BDLF3</i>	gp100–150		Unknown
<i>BILF2</i>	gp55–78		Unknown
<i>BCRF1</i>			Viral interleukin-10
<i>BHRF1<sup>ab</sup></i>			Viral <i>bcl-2</i> analogue

<sup>a</sup> Gene products involved in immortalization and/or other aspects of tumour cell phenotypes<sup>b</sup> Expressed in latently infected cells as well

EBNA, EBV nuclear antigen; LP, leader protein; LMP, latent membrane protein; ZEBRA, Z EBV replication activation; gp, glycoprotein; MHC, major histocompatibility complex

Compiled from [Liebowitz & Kieff \(1993\)](#), [Li et al. \(1995\)](#), [Nolan & Morgan \(1995\)](#), [Thompson & Kurzrock \(2004\)](#)

early genes, early genes, and late genes). Most of these genes are translated into proteins whose main proposed functions are listed in [Table 1.1](#). Several lytic genes encode for human homologues ([Table 1.2](#)). In addition, some latent genes are non-translated; this is the case for *EBV-encoded RNA (EBER)-1* and -2 ([Kieff, 1996](#); [Kieff & Rickinson, 2001](#)). EBV also encodes at least 17 micro-RNAs, arranged in two clusters: ten are located in the introns of the viral *BART* gene, and three adjacent to *BHRF1* ([Cai et al., 2006](#)).

The viral genome also contains a series of 0.5-kb terminal direct repeats at either end and internal repeat sequences that serve to divide the genome into short and long unique sequence domains that have most of the coding capacity ([Cheung & Kieff, 1982](#)). These terminal repeats are good markers to determine if EBV-infected cells are from the same progenitor: when EBV infects a cell, the viral DNA circularizes and mainly persists as a circular episome with a characteristic number of terminal repeats that depends on the number of terminal repeats in the parental genome, with some variation introduced during viral replication. If the infection is permissive for latent infection but not replication, future generations will have EBV episomes with the same number of terminal repeats ([Raab-Traub & Flynn, 1986](#)).

#### 1.1.4 Host range

Although herpesviruses are ubiquitous in nature, humans serve as the only natural host for EBV. Almost all higher primates have their own EBV-like virus. Antibodies to EBV have been detected in several primate species, probably due to the presence of cross-reactive antibodies against their own species-specific EBV homologues ([Kieff et al., 1979](#)). Infection of newborn marmosets with EBV resulted in the establishment of a long-term permissive infection, indicating similarities in the responses of marmosets and humans to EBV ([Cox et al., 1996](#)).

#### 1.1.5 Target cells

Like other gammaherpesviruses, EBV establishes latent infection in lymphocytes and can induce proliferation of the latently infected cells ([Young & Rickinson, 2004](#)). EBV infection of B cells is mediated through the interaction of the viral envelope glycoprotein gp350/220 with the cellular receptor for the C3d complement component CR2 (CD21) ([Fingeroth et al., 1984, 1988](#); [Tanner et al., 1987](#)). After binding of the viral particle to the surface of the host cell and endocytosis, the viral envelope fuses with the host-cell membrane by a mechanism involving three other viral glycoproteins: gp85, gp25, and gp42 ([Li et al., 1995](#)). It is worth noting that gp42 can bind to major histocompatibility complex (MHC) class II, and EBV uses this as a cofactor in the infection of B lymphocytes ([Li et al., 1997](#)).

It has been shown nonetheless that EBV can also infect cells, albeit at low efficiency, via CD21-independent mechanisms. Indeed, cells that do not express CD21 (as some epithelial cells) can be infected by the virus, and furthermore a virus deficient in gp350/220 was shown to be still infectious ([Imai et al., 1998](#); [Janz et al., 2000](#)).

Although EBV is considered to be a B-lymphotropic virus, it can also infect T lymphocytes or epithelial cells because it is found in some T-lymphoma cells and several important diseases of epithelial cells, including nasopharyngeal and gastric carcinomas, and oral hairy leukoplakia ([Thompson & Kurzrock, 2004](#)). Other CD21-independent pathways may be responsible for EBV infection of cells other than B lymphocytes ([Imai et al., 1998](#); [Janz et al., 2000](#)).

Current evidence suggests that EBV infection in healthy chronic virus carriers is largely restricted to B cells, although in certain situations the virus can be detected in epithelial cells. The most likely role for epithelial cells is as a site for replication and amplification of EBV rather than as a site of persistent latent infection,

**Table 1.2 Homology of EBV gene products with human proteins**

Viral gene	Human homologue	Functional similarity established
<i>BCRF1</i>	Interleukin 10	Yes
<i>BDLF2</i>	Cyclin B1	No
<i>BHRF1</i>	BCL-2	Yes
<i>BALF1</i>	BCL-2	No
<i>BARF1</i>	C-FMS receptor ICAM-1 (CD54)	Yes No

Amino acid homology between viral and human product varies from ~20% to >80%.

Adapted from [Thompson & Kurzrock \(2004\)](#)

however, this remains controversial ([Kieff, 1996](#)) (see Section 1.1.6 and Fig. 1.2).

### 1.1.6 Viral life cycle

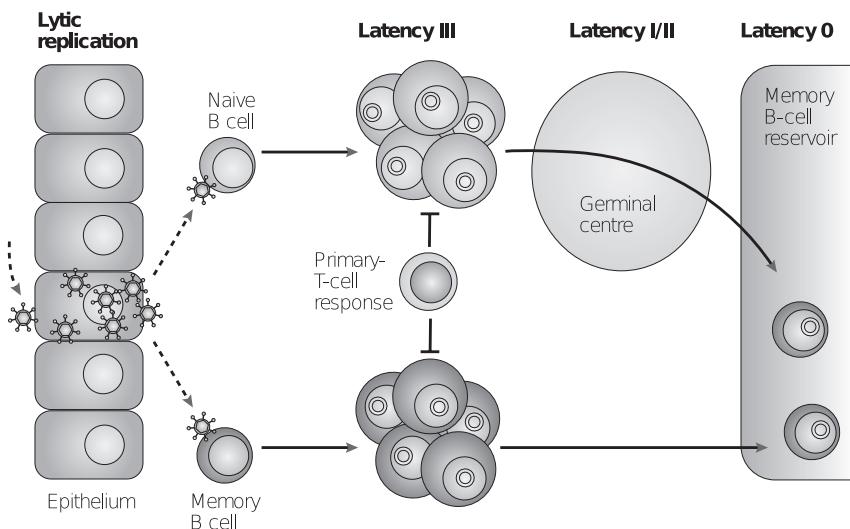
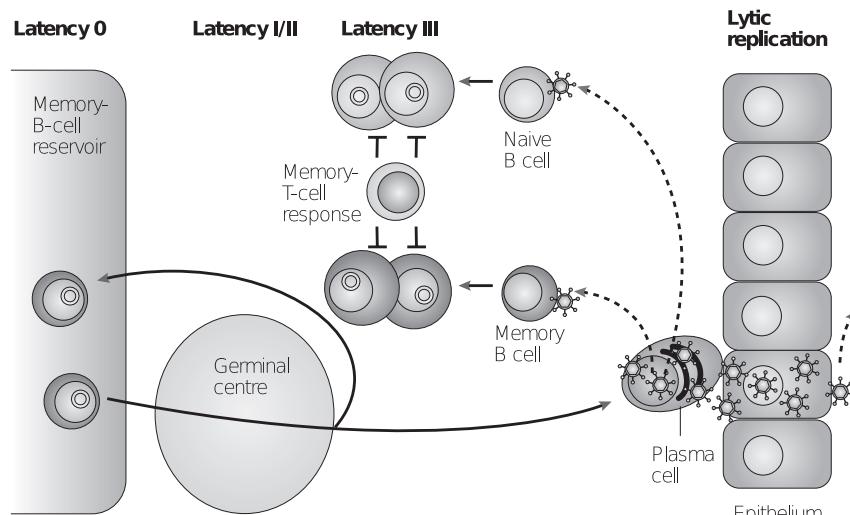
EBV, probably the most potent transforming human virus in culture, is nonetheless known to infect and persist for life in > 90% of human adults without causing disease.

Several reviews have described how EBV exploits the physiology of the normal B-cell differentiation, and uses different combinations of latent viral gene expression to progress from initial infection to long-term persistence within the memory B-cell pool of the immunocompetent host ([Thorley-Lawson & Gross, 2004](#); [Young & Rickinson, 2004](#); [Thorley-Lawson & Allday, 2008](#)).

Fig. 1.2 depicts the putative interactions between EBV and its host. EBV spreads via the saliva entering the epithelium of the Waldeyer tonsillar ring situated in the oropharynx where it probably initiates a lytic infection that leads to amplification of the virus. The virus then infects naïve B cells in the underlying lymphoid tissues, to become activated lymphoblasts using the growth transcription programme (latency III). Three of the growth-programme proteins (EBNA-3A, EBNA-3B, and EBNA-3C) negatively autoregulate the growth programme. This allows the cell to migrate into the follicle to initiate a germinal centre reaction, and to establish the default transcription programme (latency II). The default

programme provides rescue or survival signals that allow the cell to exit the germinal centre as memory B cell. Then, the latency transcription programme (latency 0) in which all viral protein expression is turned off begins in the resting memory B cells. These cells are maintained by normal memory B cell homeostasis. When they occasionally divide, they express the EBNA-1-only programme (latency I). The memory B cells eventually return to the tonsil, where they occasionally undergo plasma-cell differentiation, which triggers viral replication. The resulting virus may be released into saliva for spreading to other hosts or may infect other B cells ([Young & Rickinson, 2004](#); [Thorley-Lawson & Allday, 2008](#)).

Primary EBV infection elicits a strong cellular immune response which brings the infection under control, and newly infected cells are thought to be efficiently removed by the latent-antigen-specific T-cell response. The virus can persist for life in the host only in the resting memory B cells in which no viral proteins are expressed, and is therefore shielded from the immune system ([Thorley-Lawson & Gross, 2004](#); [Young & Rickinson, 2004](#); [Thorley-Lawson & Allday, 2008](#)).

**Figure 1.2 Putative *in vivo* interactions between EBV and host cells****a Primary infection****b Persistent infection**

a Primary infection

b Persistent infection

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**Table 1.3 The EBV transcription programmes in normal B cells**

Transcription programme	Gene products expressed	Infected normal B-cell type <sup>a</sup>	Function
Growth (latency III)	EBNA-1, -2, -3A, -3B, -3C, -LP, LMP-1, LMP-2A and LMP-2B, EBERs	Naive	Activate B cell
Default (latency II)	EBNA-1, LMP-1 and LMP-2A, EBERs	Germinal centre	Differentiate activated B cell into memory
True latency (Latency 0)	EBERs	Peripheral memory	Allow lifetime persistence
EBNA-1 only (latency I)	EBNA-1, EBERs	Dividing peripheral memory	Allow virus in latency programme cell to divide
Lytic	All lytic genes	Plasma cell	Replicate the virus in plasma cell

<sup>a</sup> Except where indicated, the cell types are primarily restricted to the lymphoid tissue of the Waldeyer ring.

Adapted from [Thorley-Lawson \(2005\)](#)

### 1.1.7 Viral gene expression

#### (a) Viral gene expression in normal cells during the viral life cycle

[Table 1.3](#) lists the different viral transcription programmes in normal B cells. The expression of EBV-encoded proteins differs depending on the type, differentiation, and activation status of the infected cell. The growth-stimulating programme is based on the expression of six nuclear and three membrane proteins. Six of these are essential for the activating and proliferation-driving effect of the virus. One virally encoded nuclear protein, EBNA-1 which is required for the maintenance of the viral episomes, is expressed to various degrees in these cells ([Thorley-Lawson, 2005](#)). In all forms of latency, EBV expresses two classes of non-coding small RNA (EBER) 1 and 2, which are highly structured RNAs of 167 and 172 nucleotides, respectively. The expression of EBER-1 and -2 is restricted to the cell nucleus where they are present at approximately  $10^7$  copies per cell ([Sample & Sample, 2008](#)). Also, EBV encodes at least 22 micro-RNAs which are expressed to various degrees in latency I, II, III ([Cai et al., 2006](#); [Grundhoff et al., 2006](#)).

#### (b) Viral gene expression in EBV-associated malignancies

Specific latency EBV-transcription programmes have been demonstrated in many human tumours, including immunoblastic lymphoma in immunosuppressed patients, Burkitt lymphoma, Hodgkin disease, and nasopharyngeal carcinoma ([Table 1.4](#)). The origins of all of these tumours can be understood as arising from specific stages in the EBV life cycle, and appear to be associated with disturbances of the immune system as shown in Fig. 1.3 ([Thorley-Lawson, 2005](#)).

Latency I is generally associated with the EBV-related Burkitt lymphoma, latency II with Hodgkin disease, T-cell non-Hodgkin lymphoma, and nasopharyngeal and gastric carcinoma; latency III occurs mainly in immunocompromised individuals, in post-transplant lymphoproliferative disorders, and HIV-associated lymphoproliferative disorders ([Liebowitz & Kieff, 1993](#); [Sbih-Lammali et al., 1996](#); [Niedobitek et al., 1997](#); [Cesarman & Mesri, 1999](#); [Kis et al., 2006](#); [Klein et al., 2007](#)).

**Table 1.4 EBV latency pattern and associated malignancies**

Latency Type	Viral products expressed	Associated malignancies
Latency I	EBNA-1	Burkitt lymphoma
	EBERs	Gastric carcinoma <sup>b</sup>
	BARFO	
Latency II	EBNA-1	Hodgkin disease
	EBERs	Nasopharyngeal carcinoma
	LMP-1	Peripheral T/NK lymphoma
	LMP-2A	
	BARFO	
Latency III	All EBNA <sup>a</sup>	AIDS-associated lymphomas
	EBERs	Post-transplant lymphoproliferative disorders
	LMP-1	
	LMP-2A	
	BARFO	

<sup>a</sup> EBNA include EBNA-1, EBNA-2, EBNA-3A (EBNA-3), EBNA-3B (EBNA-4), EBNA-3C (EBNA-6), EBNA-LP (EBNA-5 or EBNA-4)

<sup>b</sup> Gastric carcinoma have been shown to express an intermediate Latency I/II pattern including expression of EBNA-1, EBERs, LMP-2A, BARFO and some lytic infection proteins such as BARF-1, BNRF-1 ([Luo et al., 2005](#))

EBNA, EBV nuclear antigen; LMP, latent membrane protein; EBER, EBV-encoded RNA

Adapted from [Thompson & Kurzrock \(2004\)](#)

## 1.2 Epidemiology of infection

### 1.2.1 Prevalence, geographic distribution

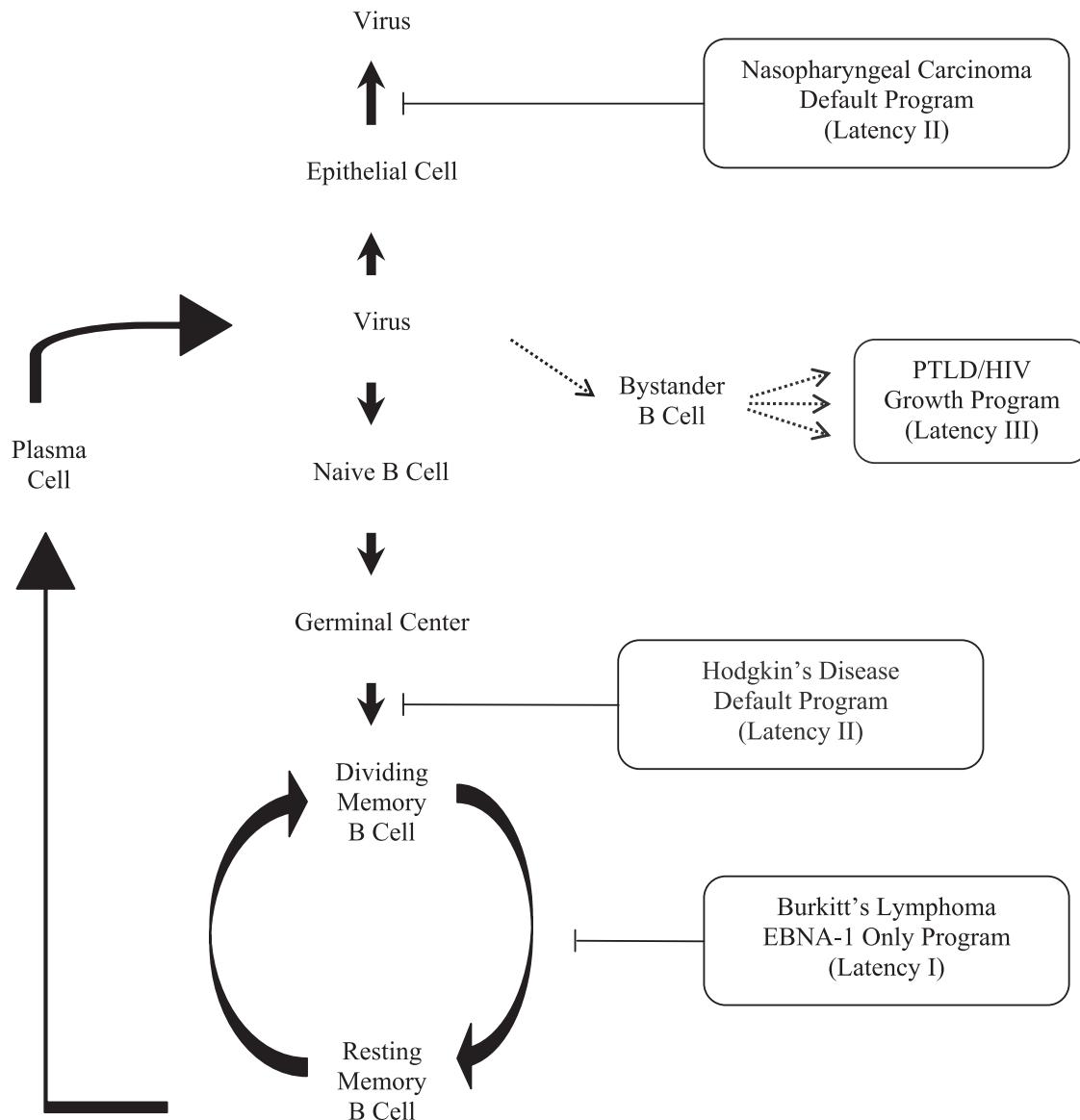
In the 1970s, IARC demonstrated that more than 90% of adults worldwide are infected with EBV, based on the detection of antibodies to EBV (especially antibodies to viral capsid (VCA) and complement-fixing soluble (CF/S) antigens) ([de Thé et al., 1975](#)). Many other epidemiological studies have shown since that EBV is highly prevalent throughout the world ([IARC, 1997](#); [Young, 2008](#)), including in remote populations ([Black et al., 1970](#); [Tischendorf et al., 1970](#)).

The age at primary infection varies substantially worldwide, and exposure to EBV is likely to be due to socioeconomic factors ([Evans, 1971](#)) such as overcrowded living conditions with poor sanitation ([de Thé et al., 1975](#)). For example, while more than 80% of children in Uganda are estimated to be seropositive for EBV by age one ([Kafuko et al., 1972](#)), this estimate is only approximately 45% in the rural United States of America ([Sumaya et al., 1975](#); [Hsu & Glaser, 2000](#)).

Although primary EBV infection during early childhood is usually subclinical or has symptoms that are similar to other respiratory illnesses, a delay in acquiring a primary EBV infection at an older age in childhood or adolescence, which usually occurs in more developed countries ([Rickinson & Kieff, 1996](#)), can manifest itself as infectious mononucleosis (occurring in approximately 25–75% of EBV-infected persons) ([Evans, 1971](#); [Sawyer et al., 1971](#); [Niederman & Evans, 1997](#); [Hsu & Glaser, 2000](#)).

In a study in the Hong Kong Special Administrative Region ([Chan et al., 2001](#)), sequential measurements for markers of EBV infection from serum samples of a group of infants demonstrated a sudden seroconversion at the age of 8 months, which may imply a protective role for persistent maternal antibodies, and also partially explain why primary EBV infection in early childhood, unlike during adolescence, is usually asymptomatic ([Chan et al., 2001](#)).

Two major types of EBV – EBV-1 and EBV-2 – have been identified and differ in geographic distribution. The role of specific EBV types in

**Figure 1.3 Putative check points in the EBV life cycle that give rise to tumours**

Events occurring normally in healthy carriers are denoted in thick arrows. EBV normally infects naive B cells in the Waldeyer ring, and can differentiate into memory cells and out of the cell cycle, and are not pathogenic. **PTLD/HIV**: If a cell other than the naive B cell in the Waldeyer ring is infected, it will express the growth programme and continue to proliferate because it cannot differentiate out of the cell cycle (thin dashed arrows) - a very rare event - highlighting how carefully controlled EBV infection is. Normally, these bystander B-cell blasts would be destroyed by CTLs, but if the CTL response is suppressed, then they grow into PTLD or AIDS-associated lymphomas. Note: a bystander-type cell could also arise if a latently infected germinal center or memory cell fortuitously switched on the growth programme. **Hodgkin disease** occurs from the default programme. **Burkitt lymphoma** evolves from a germinal-centre cell that is entering the memory compartment but is stuck proliferating. Consequently, the cell expresses EBNA-1 only. **Nasopharyngeal carcinoma** is assumed to occur from a latently infected epithelial cell blocked from terminal differentiation and viral replication.

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the etiology of different cancers is unknown. Immunocompromised patients more commonly harbour both subtypes of EBV ([Borisch et al., 1992](#); [Thompson & Kurzrock, 2004](#)). EBV-2 may be more common in Africa ([Gratama & Ernberg, 1995](#)), and in homosexual men ([van Baarle et al., 2000](#); [Higgins et al., 2007](#)). It has been hypothesized that the attenuated transforming ability of EBV-2 along with an immunosuppressive condition (HIV or malaria) may be necessary for EBV-2 to be capable of maintaining infection of B lymphocytes, and to cause transformation ([Buisson et al., 1994](#); [Thompson & Kurzrock, 2004](#)). However, other studies showing that HIV-infected haemophiliacs have lower rates of EBV-2 infection than HIV-infected homosexuals have challenged this hypothesis, and suggest that the acquisition of EBV-1 versus EBV-2 would rather be due to the opportunity for exposure ([van Baarle et al., 2000](#); [Thompson & Kurzrock, 2004](#)).

The fact that EBV is ubiquitous, and consequently causes widespread and largely asymptomatic infection, suggests that the specific geographic distribution of EBV-associated malignancies, such as endemic Burkitt lymphoma and nasopharyngeal carcinoma, is probably not due to differences in EBV infection but rather due to the activation of viral replication by additional cofactors ([Young, 2008](#)).

### *1.2.2 Transmission and risk factors for infection*

EBV infection usually occurs in individuals of a young age, with low socioeconomic status or development, from a larger than average family, and with poor hygienic standards. By their third decade of life, 80–100% of these individuals become carriers of the infection ([IARC, 1997](#)).

The oral route is the primary route of transmission of the virus; however, transmission by transfusion has been documented. In developing countries, infection is acquired in the first few

years of life. Crowding and/or the practice of pre-chewing food for infants may be contributing factors. In the developed world, infection is often delayed to adolescence, when transmission is more likely because of intimate oral exposure ([Hjalgrim et al., 2007a](#)). About 50% of primary EBV infections during young adulthood result in clinical infectious mononucleosis ([CDC, 2006](#)).

Infectious mononucleosis is usually acquired from a transfer of saliva, and in young adults, this is more likely to occur after the onset of sexual activity. However, only limited data are available to support this hypothesis ([MacSween & Crawford, 2003](#)). In a cohort study of sexually active young women, the development of detectable antibodies against EBV after primary infection increased with increasing number of sexual partners, and was greatest when a new sexual partner was encountered in the 2 years before seroconversion. In addition, transient EBV DNA loads were detected in cervical cytology samples in some of the women ([Woodman et al., 2005](#)). [The Working Group noted, however, that it is difficult to distinguish in this study whether transmission occurs through saliva or genital contact.]

### *1.2.3 Persistency, latency, and natural history of infection*

Following primary infection via transmission of cell-free virus and/or of productively infected cells in saliva, EBV will enter into the circulating B-cell pool, and then remain in most cases undetected for life in a latent state ([Young & Rickinson, 2004](#); Thorley Lawson, 2005). EBV can also infect the mucosal epithelial cells in which intermittent viral productive replication occurs ([Frangou et al., 2005](#)). The B-cell compartment, more precisely resting memory B cells, appears to be the true reservoir of the latent virus in healthy carriers. Resting memory B cells express a very restricted pattern of latent viral gene expression (see [Table 1.3](#) and [Miyashita et al., 1995](#)); this is

how these infected cells can persist in the face of efficient cytotoxic T lymphocyte (CTL) surveillance ([Masucci & Ernberg, 1994](#)). Nonetheless, cells that express the full repertoire of growth-transformation-associated antigens are likely to be generated sporadically in asymptomatic virus carriers, because memory CTLs that are reactive against most EBNAs are maintained at high levels for life (see Fig. 1.2, and further details in Section 1.1, this *Monograph*; [IARC, 1997](#)).

Primary EBV infections occurring in adolescence or early in adult life are manifested as infectious mononucleosis, which is an acute form of primary infection occurring asymptotically in early childhood.

EBV-associated malignancies are suspected to result from viral reactivation that is most likely due to interaction with additional cofactors ([Young, 2008](#)).

#### *1.2.4 Biological markers of the different status of EBV infection*

##### *(a) Antibody responses to EBV*

The detection of antibodies to EBV in biological fluids has been until recently the major means of diagnosis for EBV infection. Distinct patterns of antibody response have been identified during primary infection, latent infection of healthy carriers, viral reactivation, and in various EBV-associated diseases. Serological parameters include the detection of IgG, IgM, and occasionally IgA, directed against EBNAs, early antigens (EAs, divided into two components, EA-D (encoded by *BMRF-1*) and EA-R (a human BCL-2 homologue encoded by *BHRF-1*)), and VCAs (for a review see [IARC, 1997](#)).

##### *(i) Infectious mononucleosis*

Most information available on primary antibody response has been provided by studies on infectious mononucleosis. [Table 1.5](#) shows the variation over time of serological parameters both at and after the onset of infectious mononucleosis.

At the onset of clinical symptoms of the disease, substantial titres of IgM antibodies to VCA are detected, with rising titres of IgG to EA, and to VCA. IgA antibodies to these antigens may also appear. Whereas anti-VCA IgM titres disappear over the next few months, anti-VCA IgG titres rise to a peak that may fall slightly, and anti-EA IgG titres become either undetectable or stabilize at very low levels. Neutralizing antibodies to the major envelop glycoprotein gp350 are detected during the acute phase of infectious mononucleosis but only at very low titres, and increase to stable levels thereafter ([IARC, 1997](#)).

The serology of infectious mononucleosis for the anti-EBNA response presents an interesting pattern. For the anti-EBNA response, during the acute phase of infectious mononucleosis, patients show an IgG response to EBNA-2 (and also probably to EBNA-3A, -3B, and -3C), whereas an IgG response to EBNA-1 is not usually detected until convalescence. The production of antibodies to EBNA-1 and EBNA-2 in the course of infectious mononucleosis follows an ordered progression. Anti-EBNA-2 is the first to be detected, reaches peak titres, and then declines to a lower persistent level, and can remain undetected in about 1/3 of the cases. Anti-EBNA-1 is detected long after anti-EBNA-2, and then persists indefinitely once it has reached its concentration plateau. Therefore, within the first year following infectious mononucleosis, the ratio of anti-EBNA-1: anti-EBNA-2 is well below 1, but becomes well above 1 over time. The switch from dominant anti-EBNA-2 to dominant anti-EBNA-1 titres occurs in individual cases over a long span of time ([Table 1.5](#); [Henle et al., 1987](#); [IARC, 1997](#)).

##### *(ii) Healthy EBV-carriers*

IgG antibodies to VCA, to neutralizing anti-gp350, and to EBNA-1 are consistently detected in the serum of healthy carriers. The titre of these antibodies is usually stable over time but can markedly differ among individuals ([Henle & Henle, 1976](#)). Antibodies to EA are only detected

**Table 1.5 Serological parameters at various times after the onset of infectious mononucleosis**

	Months after onset					Healthy controls (n=38)
	0 (n=74)	2–3 (n=44)	4–12 (n=65)	13–24 (n=83)	25–48 (n=35)	
<b>IgM anti-VCA</b>						
% positive	100.0	73.1	0.0	0.0	0.0	0.0
Range of titres	80–640	10–80	<10	<10	<10	<10
<b>Anti-EA</b>						
% positive	81.8	88.5	87.8	60.9	39.4	30.0
Range of titres	10–320	10–160	10–160	10–80	10–80	10–40
<b>Anti-D</b>						
% positive	81.8	57.7	10.2	13.0	0.0	0.0
Range of titres	10–320	10–160	10–160	10–40	<10	<10
<b>Anti-R</b>						
% positive	— <sup>a</sup>	46.2	83.7	47.9	39.4	30.0
Range of titres	—	10–160	10–160	10–80	10–80	10–40
<b>Anti-EBNA-1</b>						
% positive	0.0	4.5	73.8	97.6	97.1	100.0
Geometric mean titre	<2	<2	5.3	21.2	24.8	48.2
<b>Anti-EBNA-2</b>						
% positive	0.0	93.2	87.9	60.2	71.4	71.1
Geometric mean titre	<2	7.3	11.6	4.1	4.3	3.9
<b>Ratio anti-EBNA-1/anti-EBNA-2</b>						
≤1.0	—	97.7	76.9	22.9	17.1	5.3
>1.0	—	2.3	23.1	77.1	82.9	94.7

<sup>a</sup> Anti-R can be measured only when exceeding anti-D in titre.

Adapted from [Henle et al. \(1987\)](#)

in a proportion of healthy carriers. Although persisting for life, anti-EBNA-1 and anti-VCA do not appear to have much of a protective role ([Moss et al., 1992](#)).

Virus shed can be frequently detected from throat washes of asymptomatic carriers. The levels of shedding are thought to be quite stable over many months, although with different rate depending on the individuals. A direct relationship appears to exist between the level of virus shedding in the throat and the level of virus-infected B cells in the blood. However, no obvious relationship was shown between the levels of EBV virus shedding from the throat and either anti-VCA or anti-EA titres in the serum of healthy carriers ([Yao et al., 1985](#)).

### (iii) EBV-associated malignant diseases

The major features of the humoral response to EBV in different EBV-associated malignancies have been reported ([Khanna et al., 1995](#)) but no specific pattern could be defined as useful prognostic markers for these diseases.

Although anti-VCA IgA serology was proposed as an effective and sensitive prognostic and diagnostic marker for nasopharyngeal carcinoma, more recent studies have shown high false positive rates for this antibody ([Low et al., 2000](#)). Furthermore, the follow-up of individuals with high titres of IgA to VCA demonstrated that a significant portion of those seroconverted back to normal, and did not develop nasopharyngeal carcinoma ([Lo et al., 2004](#)).

## (b) Detection of EBV in tissues and serum

## (i) Healthy carriers

In healthy carriers, EBV is mostly present in a latent form as episomal DNA in resting memory B cells. The frequency of EBV-carrying cells in peripheral blood ranges from 1 in  $2 \times 10^5$ – $10^7$  whole mononuclear cells or 1 in  $2 \times 10^4$ – $10^6$  B cells, and is quite stable in an individual over time. It was estimated that less than 1 in 40 EBV-infected cells can replicate the virus in the peripheral blood of healthy carriers ([Miyashita et al., 1995](#); [Decker et al., 1996](#)). Therefore, only very highly sensitive polymerase chain reaction (PCR) assays can detect EBV DNA in peripheral blood cells.

## (ii) EBV-associated malignancies

## - EBV DNA in tumour tissues

The EBV genome can be detected in tumour cells by PCR or in-situ hybridization assays using the BamH1 internal fragment of the viral genome as a probe. However, a major technical breakthrough has been the use, especially by in-situ hybridization, of probes specific for the small nuclear EBV-encoded RNAs, EBER-1 and EBER-2, which are highly expressed in all forms of EBV infection ([Wu et al., 1990](#)). The high sensitivity of this method allowed the determination of the incidence of EBV infection in the very scarce neoplastic Hodgkin and Reed-Sternberg cells (HRC) in biopsies from Hodgkin disease patient ([IARC, 1997](#)).

Detection of EBV genomic DNA by PCR using EBV genes (e.g. *EBNA-1*, *EBNA-2*, and *LMP-1*) as targets in tissue obtained from nasopharyngeal biopsy and fine-needle aspirate samples have also been shown as being reliable and accurate methods for the diagnosis of nasopharyngeal carcinoma ([Yap et al., 2007](#)). In addition, nasopharyngeal carcinoma patients have a very high load of EBV DNA as collected in non-invasive nasopharyngeal brushing. EBNA1 and BARF1 mRNAs are detected at even higher levels in such samples, whereas no EBV mRNA is

detected from nasopharyngeal brushing samples of healthy donors ([Stevens et al., 2006](#)).

## - Cell-free EBV DNA in serum

Cell-free EBV DNA has been detected in the plasma and serum of patients with several EBV-associated malignant diseases: Hodgkin disease, post-transplant lymphoproliferative diseases, NK/T-cells lymphoma, Burkitt lymphoma, nasopharyngeal carcinoma, and EBER-positive gastric carcinoma; and, this detection correlated with the EBV status in tumours. In contrast, cell-free EBV DNA was not detected in any of the healthy control subjects ([Lei et al., 2000, 2002](#); [Lo et al., 2001](#); [Musacchio et al., 2006](#)). Plasma EBV DNA, as measured by real-time quantitative PCR, has been proposed as a sensitive and specific tumour marker for diagnosis, disease monitoring, and prediction of outcome for several of the EBV-associated diseases ([Lo et al., 2001](#); [Lei et al., 2002](#); [Shao et al., 2004](#)).

Low-level EBV DNA positivity in serum has been reported to occur relatively frequently after stem-cell transplantation, and may subside without specific treatment. However, high EBV DNA levels (i.e.  $> 50\,000$  copies/mL) are strong predictors for the development of post-transplantation lymphoproliferative disease ([Aalto et al., 2007](#)).

The lack of detectable viral DNA in the serum of healthy carriers indicates that although most of these individuals are expected to be carrying EBV DNA in their lymphocytes, EBV DNA is not usually found in serum in the absence of active EBV disease. It is likely, however, that the viral DNA in serum is present in cases of EBV reactivation as well as in cases of primary infection, and tests for viral DNA can not discriminate between the two cases unless they are used in conjunction with serology. EBV reactivation is particularly relevant in immunocompromised patients ([Chan et al., 2001](#)).

## 2. Cancer in Humans

In the previous *IARC Monograph*, EBV infection was associated with several cancer types: Burkitt lymphoma, immunosuppression-related non-Hodgkin lymphoma, extranodal NK/T-cell lymphoma (nasal type – [Swerdlow et al., 2008](#); previously known as angiocentric T-cell lymphoma – [IARC, 1997](#)); Hodgkin lymphoma, and nasopharyngeal carcinoma ([IARC, 1997](#)). The following text comprises updated relevant data from case-control and cohort studies for several cancer types in relation to infection with EBV.

### 2.1 Virus-associated B-cell lymphoma

#### 2.1.1 Burkitt lymphoma

There are three subtypes of Burkitt lymphoma: endemic Burkitt lymphoma, sporadic Burkitt lymphoma, and immunodeficiency-associated Burkitt lymphoma. Endemic Burkitt lymphoma is defined as affecting children in equatorial Africa and New Guinea, sporadic Burkitt lymphoma affects children and young adults throughout the world, and immunodeficiency-associated Burkitt lymphoma is primarily associated with HIV infection. The majority of endemic Burkitt lymphoma, sporadic Burkitt lymphoma, and immunodeficiency-associated Burkitt lymphoma form three distinct clinical entities. It has been reported that EBV is detected in the tumour tissue of almost 100% of the cases of endemic Burkitt lymphoma, this proportion is less in cases of sporadic and immunodeficiency-associated Burkitt lymphoma ([Carbone et al., 2008](#)).

With regard to endemic Burkitt lymphoma, two new studies ([Carpenter et al., 2008](#); [Mutalima et al., 2008](#)) from Uganda and Malawi (with 325 and 148 cases, respectively) add to the evidence from five case-control studies (including 431 cases in total) ([Henle et al., 1969, 1971b](#); [Klein](#)

[et al., 1970](#); [Hirshaut et al., 1973](#); [Nkrumah et al., 1976](#)), and one cohort study (with 16 cases) ([Geser et al., 1982](#)) outlined in the previous *IARC Monograph* ([IARC, 1997](#)). Both studies ([Carpenter et al., 2008](#); [Mutalima et al., 2008](#)) demonstrate there is a relationship between an increase in the titre of antibodies against EBV-VCA and an increase in risk for endemic Burkitt lymphoma (see Table 2.1 available at <http://monographs.iarc.fr/ENG/Monographs/vol100B/100B-01-Table2.1.pdf>). There are no new data available relating to sporadic Burkitt lymphoma to add to the 90 cases in the four studies previously reported ([Hirshaut et al., 1973](#); [Ablashi et al., 1974](#); [Gotleib-Stematsky et al., 1976](#); [Çavdar et al., 1994](#)).

#### (a) Cofactors for endemic Burkitt lymphoma

A thorough review of potential cofactors for endemic Burkitt lymphoma (e.g. malaria, sickle-cell trait, *Euphorbia tirucalli*, and other medicinal plants) is outlined in the previous *IARC Monograph* ([IARC, 1997](#)), and, with the exception of malaria, no new data were available to the Working Group. Previously, the relationship between malaria and endemic Burkitt lymphoma was based mostly on ecological data – for example, geographic correlations between the prevalence of malaria and the reported incidence of endemic Burkitt lymphoma, and apparent declines in the incidence following widespread malaria eradication programmes. One intervention study ([Geser et al., 1989](#)) in the United Republic of Tanzania confirmed the relationship between malaria prevalence and the incidence of Burkitt lymphoma. More recently, two case-control studies have demonstrated an increasing risk of endemic Burkitt lymphoma in relation to an increase in the titre of antibodies against malaria, and also suggested that EBV and malaria act synergistically in the etiology of the disease ([Carpenter et al., 2008](#); [Mutalima et al., 2008](#)). In addition, the use of insecticides or bed nets in the home was associated with substantially

lower risks of endemic Burkitt lymphoma. There is evidence that malaria reduces T-cell-mediated immunosurveillance of EBV-infected cells, and is linked to an increased viral load of EBV ([Moormann et al., 2005, 2007, 2009](#)).

### 2.1.2 Hodgkin lymphoma

EBV is more commonly associated with classic Hodgkin lymphoma, especially the mixed-cellular subtypes. The non-classical nodular lymphocyte-predominant Hodgkin lymphoma cases are very rarely associated with EBV ([Khalidi et al., 1997](#)). Developing countries have an increased incidence of EBV-positive cases, which may be attributed to the existence of underlying immunosuppression ([Jarrett et al., 1991](#); [Murray & Young, 2005](#)). A bimodal age distribution has been recognized for EBV-positive Hodgkin lymphoma patients; children (< 15 years) and older-age groups tend to have much higher rates than young adults ([Flavell & Murray, 2000](#)). In western populations, the EBV genome has been detected in the tumour tissue of 40–50% of Hodgkin lymphoma cases ([Weiss, 2000](#)). The subclassification of Hodgkin lymphoma cases as EBV-positive or EBV-negative provides the potential to identify etiological subgroups ([Alexander et al., 2000](#)). EBV has been identified as a cause of infectious mononucleosis, a potential risk factor for Hodgkin lymphoma, which results from hyperproliferation of EBV-containing B cells, and a reactive T-cell response ([Henle et al., 1968](#)).

Previously, epidemiological data on the association between Hodgkin lymphoma and EBV were derived from several sources:

- Investigations of the relationship between infectious mononucleosis and Hodgkin lymphoma from six case-control studies (odds ratios (ORs) ranging from 1.0–8.2) (Table 18, [IARC, 1997](#); [Henderson et al., 1979](#); [Gutensohn & Cole, 1981](#); [Gutensohn, 1982](#); [Evans & Gutensohn, 1984](#); [Bernard et al., 1987](#); [Serraino et al., 1991](#)) and

six cohort studies (ORs ranging from 2.0–5.0) (Table 22, [IARC, 1997](#); [Miller & Beebe, 1973](#); [Connelly & Christine, 1974](#); [Rosdahl et al., 1974](#); [Carter et al., 1977](#); [Muñoz et al., 1978](#); [Kvåle et al., 1979](#)). A 2–4-fold increased risk for Hodgkin lymphoma within the first 3 years following infectious mononucleosis was also demonstrated ([Rosdahl et al., 1974](#); [Muñoz et al., 1978](#))

- 41 case-control studies of Hodgkin lymphoma in which there was evidence of antibodies against EBV-VCA (22 studies; ORs, 0.8–79; Table 19, [IARC, 1997](#)), EA (11 studies; ORs, 1.2–infinity; Table 20, [IARC, 1997](#)), and EBNA (eight studies; five with equivocal results, and three with ORs ranging from 1.7–infinite; Table 21, [IARC, 1997](#))
- Two large cohort studies, both reporting statistically significant excess risks associated with antibodies against EBV ([Mueller et al., 1989](#); [Lehtinen et al., 1993](#))

One further cohort study ([Hjalgrim et al., 2000](#)) of patients with infectious mononucleosis (reporting 46 cases of Hodgkin lymphoma; OR, 2.6; 95%CI: 1.9–3.4), together with seven case-control studies ([Gallagher et al., 1999](#); [Alexander et al., 2000, 2003](#); [Glaser et al., 2005](#); [Berrington de González et al., 2006](#); [Musacchio et al., 2006](#); [Dinand et al., 2007](#); [Hjalgrim et al., 2007b](#)) addressed the association between EBV and Hodgkin lymphoma (see Table 2.2 available at <http://monographs.iarc.fr/ENG/Monographs/vol100B/100B-01-Table2.2.pdf> and Table 2.3 available at <http://monographs.iarc.fr/ENG/Monographs/vol100B/100B-01-Table2.3.pdf>). In two studies, one in the United Kingdom ([Gallagher et al., 1999](#)) and one in Brazil ([Musacchio et al., 2006](#)), examining the association between EBV DNA (*Bam*H1-W) in serum and risk for Hodgkin lymphoma, cases of both EBV-positive and EBV-negative Hodgkin lymphoma had a much higher proportion with detectable EBV DNA

in serum/plasma than that of healthy controls. In a case-control study involving 145 Hodgkin lymphoma cases and 25 follicular hyperplasia controls in India, EBV DNA was detected in the lymph nodes of 140 (96.6%) Hodgkin lymphoma cases and but not in controls (0%) ([Dinand et al., 2007](#)). One case-control study from South Africa did not find a positive association between levels EBV antibody in serum and Hodgkin lymphoma ([Berrington de González et al., 2006](#)). In case-control studies on the association between a history of infectious mononucleosis and risk for Hodgkin lymphoma, a significant association was observed in EBV-positive Hodgkin lymphoma cases, particularly in those with young age at onset in the United Kingdom ([Alexander et al., 2000, 2003](#)), and Denmark and Sweden ([Hjalgrim et al., 2007b](#)). However, no significant association between a history of infectious mononucleosis and Hodgkin lymphoma was found in the USA ([Glaser et al., 2005](#)). A case-case analysis in a population-based case-control study compared 95 EBV-positive and 303 EBV-negative Hodgkin lymphoma cases ([Chang et al., 2004](#)). EBV antibody titres were significantly higher in the EBV-positive cases, including anti-VCA IgG and IgA, EA and an EBNA-1:EBNA-2 ratio  $\leq 1$ . With mutual adjustment, the odds ratios for elevated VCA IgG were 3.6 (95%CI: 1.4–8.7), and for low EBNA-1:EBNA-2 ratio, 3.2 (95%CI: 1.1–9.0).

### 2.1.3 Lymphomas in immunosuppressed individuals

#### (a) Post-transplant lymphoproliferative disorders

Since the original reports of post-transplant lymphoproliferative disorders in 1969 ([McKhann, 1969; Penn et al., 1969](#)), a higher incidence of lymphoproliferative disorders in transplant recipients of both a solid organ and bone marrow has been observed ([Carbone et al., 2008](#)). According to the WHO classification ([Swerdlow et al., 2008](#)), post-transplant lymphoproliferative

disorders may be classified into: a) early lesions, generally represented by EBV-driven polyclonal lymphoproliferations; and, b) true monoclonal diseases, including polymorphic post-transplant lymphoproliferative disorders and monomorphic post-transplant lymphoproliferative disorders.

In addition to data presented previously ([IARC, 1997](#)), a case-control study of EBV DNA in plasma samples of four cases of post-transplant EBV-associated nasal NK/T-cell lymphoma, two cases of post-transplant lymphoproliferative disorders, and 35 healthy controls in the Hong Kong Special Administrative Region ([Lei et al., 2000](#)) were considered – all six cases (100%) and no control (0%) had EBV DNA (*BamHI-W*) levels in plasma (see Table 2.4 available <http://monographs.iarc.fr/ENG/Monographs/vol100B/100B-01-Table2.4.pdf>).

#### (b) HIV-associated lymphoproliferative disorders

HIV-associated lymphoproliferative disorders are a heterogeneous group of diseases that occur in the presence of HIV-associated immunosuppression. These aggressive disorders include both central nervous system and systemic lymphomas. Primary effusion lymphoma (reviewed in this volume, see *Monograph* on Kaposi Sarcoma Herpes Virus (KSHV)) also occurs and often involves EBV in addition to KSHV. The categories of HIV-associated non-Hodgkin lymphoma (HIV-NHL) confirmed in the latest WHO Classification of Tumours ([Raphael et al., 2001, 2008](#)) are grouped as follows ([Carbone et al., 2008](#)):

- Lymphomas also occurring in immunocompetent patients. Most of these HIV-NHLs belong to three high-grade B-cell lymphomas: Burkitt lymphoma, diffuse large B-cell lymphoma with centroblastic features, and diffuse large B-cell lymphoma with immunoblastic features. According to the site of involvement, the present spectrum of HIV-NHL includes extranodal/nodal lymphomas, and

- primary central nervous system lymphomas ([Carbone et al., 2008](#));
- Unusual lymphomas occurring more specifically in HIV-positive patients – these lymphomas include two rare entities, namely, primary effusion lymphoma ([Cesarman et al., 1995](#)), and plasmablastic lymphoma of the oral cavity ([Delecluse et al., 1997; Carbone et al., 1999](#));
  - Lymphomas also occurring in other immunodeficient states ([Carbone et al., 2008](#)).

One nested case-control study of non-Hodgkin lymphoma among HIV-infected people identified an association between anti-VCA antibodies and risk of disease, although no division by histological subtype of lymphoma was possible ([Newton et al., 2006](#); see Table 2.5 available <http://monographs.iarc.fr/ENG/Monographs/vol100B/100B-01-Table2.5.pdf>).

#### *2.1.4 EBV-positive diffuse large B-cell lymphoma of the elderly*

This is defined as an EBV-containing diffuse large B-cell lymphoma occurring in patients over 50 years of age without any prior lymphoma or other known immunodeficiency. These EBV-positive lymphomas account for 8–10% of diffuse large B-cell lymphomas in Asian countries, but there are little data from western countries ([Oyama et al., 2007; Swerdlow et al., 2008](#)).

## **2.2 Virus-associated T-cell and NK-cell lymphomas**

EBV is an established cause of extranodal NK/T-cell lymphoma (nasal type); previously called angiocentric T-cell lymphoma ([IARC, 1997; Chan et al., 2001b; Swerdlow et al., 2008](#)). A higher incidence of extranodal NK/T-cell lymphoma (nasal type) has been described in central and south America, and in several east Asian countries ([Suzuki et al., 2008](#)). Several

recent large case series confirm the presence of EBV in tumour cells in nearly 100% of cases ([Barrionuevo et al., 2007; He et al., 2007](#)). In a case-control study of anti-EBV VCA, EA, and EBNA in serum samples of 100 cases of peripheral NK/T-cell proliferative disease/lymphoma, and 100 age- and sex-matched controls in Thailand ([Mitarnun et al., 2002](#)), elevated serum levels of anti-EBV VCA IgG and EA IgG were associated with an increased risk of the disease (see Table 2.4 on-line). In another case-control study of seven cases of peripheral NK/T-cell proliferative disease, 38 cases of peripheral NK/T-cell lymphoma, and 45 age- and sex-matched healthy controls in Thailand ([Suwiwat et al., 2007](#)), 37 (82.2%) cases and no control had detectable EBV DNA levels (*BamHI-W*) in their plasma.

Other T-cell lymphoproliferative disorders that have been reported to be associated with EBV include a subset of peripheral T-cell lymphomas ([Dupuis et al., 2006a, b; Tan et al., 2006](#)), enteropathy-type T-cell lymphomas ([de Bruin et al., 1995; Quintanilla-Martínez et al., 1998; Isaacson et al., 2001](#)),  $\gamma\delta$  T-cell lymphomas (hepatosplenlic and non-hepatosplenlic) ([Arnulf et al., 1998; Ohshima et al., 2000](#)), T-cell lymphoproliferative disorders after chronic EBV infection ([Quintanilla-Martínez et al., 2000](#)), EBV-associated cutaneous T-cell lymphoproliferative disorders (especially in Asia) ([Chan et al., 2001a; Kim et al., 2006](#)), and aggressive NK-cell leukaemias/lymphomas ([Chan et al., 2001b](#)).

Angioimmunoblastic T-cell lymphoma is a distinct entity of peripheral T-cell lymphoma ([Dupuis et al., 2006b](#)). Angioimmunoblastic T-cell lymphomas are also nearly always associated with EBV; however, the cellular origins remain unknown. The virus is present in B cells, rather than in the neoplastic T cells, suggesting an indirect role, hypothetically through antigenic stimulation ([Knecht et al., 1990; Dupuis et al., 2006b; Dunleavy et al., 2007](#)).

### 2.2.1 Other non-Hodgkin lymphoma

Two cohort studies including a total of 115 cases of non-Hodgkin lymphoma occurring in apparently immunocompetent individuals reported no excess risk in relation to anti-VCA antibody titres ([Mueller et al., 1991](#); [Lehtinen et al., 1993](#); [IARC, 1997](#)). Since then, four case-control studies have investigated the serological evidence of infection with EBV: two reported no associations ([Hardell et al., 2001a](#); [Berrington de González et al., 2006](#)), one reported a borderline increased association ([Hardell et al., 2001b](#)), and the other showed a significant association between abnormal reactive EBV antibody patterns and non-Hodgkin lymphoma (OR, 1.4; 95%CI: 1.2–1.7; based on 1085 cases; [de Sanjose et al., 2007](#)).

## 2.3 Cancers of the nasopharynx, stomach, and lymphoepithelium

### 2.3.1 Cancer of the nasopharynx

Cancer of the nasopharynx is rare in most populations around the world but common in South-East Asia ([Ferlay et al., 2010](#)). According to the WHO Classification of Tumours, cancers of the nasopharynx are classified into three types: keratinizing squamous cell carcinoma (Class I), non-keratinizing carcinoma (Class II), and basaloid squamous cell carcinoma (Class III) ([Chan et al., 2005](#)). Most cancers of the nasopharynx diagnosed in the high-risk areas belong to Class II. In the previous *IARC Monograph*, an increased risk of cancer of the nasopharynx was demonstrated in five case-control studies in which all 671 cases had evidence of infection with EBV ([de Thé et al., 1978b](#); [Lanier et al., 1980a](#); [Pearson et al., 1983b](#); [Chen et al., 1987](#); [Zheng et al., 1994a](#)), and one cohort study in which all seven cases had evidence of infection with EBV ([Chan et al., 1991](#)). Since then, two cohort ([Chien et al., 2001](#); [Ji et al., 2007](#)) and eight case-control

studies ([Mutirangura et al., 1998](#); [Lo et al., 1999](#); [Chen et al., 2001](#); [Lin et al., 2001, 2004](#); [Fan et al., 2004](#); [Leung et al., 2004](#); [Tiwawech et al., 2008](#)) on the association between EBV and cancer of the nasopharynx have been reported.

The prospective cohort studies on the association between EBV biomarkers and the development of nasopharyngeal carcinoma are shown in Table 2.6 (available at <http://monographs.iarc.fr/ENG/Monographs/vol100B/100B-01-Table2.6.pdf>) and include 22 and 131 cases ([Chien et al., 2001](#); [Ji et al., 2007](#), respectively). In one study ([Chien et al., 2001](#)), the relative risks of developing nasopharyngeal carcinoma were 22.0 (95%CI: 7.3–66.9) for anti-EBV VCA IgA-seropositivity, and 3.5 (95%CI: 1.4–8.7) for anti-EBV DNase-seropositivity. Compared with those who were seronegative for both anti-EBV markers as the referent group, the adjusted relative risk was 32.8 (95%CI: 7.3–147.2) for those who were seropositive for both anti-EBV markers ([Chien et al., 2001](#)). In the other study ([Ji et al., 2007](#)), seropositivity of anti-EBV VCA IgA was associated with an increased risk of nasopharyngeal carcinoma during follow-up with a crude relative risk of [9.4; 95%CI: 6.8–13.5]. For seven case-control studies (see Table 2.7 available at <http://monographs.iarc.fr/ENG/Monographs/vol100B/100B-01-Table2.7.pdf>), the odds ratios for nasopharyngeal carcinoma in relation to the evidence of infection with EBV ranged from 20.6 to infinity ([Mutirangura et al., 1998](#); [Lo et al., 1999](#); [Lin et al., 2001, 2004](#); [Chen et al., 2001](#); [Fan et al., 2004](#); [Leung et al., 2004](#)). For only one study were the reported odds ratios below 3 ([Tiwawech et al., 2008](#)).

Since the previous *IARC Monograph*, no new cofactors for cancer of the nasopharynx have been identified.

### 2.3.2 Lymphoepithelioma-like carcinomas

Rare carcinomas with a histological similarity to nasopharyngeal carcinoma (in that both have lymphoid stroma) are called lymphoepithelial-like carcinomas ([Tsang & Chan, 2005](#)). These can occur in multiple organ sites with epithelial lining, and have been reported most frequently in the salivary glands, and in the stomach. In the previous *IARC Monograph* (Table 30, [IARC, 1997](#)), 19 case series reported on the association of EBV with cancers of the stomach. These included a total of 102 lymphoepithelial cancers of which 90 had evidence of infection with EBV. In addition, five case series of lymphoepithelial cancers of the salivary gland indicated that 25/27 reported cases had evidence of infection with EBV (Table 28, [IARC, 1997](#)). More recently, in a case-control study of lymphoepithelial cancer of the salivary gland, 16/16 cases had evidence of EBV DNA in tumour tissue compared to 0/12 salivary gland tumours of other histology ([Wang et al., 2004](#); see Table 2.8 available at <http://monographs.iarc.fr/ENG/Monographs/vol100B/100B-01-Table2.8.pdf>). More recently, multiple case series including 209 cases of parotid gland lymphoepithelial carcinoma reported that EBV DNA was present in the tumour cells of 208 cases ([Leung et al., 1995](#); [Kim et al., 1999](#); [Squillaci et al., 2000](#); [Białas et al., 2002](#); [Saku et al., 2003](#); [Wu et al., 2004](#); [Jen et al., 2005](#); [Hsu et al., 2006](#); [Saqui-Salces et al., 2006](#)). Therefore, the evidence of an association between EBV infection and lymphoepithelioma-like carcinomas has become substantially stronger since the previous *IARC Monograph* ([IARC, 1997](#)).

### 2.3.3 Cancer of the stomach

In 19 case series of cancer of the stomach reported in 1997 (Table 30, [IARC, 1997](#)), 115/1322 (9%) of cases had evidence of EBV DNA in tumour tissue. None of these studies provided information on possible infection with *H. pylori*. Since then,

three case-control studies have been published in which two include a total of 69/174 cases with evidence of EBV DNA in tumour tissue ([Shinkura et al., 2000](#); [Lo et al., 2001](#); see Table 2.9 available at <http://monographs.iarc.fr/ENG/Monographs/vol100B/100B-01-Table2.9.pdf>), while the other, a nested case-control study, considered only serological responses against EBV in relation to all cancers of the stomach ([Koshiol et al., 2007](#); see Table 2.10 available at <http://monographs.iarc.fr/ENG/Monographs/vol100B/100B-01-Table2.10.pdf>). Recent findings indicate that people with EBV-positive tumours tend to have higher antibody titres against EBV ([Shinkura et al., 2000](#)) or higher EBV viral loads ([Lo et al., 2001](#)) than people with EBV-negative tumours or controls without cancer of the stomach. A recent review of over 30000 cancers of the stomach identified evidence of EBV DNA in 8% of the patients ([Sousa et al., 2008](#)). It is important to note that EBV DNA is present within tumour cells and not in the surrounding epithelium, and that virus monoclonality has been demonstrated in tumour cells only ([Sousa et al., 2008](#)). [The Working Group noted that the interaction of *H.pylori* and EBV in the etiology of cancer of the stomach needs further clarification.]

## 2.4 Other cancers

Several other studies investigating the evidence of infection with EBV in relation to cancers of the oral cavity, breast, cervix, testis, prostate, and multiple myeloma and leukaemia show limited or no evidence of an association.

See Table 2.11 available online at <http://monographs.iarc.fr/ENG/Monographs/vol100B/100B-01-Table2.11.pdf>, Table 2.12 available at <http://monographs.iarc.fr/ENG/Monographs/vol100B/100B-01-Table2.12.pdf>, and Table 2.13 available at <http://monographs.iarc.fr/ENG/Monographs/vol100B/100B-01-Table2.13.pdf>.

### 3. Cancer in Experimental Animals

In this volume, the Working Group decided not to include a separate section on “Cancer in Experimental Animals” in the *Monographs* on viruses but rather to include description of such studies under Section 4 (below). The reasoning for this decision is explained in the General Remarks.

### 4. Other Relevant Data

The mechanistic evidence for EBV-associated oncogenesis was thoroughly reviewed in the previous *IARC Monograph* ([IARC, 1997](#)), and is based on the following:

- The ability of EBV to immortalize human B lymphocytes *in vitro* ([Nilsson et al., 1971](#))
- Other effects of EBV infection of human cells *in vitro* affecting their phenotype – migration and invasion ([Pegtel et al., 2005; Dawson et al., 2008](#))
- Convincing links of these phenotypic effects on cell proliferation, apoptosis, and cell migration to single EBV proteins or combinations thereof, primarily by the expression of or “knock down” of single proteins ([Klein & Ernberg, 2007](#))
- Induction of EBV-positive lymphoproliferative diseases or lymphomas by infection of animals (New World monkeys) with EBV, or transplantation of infected human B lymphocytes to immunosuppressed mice (SCID or nude; [Mosier et al., 1988; Young et al., 1989](#))

Although more circumstantial, *in vivo* evidence is also strong.

The EBV genome and the constant expression of viral proteins detected in a wide spectrum of human malignancies strongly support a role for EBV in carcinogenesis. EBV-associated tumours

include diffuse large B-cell lymphomas that occur in immunocompromised persons, such as transplant recipients, certain congenitally immunocompromised individuals, and HIV-infected persons. EBV is also involved in the pathogenesis of Burkitt lymphoma, Hodgkin lymphoma, some T/NK-cell lymphomas, cancer of the nasopharynx, and some cancers of the stomach ([Ambinder & Ceserman, 2007; Table 4.1](#)).

In several of the high-risk groups for EBV-associated cancers, the EBV-genome load found in the peripheral blood lymphocytes or plasma precedes the development of malignancy ([Lin et al., 2004](#)).

Further convincing evidence comes from the successful prevention or regression of EBV-carrying lymphoid tumours in humans by the adoptive transfer of EBV-specific cytotoxic T cells ([Heslop & Rooney, 1997; Gustafsson et al., 2000; Bolland et al., 2007; Merlo et al., 2008](#)).

#### 4.1. Transforming capacity of EBV

*In vitro*, EBV has the unique ability to transform resting B cells into permanent, latently infected lymphoblastoid cell lines, a system that has provided an invaluable, albeit incomplete, model of the lymphomagenic potential of the virus ([Young & Rickinson, 2004](#)). EBV can infect human B lymphocytes from any human donor as long as they express the CD21 consensus receptor for the attachment of EBV particles, and entry into the host cell (see Section 1.1). The B lymphocytes of several differentiation stages can be infected. The most efficient infection – and immortalization – has been seen in mature virgin B lymphocytes, which are IgM- and IgD-positive. The virus establishes a ‘latent’ blastogenic “growth programme” (latency III) expressing nine latency-associated proteins and several non-translated genes, the two EBERs, micro-RNAs and BARF-transcripts. The infection results in the immortalization of the target

**Table 4.1 EBV-associated tumours**

Tumour type	Approx % EBV positivity
<b>Lymphoid tissues</b>	
<i>Burkitt lymphoma</i>	
Endemic (Subequatorial Africa)	>95 <sup>a</sup>
Sporadic (Other countries)	20–80 <sup>b</sup>
AIDS	30–50
<i>AIDS-related DLBCL</i>	
Immunoblastic	70–100
Non-immunoblastic	10–30
<i>CNS lymphomas</i>	>95
Plasmablastic lymphoma	60–75 <sup>c</sup>
<i>Primary effusion lymphoma</i>	70–90
<i>Post-transplant lymphoproliferative disorders</i>	>90
<i>Primary effusion lymphoma</i>	70–90
<i>Hodgkin lymphoma</i>	20–90 <sup>d</sup>
<i>EBV-positive diffuse large B cell lymphoma of the elderly</i>	100
<i>Extranodal T/NK cell lymphoma, nasal type</i>	100
<b>Epithelial tissues</b>	
Nasopharyngeal carcinoma	100
Lymphoepithelioma-like carcinoma	>80 <sup>e</sup>
Gastric carcinoma	5–10
<b>Other tissues</b>	
Leiomyosarcoma in immunodeficient individuals	100

<sup>a</sup> The small fraction of EBV-negative cases in endemic regions may represent cases of sporadic Burkitt lymphoma.

<sup>b</sup> The proportion of positive cases varies widely with geographic distribution, ranging from 20% in some US and European studies to close to 80% in India, Egypt, and Northeastern Brazil.

<sup>c</sup> EBV positivity in plasmablastic lymphoma of the oral cavity in AIDS patients is close to 100%.

<sup>d</sup> The proportion of positive cases varies widely with geographic distribution, age, histological type and immunocompetence status. It is more frequent in low-resource areas, in very young or older patients, in the mixed cellularity subtypes, and approaches 100% in patients with AIDS.

<sup>e</sup> Stomach, parotid and liver

Compiled by the Working Group mainly from two reviews ([Ambinder & Ceserman, 2007](#); [Cesarman & Chadburn, 2007](#))

cells with a high efficiency, resulting in proliferation with a 30–40-hour extended cell cycle ([Einhorn & Ernberg, 1978](#)).

*In vivo*, mucosal epithelial cells can also be infected (see Section 1.1). By contrast, the infection of epithelial cells *in vitro* does not activate the full growth-transforming programme of the virus, and rarely – if ever – achieves full lytic replication ([Young & Rickinson, 2004](#)).

## 4.2 Biochemical and biological properties of EBV gene products

This section provides an overview of the properties of the EBV-encoded latency-associated gene products, and their mechanism of action relevant to transformation and tumorigenesis.

### 4.2.1 The latent EBV nuclear gene products (EBNAs)

The main known functions of the six nuclear proteins, EBNA-1, -2, -3A, -3B, -3C and -LP are summarized in [Table 4.2](#).

#### (a) EBNA-1

EBNA-1, encoded by the ORF BKRF1, is a protein of highly variable size (60–100 kDa) due to a glycine–alanine repetitive sequence ([Hennessy et al., 1983](#)). With the possible exception of latently infected resting B cells, EBNA-1 is expressed in most EBV-carrying cells, irrespective of the cell phenotype, level of differentiation or, in the case of lymphocytes, activation status (Section 1.1.7; [Tables 1.3](#) and [1.4](#)). EBV-positive B cells that only express EBNA-1 are poorly recognized by CTLs. The glycine–alanine repeat of EBNA-1 inhibits its processing through the ubiquitin–proteasome system and the subsequent MHC-class 1 association of the derived peptides, a prerequisite for recognition by CD8-positive CTLs ([Levitskaya et al., 1995](#)). This results in a dramatically extended half-life of EBNA-1 to more than 2 weeks, and may contribute to its

likely presence in resting B cells without *de novo* synthesis.

EBNA-1 is a DNA-binding protein that can bind to three different specific palindromic target sites on the viral DNA, each of which occurs multiple times in the viral genome, and is involved in the control of episomal maintenance, DNA replication, and viral gene expression in latency. Twenty binding sites are located in the family of repeats (FR) element, four in the dyad symmetry (DS) element, both these elements being localized to the origin of replication (OriP), and finally, two EBNA-1-binding sites are situated downstream of the Q promoter ([Ambinder et al., 1990](#); see also the map of the EBV genome depicted in Fig. 1.1). The dyad symmetry element controls S-phase-associated viral DNA replication. EBNA-1 regulates viral promoters via its multiple binding sites. The family of repeats element acts as an enhancer for the C promoter, directing transcription for all six EBNAs, and the Q-promoter elements are negative regulators of Q-promoter-driven EBNA-1 transcription through a negative autoregulatory feedback loop.

EBNA-1 acts as a transcriptional regulator of viral programmes, and maintains the viral genomes in the host cell. It is therefore necessary for cell transformation. EBNA-1 might also directly contribute to the tumorigenic process as it is expressed in all tumour types ([Sample & Sample, 2008](#)). It has been shown to exert an anti-apoptotic effect. Blocking its function with dominant negative mutants induces apoptosis in Burkitt lymphoma cell lines ([Kirchmaier & Sugden, 1997](#)).

EBNA-1 induces the specific recombinases RAG-1 and -2, which could contribute to genomic instability or even specific translocations ([Tsimbouri et al., 2002](#)). EBNA-1 also induces genomic instability involving increases in the levels of reactive oxygen species ([Gruhne et al., 2009](#)).

Recently, it was shown that EBNA-1 can also physically bind to cellular promoters, but whether

**Table 4.2 Overview of the EBNA proteins: Functions and Interactions**

Name (alternative nomenclature)	Functions	Interaction with cellular proteins	Expression in B cells	Evidence for role in B-cell immortalization
EBNA-1	- Viral episome maintenance - Viral DNA replication - Regulation of viral promoters	Karyopherins 2 α and β; TAP/p32; USP7 (HAUSP); RPA	Latency I, II, and III	Yes
EBNA-2	Activation of viral and cellular promoters	PU.1; hSNF5; Spi-B; CBF1/ RBP-J kappa; p300/CBP; DP103; p100; TFIIE; TFIIF; TFIIB; TAF40; Myb; TBP	Latency III	Yes
EBNA-3A (EBNA-3)	Repression of the CBF1/ RBP-J kappa dependent transcription	CBF1/RBP-J kappa; RBP-2N; CtBP; epsilon-subunit of TCP-1; XAP-2 ; F538 (UK/ UPRT); AhR	Latency III	Yes
EBNA-3B (EBNA-4)		CBF1/RBP-J kappa; RBP-2N	Latency III	No
EBNA-LP (EBNA-5)	Co-activation of EBNA-2-dependent transcription	Hsp27; Hsp70 (Hsp72); Hsc70 (Hsp73); HAX-1; HA95; alpha & beta tubulins; prolyl-4-hydroxylase alpha-1 subunit; p14ARF; Fte-1/S3a	Latency III	Yes
EBNA-3C (EBNA-6)	Repression of the CBF1/ RBP-J kappa dependent transcription	CBF1/RBP-J kappa; RBP-2N; DP103; ProT- alpha; SMN; NM23-H1; pRB	Latency III	Yes

Adapted from [Klein & Ernberg \(2007\)](#)

or not this binding plays a role in regulating the transcription of these genes *in vivo* remains to be demonstrated ([Dresang et al., 2009](#)).

### (b) EBNA-2

EBNA-2 is a phosphoprotein of about 82 kDa, and is among the earliest viral protein expressed in newly infected B cells. EBNA-2 is a potent transactivator of many cellular and viral genes but does not bind directly to DNA. It influences the responding promoters through its interaction with CBF1/RBP-Jk, PU1, and other cellular proteins. The EBNA-2 protein complexes formed induce chromatin remodelling. Elements for EBNA-2 responsiveness have been found in the EBV-Cp, LMP-1, LPMP-2, and CD23 promoters ([Klein & Ernberg, 2007](#)).

EBNA-2 is essential for the transformation of B cells into immunoblasts, and for the derivation of lymphoblastoid cell lines. EBNA-2-defective viral substrains cannot immortalize B cells. EBNA-2 is the EBV-encoded oncoprotein that differs most extensively between EBV types 1 and 2. EBV type 1 is a more efficient transformer of primary B lymphocytes than is type 2 ([Rickinson et al., 1987](#)). Recruitment of EBNA-2 to DNA is essential for the transforming activity of EBV, and CBF1/RBP-Jk is its most extensively studied partner. CBF1/RBP-Jk functions as a downstream target of the Notch cell-surface receptor. Notch genes encode cell-surface receptors that regulate the developmental processes in a wide variety of organisms. The cleaved product of Notch is targeted to the nucleus where it binds to CBF1/RBP-Jk, and can activate transcription

([Strobl et al., 1997](#)). EBNA-2 is regarded as a constitutively active homologue of Notch. However, Notch can only partially substitute for EBNA-2 in B-cell transformation experiments, probably owing to its inability to upregulate the transcription of LMP1 or c-myc. Thus, the functional homology is partial ([Höfelmayr et al., 2001](#)).

EBNA-2 induces a variety of activation markers and other cellular proteins in B cells, including CD23, CD21, c-fgr, and c-myc. It is required for the expression of EBV-encoded LMP-1 and LMP-2A in immunoblastic cells ([Wang et al., 1990a, b](#); [Kaiser et al., 1999](#); [Klein & Ernberg, 2007](#)). The essential role of EBNA-2 in the immortalization of B cells is therefore due to its role in the transactivation of viral promoters (Cp, LMP-1 and -2) and of cellular genes associated with B-cell activation and growth, among them c-myc. C-myc activation in lymphocytes, in turn, induces protein synthesis (e.g. D-type cyclins and cyclin E) but also the downregulation of the inhibitors p21 and p27. The induction of c-myc is regarded as a major link between EBV infection and cell-cycle control ([Kaiser et al., 1999](#)). EBNA-2 is required to maintain the EBV-driven proliferation of B cells. EBNA-2 can be replaced by the constitutive expression of exogenous c-myc. The switch from the EBNA-2-driven to the c-myc-driven state is accompanied by a phenotypic change of the lymphoblastoid cell line-like cell to a more Burkitt lymphoma-like cell, resembling dividing germinal centre B cells ([Polack et al., 1996](#)).

#### (c) EBNA-LP

EBNA-LP (also known as EBNA-5) is a nuclear phosphoprotein. Together with EBNA-2, EBNA-LP is the earliest viral protein expressed in freshly infected B cells. Co-expression of EBNA-LP with EBNA-2 enhances EBNA-2-mediated transcriptional activation ([Klein & Ernberg, 2007](#)). The two proteins can induce the entry of resting B cells into the G1-phase ([Sinclair](#)

[et al., 1994](#)). EBNA-LP is tightly associated with the nuclear matrix, and often accumulates in the nuclear promyelocytic leukaemia bodies. EBNA-LP is also necessary for immortalization ([Pokrovskaja et al., 2001](#)). EBNA-LP was shown *in vitro* to exert an inhibitory effect on the p53–Rb axis by targeting the p53 regulator p14 ARF. The latter can bind MDM2, suppress its ability to mediate in the degradation of p53, and thereby increase the expression level of p53. It was suggested that EBNA-LP participates in the elimination of the p14 ARF–MDM2–p53 complexes and contributes to the downregulation of p14 ARF and p53 protein levels in EBV-infected B cells ([Kashuba et al., 2003](#)).

#### (d) EBNA-3

The EBNA-3 family – EBNA-3A (ORF: BLRF3 + BERF1), EBNA-3B (or EBNA-4, ORF: BERF2a + BERF2b), and EBNA-3C (or EBNA-6, ORF: BERF3 + BERF4) – comprises three large nuclear phosphoproteins in sizes ranging from 140–180 kDa. All three proteins are stable proteins that accumulate in intranuclear clumps, sparing the nucleolus ([Klein & Ernberg, 2007](#)).

All EBNA-3 proteins share a limited homology in a region near the N terminus, and this conserved domain mediates the binding to CBF1/RBP-Jk. This is how they all act as repressors of EBNA-2-mediated transactivation of the CBF1/RBP-Jk-dependent Cp, LMP-2A, and LMP-1 promoters. EBNA-3C also physically associates with histone deacetylase HDAC1, and can repress transcription through the Notch signalling pathway ([Radkov et al., 1999](#)).

EBNA-3C (but not EBNA-3A or -3B) can also activate the transcription of both cellular and viral genes (e.g. *CD21*, *CD23*, and *LMP-1*). This activation is clearly distinct from the interaction of EBNA-3C with CBF1/RBP-Jk, and requires an intact Spi binding site as well as a fully functional EBNA-2 protein ([Zhao & Sample, 2000](#)). EBNA-3C disrupts cell-cycle checkpoints at several levels. One is by recruiting the SCF<sup>skp2</sup>

ubiquitin ligase complex, which mediates the ubiquitination and degradation of pRB ([Knight et al., 2005](#)).

EBNA-3A and EBNA-3C, but not EBNA-3B, are necessary for *in vitro* immortalization ([Tomkinson et al., 1993](#)).

#### 4.2.2 The latent membrane proteins

EBV expresses three latent membrane proteins (LMPs) during latency II and III in immunoblasts as well as in derived tumours and cellines: LMP-1, LMP-2A, and LMP-2B. All three proteins are also detected in epithelial tumours of the nasopharynx, and during the early stages of oral hairy leukoplakia ([Webster-Cyriaque & Raab-Traub, 1998](#)) (see Section 1.1; [Table 1.4](#)). LMP-2A transcripts can also be expressed in resting virus-carrying B lymphocytes in healthy individuals – the reservoir of persistently latent EBV ([Chen et al., 1995](#)).

LMP-2A together with LMP-1 are necessary for continued lymphoma cell survival via TRAF2 regulation of NF-κB ([Guasparri et al., 2008](#)).

The three LMP proteins are highly multifunctional and interact with several cellular signalling pathways ([Table 4.3](#)). They are expressed at the cell surface membrane as well as in intracellular membranes of the Golgi and endoplasmic reticulum ([Hennessy et al., 1984](#); [Lynch et al., 2002](#)).

##### (a) LMP-1

LMP-1 is a 356-amino-acid protein which consists in a short *N*-terminal cytoplasmic domain, six membrane-spanning domains, and a *C*-terminal cytoplasmic domain 200 amino-acid long ([Liebowitz et al., 1986](#)).

LMP-1 is essential although not mandatory for the transformation of B lymphocytes into lymphoblastoid cell lines, and EBV mutants lacking LMP-1 fail to efficiently immortalize B cells ([Dirmeier et al., 2003](#)).

LMP-1 can induce lymphomas and epithelial tumours in transgenic mice, acting as tumour promoter after chemical initiation ([Curran et al., 2001](#)).

LMP-1 as an integral membrane protein acts like a constitutively activated receptor. It almost completely mimics the CD40-mediated signalling, and is thus functionally homologous to the TNF-receptor (TNFR)-family of proteins in B lymphocytes and epithelial cells. Indeed, it constitutively activates major signalling systems such as NF-κB (canonical and non-canonical), JNK-kinase, and JAK/STAT-pathways. Protection from apoptosis is one of its major downstream effects ([Lam & Sugden, 2003a, b](#)).

LMP-1 has been shown to interact with several proteins of the TNFR-signalling pathway through its C-terminal activation region (CTAR) 1 and 2 ([Table 4.3](#); [Lam & Sugden, 2003b](#)). These interactions result in the NF-κB-dependent upregulation of several genes. LMP-1 can block apoptosis due to the upregulation of several anti-apoptotic proteins, including A20 and Bcl-2, and the block of p53-mediated apoptosis by the latter ([Henderson et al., 1991](#); [Hatzivassiliou & Mosialos, 2002](#)). It may also alter the ratio of caspase-8, an initiator caspase, and its competitor FLIP (FLICE inhibitor protein) ([Tepper & Seldin, 1999](#)).

LMP-1 also activates JNK-kinase ([Eliopoulos et al., 1999](#); [Kieser et al., 1999](#)). It can also induce telomerase activity ([Terrin et al., 2008](#)).

Through its interference with several major signalling pathways in B cells and epithelial cells, LMP-1 mediates deregulation of several hundred cellular proteins. LMP-1 induces the expression of adhesion molecules such as ICAM-1 and LFA, and also MHC Class I and II ([Hatzivassiliou & Mosialos, 2002](#)).

Moreover, LMP-1 expressed in epithelial cell lines *in vitro* inhibits DNA repair and induces micronuclei formation, chromosomal aberrations, and consequent genomic instability ([Liu et al., 2004](#)).

**Table 4.3 Overview of the EBV latent membrane proteins: functions and interactions**

Name	Functions	Major protein interactions	Expression in B cells	Evidence for role in oncogenesis
LMP-1	Mimics CD 40 Activation of NFkB, JNK kinase, JAK/STAT, MAP kinase, Akt Cell survival Induction of adhesion and immune regulatory membrane proteins	RAF 1,2,3, TRADD BRAM 1 LMP-2A	Latency II-III	<b>Yes</b> - Anti-apoptotic - Survival of lymphoma cells - Transformation of primary rat embryo fibroblasts - Tumours in transgenic animals
LMP-2A	Interacts with phosphotyrosine kinases including Src-family and PI3-kinase Blocks lytic cycle Block BCR activation	Src, Lyn, Lck ZAP-70, Syk, AIP4/Nedd4	Latency I-III	<b>Yes</b> - Survival of B-cells and lymphoma cells - Cell migration and invasion
LMP-2B	Modulates function of LMP-2A	LMP-2A	Latency III	<b>No</b>

Adapted from [Klein & Ernberg \(2007\)](#)

### (b) LMP-2A and LMP-2B

LMP-2A contains 12 trans-membrane domains, and two intracellular tails: a 27-amino-acid C-terminal tail, important for protein aggregation, and a 119-amino-acid N-terminal tail that confers the capacity of LMP-2A to activate signal cascade. LMP-2A has been reported to aggregate into ‘cap-like’ structures at the plasma membrane and specifically associate with lipid rafts, sites enriched for signalling molecules ([Dykstra et al., 2001](#); [Higuchi et al., 2001](#)).

LMP-2A signalling mimics signalling through the B-cell receptor with which it shares structural and functional similarities. The N-terminal tail of LMP-2A contains eight phosphotyrosine motifs that interact with SH2-domain-containing proteins such as the immunoglobulin-receptor (IgR)-induced kinases Lyn. In addition, LMP-2A also possesses an Immunoglobulin Transactivation Motif (ITAM) with complete homology to the corresponding IgR-ITAM-motif of its gamma-chain that binds the Syk kinase in its activated phosphorylated state ([Klein & Ernberg, 2007](#)).

When expressed as a B-lineage-specific transgene in mice, it can both drive B-cell

development, and promote the survival of mature B-cells in the absence of surface immunoglobulin expression ([Merchant et al., 2000](#)). EBV mutants with a deleted LMP-2A gene fail to allow germinal centre B cells to survive; it is thus essential for growth transformation of these B cells ([Mancao & Hammerschmidt, 2007](#)).

If LMP-2A mimics B-cell receptor signalling, there is evidence however that expression of LMP-2A in B lymphocytes also attenuates normal activation through B-cell receptors. It was shown that LMP-2A blocks both B-cell receptor signalling and antigen-processing function in lymphoblastoid cell lines ([Dykstra et al., 2001](#)). It inhibits apoptosis pathways that are normally activated by B-cell receptor activation in Ramos and Akata cells, and prevents EBV reactivation in these cells. Thus, LMP-2A has an important role in maintaining viral latency.

LMP-2A has also been shown to activate PI3 kinase and the downstream phosphorylation of Akt in epithelial cells and B cells. This may modulate cell growth and apoptosis ([Swart et al., 2000](#); [Moody et al., 2005](#)). It was shown to induce cell mobility and invasion in epithelial cells ([Pegtel et al., 2005](#)).

LMP-2A can also associate with Nedd4-ubiquitin ligases via its PPPPY-motif located at its AA-terminus. It is conceivable that the binding of LMP-2A to the Nedd4 family of proteins can result in fast destruction of LMP-2A itself and LMP-2A-associated kinases, by guiding the complex to the ubiquitin–proteasome system ([Winberg et al., 2000](#)).

A major role of LMP-2A in relation to latent EBV infection may stem from its ability to inhibit the activation of lytic EBV replication in infected B cells by cell-surface-mediated signal transduction ([Miller et al., 1994](#)). This may prevent lytic replication in latently infected B cells as they circulate in the blood, bone marrow or lymphatic tissues, where they might encounter antigens or other ligands capable of engaging B-cell receptors and activating the viral cycle.

LMP-2B is a splice variant of LMP-2A which lacks the *N*-terminal tail with its kinase-interacting domains. It is thought to interact with LMP-2A, and thereby modulates its functions ([Rovedo & Longnecker, 2007](#)).

#### 4.2.3 The non-coding RNAs

##### (a) The EBV-encoded RNAs (EBERs)

The EBERs are two non-coding, non-polyadenylated RNAs, EBER-1 (166 nucleotide long) and EBER-2 (172 nucleotides long), which are always expressed in very high abundance ( $10^5$ – $10^6$  copies/cell) in latently EBV-infected cells irrespective of cell phenotype. Structural predictions suggest that they can form a compact structure with five major hairpin structures. They act as regulators of signalling and transcription factors, resulting in the production of interferons and cytokines ([Samanta et al., 2008](#)). The EBERs were shown to induce the anti-inflammatory cytokine IL10 as an autocrine growth factor in Burkitt lymphoma cells. This effect is produced via retinoic-acid-inducible gene I (*RIG-I*, a sensor of innate immunity)-mediated activation of IRF-3. In cell lines derived from nasopharyngeal carcinoma,

the EBERs induce insulin-like growth factor 1 (IGF-1), which also acts as an autocrine growth factor. This is corroborated *in vivo* because nasopharyngeal carcinoma biopsies consistently express IGF-1 ([Wu et al., 2007](#); [Samanta et al., 2008](#)).

EBERs may also contribute to B-cell transformation; this was shown for EBER-2 RNA via its efficient induction of IL6 ([Wu et al., 2007](#)).

##### (b) The EBV micro-RNAs

Micro-RNAs are small non-coding RNAs, generally 20–24 nucleotides in length, that can transcriptionally downregulate the expression of mRNAs, bearing complementary sequences. EBV encodes at least 22 micro-RNAs which are expressed to various degrees in all forms of latency, and in tumour tissues ([Pfeffer et al., 2004](#); [Cai et al., 2006](#); [Grundhoff et al., 2006](#)). All EBV tumours that have been studied express at least some of the EBV-encoded micro-RNAs. They have been shown to target several interesting cellular genes, and thus they may very well turn out to play a central role in the tumorigenesis of EBV. Target genes identified so far include PUMA of the p53 pathway, and the chemokine CXCL11 ([Choy et al., 2008](#); [Xia et al., 2008](#)).

### 4.3 In vivo and in vitro evidence for a role of EBV in human malignancies

#### 4.3.1 EBV-associated B-cell lymphomas

There are three histologically and clinically distinct types of EBV-associated B-cell lymphomas that show different patterns of latent gene expression and seem, from the immunoglobulin gene sequencing, to derive from cells at different position in the B-cell differentiation pathway (Fig. 1.3).

(a) *Lymphomas in immunosuppressed individuals*

T-cell-immunocompromised patients — organ transplant recipients, congenitally immunocompromised individual, particularly the X-linked lymphoproliferative syndrome (XLP) and AIDS patients — are at a high risk of developing B-cell lymphomas.

Most post-transplant lymphoproliferative diseases occur as polyclonal or monoclonal lesions within the first year of allografting, when immunosuppression is most severe. Almost all of these early onset tumours are EBV-positive, and express the full latency III programme, which identifies them as virus-transformed B cells that grow out in the absence of effective T-cell surveillance. Some of the lymphomas that are seen in highly immunocompromised AIDS patients, particularly central nervous system lesions, show essentially the same phenotype. ([Young & Rickinson, 2004](#)). The EBV proteins expressed include the highly immunogenic members of the EBNA-3 triad (EBNA-3A, -3B, and -3C), which is why passive immunotherapy with *in vitro*-expanded EBV-antigen-specific CD8-positive CTLs can bring about dramatic regression, even of widely disseminated tumours ([Rooney et al., 1995](#); [Khanna et al., 2001](#)).

(b) *Burkitt lymphoma*

EBV is associated with almost all of the paediatric Burkitt lymphomas in high endemicity areas, but only with a fraction of sporadic or AIDS-associated Burkitt lymphomas ([Kelly & Rickinson, 2007](#)).

In equatorial African endemic areas, high EBV VCA antibody titres are regularly detected in children as early as 4 years before tumour development, which indicates an early infection and a high viral load ([Geser et al., 1982](#)). In these countries, malaria is holoendemic and this infection appears to be a strong risk factor of Burkitt lymphoma (reviewed in [Rochford et al., 2005](#)).

EBV gene expression in Burkitt lymphoma is strictly latent and very constrained. Most cells express only EBNA-1 and the EBERs (latency I programme). The BARTs have also been detected in Burkitt lymphoma samples by PCR. In addition, some genes traditionally thought to be confined to expression during the lytic cycle can also be expressed in Burkitt lymphoma cells, by alternative splicing of transcripts driven from latent promoters. The expression of the bcl-2 homologue *BHRF-1* by this mechanism might be particularly significant ([Kelly et al., 2006](#)).

All Burkitt lymphomas, irrespective of form or EBV status, carry *c-myc* translocations to one of the immunoglobulin loci, the heavy chain locus on chromosome 14 or the light chain loci on chromosomes 2 or 22. These translocations are the hallmark of all Burkitt lymphomas. This may reflect the timing of the initiation of the lymphoma in relation to B-cell differentiation and at the time of the immunoglobulin gene rearrangement. As a result, the *c-myc* gene is in these cells under the control of a highly active immunoglobulin gene promoter leading to constitutive expression of *c-myc* ([Klein, 1983](#)).

Apart from the possible role of expanding the lifespan of the EBV-carrying B cells before lymphomagenesis, thus increasing the likelihood of secondary genetic events (such as the *c-myc* translocation), EBV can also play a direct role in lymphoma initiation. First, the expression of dominant-negative EBNA-1 mutants in Burkitt lymphoma cells *in vitro* induces apoptosis, which points to the requirement of EBNA-1 for the continued survival of EBV-positive Burkitt lymphoma cells ([Kirchmaier & Sugden, 1997](#)). In addition, genomic instability induced by EBNA-1 could be another possible mechanism ([Tsimbouri et al., 2002](#); [Gruhne et al., 2009](#)).

It has also been proposed that EBV, by virtue of its anti-apoptotic *BHRF1* gene, provides protection against apoptosis induced by deregulated *c-myc* expression ([Kelly et al., 2006](#)). Another

possibility is apoptotic protection by the EBERs ([Takada, 2001](#)).

### (c) *Hodgkin lymphoma*

Hodgkin lymphoma is characterized by an expansion of Reed-Sternberg cells, which are now postulated to be of B-cell lineage. Several lines of evidence link EBV to Hodgkin lymphoma:

- A 4-fold increase in risk in individuals with a past history of infectious mononucleosis;
- Increased antibody titres to EBV viral capsid antigen; and,
- The detection of monoclonal EBV genomes in the HRCs.

Almost half of the Hodgkin lymphoma cases in Western countries carry EBV-positive HRCs that express the latency II pattern with EBNA-1, LMP-1, LMP-2A, LMP-2B, and the EBERs being expressed (reviewed in [Thompson & Kurzrock, 2004](#)).

## 4.3.2 EBV-associated epithelial cancers

### (a) *Cancer of the nasopharynx*

EBV is consistently detected in patients with cancer of the nasopharynx, with a stronger association with non-keratinizing carcinoma than with keratinizing carcinoma ([Maeda et al., 2009](#)). Regardless of whether the patient with nasopharyngeal carcinoma lives in an area of endemic or sporadic incidence (see Section 1.2), all tumour cells contain EBV DNA as multiple clonal episomes as shown by terminal repeats analysis. The clonality of EBV DNA suggests that nasopharyngeal carcinoma occurs from the clonal expansion of a single EBV-infected cell, and that EBV infection is an early, possibly initiating, event in the development of nasopharyngeal carcinoma ([Raab-Traub & Flynn, 1986](#)). This is further supported by studies showing that preneoplastic and preinvasive lesions of the nasopharynx are also infected by EBV, and express

the same latency programme ([Pathmanathan et al., 1995](#)).

Nasopharyngeal carcinoma cells express an EBV-latency II pattern (see Section 1.1) including the expression of EBNA-1, LMP-1, LMP-2A, LMP-2B, the EBERs, and micro-RNAs.

Several genes relevant for the tumorigenic phenotype of the nasopharyngeal carcinoma cell are induced by LMP-1 ([Thornburg & Raab-Traub, 2007](#)).

However, only about two-thirds of nasopharyngeal tumours express LMP-1 *in vivo* as measured by Western blot or in-situ staining. Clinical and follow-up data from 74 cases of nasopharyngeal carcinoma showed that LMP-1-positive nasopharyngeal carcinoma grew faster and more expansively than LMP-1-negative tumours, in a short two-year follow-up ([Hu et al., 1995](#)). In the LMP-1 non-expressing tumours, the promoter region of the LMP-1 gene is hypermethylated ([Hu et al., 1991](#)).

In a few early precancerous lesions *in situ* that could be studied, LMP-1 is always expressed ([Pathmanathan et al., 1995](#)). It might thus have an important role in the early process, but its functions can later be replaced by cellular genes.

LMP-2A induces migration and invasion of epithelial cells including nasopharyngeal-carcinoma-derived cell lines, which could affect the *in vivo* phenotype of the tumour ([Allen et al., 2005](#)).

Both EBERs and some of the EBV microRNAs are expressed in nasopharyngeal carcinoma, but their respective role in tumorigenesis has not yet been addressed ([Cosmopoulos et al., 2009](#)).

### (b) *Cancers of the stomach*

EBV is detected in 5–10% of gastric carcinomas worldwide (see Section 1.2). It has been suggested that EBV-positive gastric carcinoma belongs to a separate clinico-histopathological entity, distinguishable from most gastric carcinomas as this occurs at younger age, with a distinct histopathology (ranging from

adenocarcinoma with lymphoid infiltration to lymphoepithelioma-like), and with a more proximal location ([Fukayama et al., 2008](#)). In these tumours, the EBV genome is present in (almost) all cells and is monoclonal, suggesting that the infection takes place at tumour precursor cell state. The latent pattern of EBV in gastric carcinoma corresponds to an intermediate latency I / II programme, with EBNA-1, EBERs, BARF-0, LMP-2A, and micro-RNAS. In addition, some lytic infection genes such as *BARF-1* and *BHRF-1* have also been detected in these tumours. All tumour cells express the EBERs as shown by PCR and by *in-situ* hybridization, while expression of LMP-2A and the lytic genes is variable ([Luo et al., 2005](#)).

In gastric carcinoma cells in culture, EBV expresses a latency pattern that is similar to gastric carcinoma *in vivo*, including the viral micro-RNAs. In these cells, EBV uses LMP-2A to activate the NF-κB-surviving pathway which confers some resistance to apoptosis induced by serum deprivation ([Hino et al., 2008](#)). In parallel with the results *in vitro*, the NF-κB-surviving pathway has been shown to be highly activated in nearly all EBV-associated gastric carcinomas in the advanced stage, and the frequency is significantly higher than that in EBV-negative gastric carcinomas ([Luo et al., 2005](#)). Various viral proteins (e.g. HTLV-1 Tax, HPV-16E6, HBx) are known to upregulate surviving protein expression in human neoplasms; this may be a common denominator in the mechanisms of human viral oncogenesis ([Hino et al., 2008](#)).

#### (c) Other carcinomas

Carcinomas showing morphological features that are similar to undifferentiated nasopharyngeal carcinomas or EBV-related gastric carcinomas, so-called lymphoepithelial carcinomas, can occur at other sites. Lymphoepithelial carcinomas of the salivary glands, of the lungs, and possibly of the thymus are frequently associated with EBV infection ([IARC, 1997](#)), but there is no

mechanistic data demonstrating a specific role of EBV in these tumours.

### 4.4 Interaction between EBV and other agents; mechanisms involved in EBV reactivation

EBV coexists for a lifetime in a latent state in most human hosts without overt serious consequences. This strongly suggests that cofactors able to reactivate EBV viral replication may potentially be required for EBV-associated carcinogenesis.

EBV can be reactivated from its latent state by several means, and its reactivation could potentially lead to the development of EBV-related pathology.

#### (a) Foreign antigen

In healthy carriers, EBV remains silent and expresses only EBERs in infected resting memory B cells. Viral replication can only occur in dividing cells; this is the case when memory B cells divide for cell maintenance or when they differentiate into plasma cells following activation by the presence of a foreign antigen ([Laichalk & Thorley-Lawson, 2005](#)). This means that any additional infection may potentially reactivate EBV in these cells.

#### (b) Immunodeficiency

EBV infection is strictly kept under very tight control by cell-mediated immunity in immunocompetent individuals (see Fig. 1.2). Immunodeficiency (iatrogenic as in transplant recipients, congenital, or HIV-related) allows the spread of uncontrolled reactivated EBV from infected memory B cells, which can give rise to various lymphoproliferative disorders (Fig. 1.3 and Section 4.3.1).

(c) *Malaria*

Infection with both EBV and *Plasmodium falsiparum* are recognized to be required for the genesis of endemic Burkitt lymphoma. Children living in areas endemic for malaria have an elevated EBV load, and have diminished EBV-specific T-cell immunosurveillance between the ages of 5–9 years, which coincides with the peak age incidence of the diseases ([Moormann et al., 2005, 2007](#)). In addition, acute malaria infection leads to increased levels of circulating EBV that are cleared following anti-malaria treatment ([Rasti et al., 2005; Donati et al., 2006](#)). A direct molecular mechanism of interaction has been demonstrated between *P. falsiparum* and EBV. CIDR1α, a cystein-rich domain of the *P. falsiparum* membrane protein 1 was shown to act as a polyclonal B-cell activator, and to induce the EBV lytic cycle ([Chêne et al., 2007](#)).

(d) *Food*

In the southern region of the People's Republic of China, where nasopharyngeal carcinoma is a very common malignancy, the ingestion of salted fish especially during weaning has been shown to be an important risk factor for the condition ([Yuan et al., 2000](#)). Other preserved food preparations such as the spiced mixture "harissa" in Tunisia have also been identified as potential risk factors for nasopharyngeal carcinoma ([Jeannel et al., 1990](#)). Using Raji cells, an *in vitro* study demonstrated a strong EBV reactivation activity in aqueous extracts of some Cantonese salted fish from China, and harissa, and to a lesser extent qaddid (dry mutton preserved in olive oil) from Tunisia ([Shao et al., 1988](#)).

(e) *Inflammation*

TGFβ-1, a multifunctional cytokine, induces EBV reactivation in EBV-infected gastric carcinoma cell lines *in vitro* as shown by the induction of EBV-early immediate *BZLF-1* RNA, and its protein product ZEBRA ([Fukuda et al., 2001](#)).

[The Working Group noted that TGFβ is highly expressed during inflammation suggesting that chronic inflammation may potentially reactivate latent EBV infection *in vivo*; this hypothesis still needs to be demonstrated.]

(f) *Chemical agents and drugs*

Stimuli that can activate the latency-to-lytic switch in cultured cell lines, include phorbol esters, which are protein kinase C agonists; sodium butyrate and trichostatin A, which are histone deacetylase inhibitors; 5-aza-2-deoxycytidine, which is a DNA methyltransferase inhibitor; and anti-immunoglobuline G, which activates the B-cell antigen receptor. While operating by different modes of action, these agents all lead to the expression of the EBV lytic activator genes *BZLF-1* and *BRLF-1*, which encode ZEBRA and Rta ([Countryman et al., 2009](#)).

## 4.5 Transgenic models for EBV-associated cancers

Transgenic mice models expressing EBNA-1 and LMP-1 under various tissue-specific promoters are available. They show dysregulation of the haematopoietic and epithelial compartments, depending on which tissue the transgene is directed to. Transplantable tumours occur with an elevated frequency in LMP-1-transgenic mice ([Kulwichit et al., 1998; Curran et al., 2001; Shair et al., 2007](#)). EBNA-1-transgenic mice have also been shown to develop tumours ([Wilson et al., 1996](#)).

A humanized mouse model in which the functional human immune system (including T, B, and natural killer lymphocytes) is reconstituted, can simulate key aspects of EBV infection. Inoculation of EBV in these mice causes B-cell lymphoproliferative disorder, with histopathological findings and latent EBV gene expression that are similar to that in immunocompromised patients ([Yajima et al., 2008](#)).

## 4.6 Synthesis

Mechanistic data that strongly support an oncogenic role of EBV in human cancer can be summarized as follows:

- EBV immortalizes normal B cells in culture.
- One or several EBV gene products are expressed in all EBV-associated cancers.
- At the molecular level, these EBV-encoded gene products associated with latent viral infection induce cell proliferation, block apoptosis, induce genomic instability or modulate cell migration. These events occur before or during tumour initiation. Several of these gene products are also involved in mechanisms contributing to continued tumour maintenance, cell growth, and progression.

Mechanistic data strongly support an oncogenic role of EBV in diffuse large B-cell lymphomas in immunocompromised individuals (post-transplant patients, XLP, AIDS). In these tumours, EBV adopts the growth-proliferative programme seen in EBV-infected human B cells, which is solely driven by the virus.

Mechanistic data strongly support an oncogenic role of EBV in Burkitt lymphoma, where EBV promotes the survival of B cells that have undergone the pro-apoptotic *myc*-translocation.

Mechanistic data strongly support an oncogenic role of EBV in Hodgkin lymphoma and nasopharyngeal carcinoma, where LMP-1 can act as a transforming viral protein.

There is positive mechanistic data for a role of the virus in EBV-positive gastric carcinoma.

Regarding the role of EBV in EBV-positive T/NK-cell lymphomas, *in vitro* model systems still require optimization, and as a result, only weak mechanistic evidence is available to support a role of EBV in these types of cancer.

At the time of writing, no mechanistic studies have been published that directly investigate a

role of EBV in lymphoepitheliomas of the salivary gland.

## 5. Evaluation

There is *sufficient evidence* in humans for the carcinogenicity of EBV. EBV causes Burkitt lymphoma, immunosuppression-related non-Hodgkin lymphoma, extranodal NK/T-cell lymphoma (nasal type), Hodgkin lymphoma, and cancer of the nasopharynx. Also, a positive association has been observed between exposure to EBV and lymphoepithelioma-like carcinoma.

In the case of gastric carcinoma, there is insufficient epidemiological evidence for the involvement of EBV. However, the fact that the EBV genome is present in the tumour cell in a monoclonal form, and that transforming EBV proteins are expressed in the tumour cell provides a mechanistic explanation of how EBV might cause a proportion of gastric cancer.

EBV is *carcinogenic to humans (Group 1)*.

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# HEPATITIS B VIRUS

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The hepatitis B virus was considered by a previous IARC Working Group in 1993 ([IARC, 1994](#)). Since that time, new data have become available, these have been incorporated in the *Monograph*, and taken into consideration in the present evaluation.

## 1. Exposure Data

### 1.1 Taxonomy, structure, and biology

#### 1.1.1 Taxonomy

The hepatitis B virus (HBV) is the prototype member of a family of hepatotropic DNA viruses, the *Hepadnaviridae*, that replicate by reverse transcription of an RNA pregenome. HBV infects humans, whereas other hepadnaviruses infect mammals (orthohepadnaviruses) or birds (avihepadnaviruses) ([Schaefer, 2007a](#)).

HBV comprises eight genotypes (A to H) with distinct virological characteristics and geographic distributions ([Kramvis et al., 2005](#); [Schaefer, 2007a](#); see Section 1.2.3). Each genotype differs from the others by more than 8% of its nucleotide sequence. Genotypes may influence the disease caused, although further analysis of this association is required. The variability of the HBV genome may be further increased by recombination among genotypes, especially B/C and A/D.

HBV genotypes, with the exception of genotypes E and G, are divisible into subgenotypes. Each subgenotype differs from the others by more than 4% of its nucleotide sequence. The number of subgenotypes per genotype described

to date ranges from three to five ([Kramvis et al., 2005](#); [Schaefer, 2007a](#)).

#### 1.1.2 Structure of the virion

HBV is an enveloped virus, measuring 42–47 nm in diameter, with an icosahedral nucleocapsid that encloses a partially double-stranded relaxed-circular (rc) DNA genome covalently bound to the viral polymerase. The envelope comprises a small amount of lipid of cellular origin and three hepatitis B surface proteins (HBs): large (LHB), medium, (MHB), and small (SHB), which form disulfide-linked homo- and heterodimers. The serum of infected individuals contains, in addition, two types of subviral particles: small spherical particles with a diameter of approximately 20 nm and filamentous particles also with a diameter of about 20 nm but of variable length. These non-infectious subviral particles lacking genomic DNA greatly outnumber the infectious viral particles, and have a composition similar to that of the viral envelope ([Kann, 2002](#)).

The nucleocapsid is formed by multiple copies of core protein. Of the total 183–185 amino acids (depending upon genotype), the N-terminal 149–151 amino acids are responsible for self-assembly of the nucleocapsid. Although the steps in its assembly remain to be clarified, the

first step is the formation of homodimers linked by disulfide bridges. The nucleocapsid contains pores that allow the diffusion of nucleotides during the synthesis of the DNA genome. The C-terminal amino acids of the core protein play a role in the packaging of the pregenome–polymerase complex within the nucleocapsid ([Bruss, 2007](#)).

### 1.1.3 Structure of the viral genome

HBV has a partially double-stranded but not covalently closed circular (ccc) DNA genome composed of between 3182–3248 nucleotides, depending on the genotype. The genome consists of a complete minus-DNA strand with a short-terminal redundancy, and a shorter plus-DNA strand that leaves a single-stranded gap of variable length in mature nucleocapsids and released viruses ([Kann, 2002](#); [Jilbert et al., 2002](#)). Base-pairing of plus- and minus-strands in the cohesive overlap region of the genome maintains the circular configuration. The 5'end of the minus-strand is covalently linked to the N-terminal portion of the viral polymerase. At its 5'end, the plus-strand is linked to a capped RNA oligonucleotide that is derived from the 5'end of the RNA pregenome, and serves as the primer for plus-strand-DNA synthesis.

The genome consists of four partially overlapping open reading frames (ORFs) ([Kann, 2002](#)) that express surface, precore/core, polymerase, and X proteins. Each ORF overlaps at least one other ORF, with the polymerase ORF overlapping all of the others, and every nucleotide is part of at least one ORF. Translation of preS1, preS2, and S ORFs leads to the expression of the surface proteins, LHB, MHB, and SHB, respectively (Fig. 1.1).

Four promoters (preC/C, preS1, S, and X) and two enhancers (Enh1 and Enh2) overlap the ORFs ([Kann, 2002](#)). The promoters initiate the transcription of messenger (m) RNAs of 3.5, 2.4, 2.1 and 0.9 kb that allow, by the use of different

start codons, the expression of seven proteins. All are of positive orientation, possess a 5'cap, are polyadenylated at their 3'ends, and serve as mRNAs for viral gene products. Enh1, which stimulates the transcription of all viral RNAs, is located between the S and X ORFs, and Enh2, a less potent enhancer, overlaps the preC/C promoter.

In addition to the enhancers, other regulatory elements have been identified: a glucocorticoid responsive element (GRE) is located between Enh1 and Enh2; a CCAAT element regulates the transcription of the upstream preS1 promoter, and activates the transcription of S mRNA; and a negative regulatory element (NRE) appears to inhibit only the precore/core mRNA ([Kann, 2002](#)).

### 1.1.4 Host range

HBV primarily infects humans, although chimpanzees, Chacma baboons, and tree shrews are also susceptible to infection ([Hu et al., 2000](#); [Cao et al., 2003](#)).

### 1.1.5 Target cells

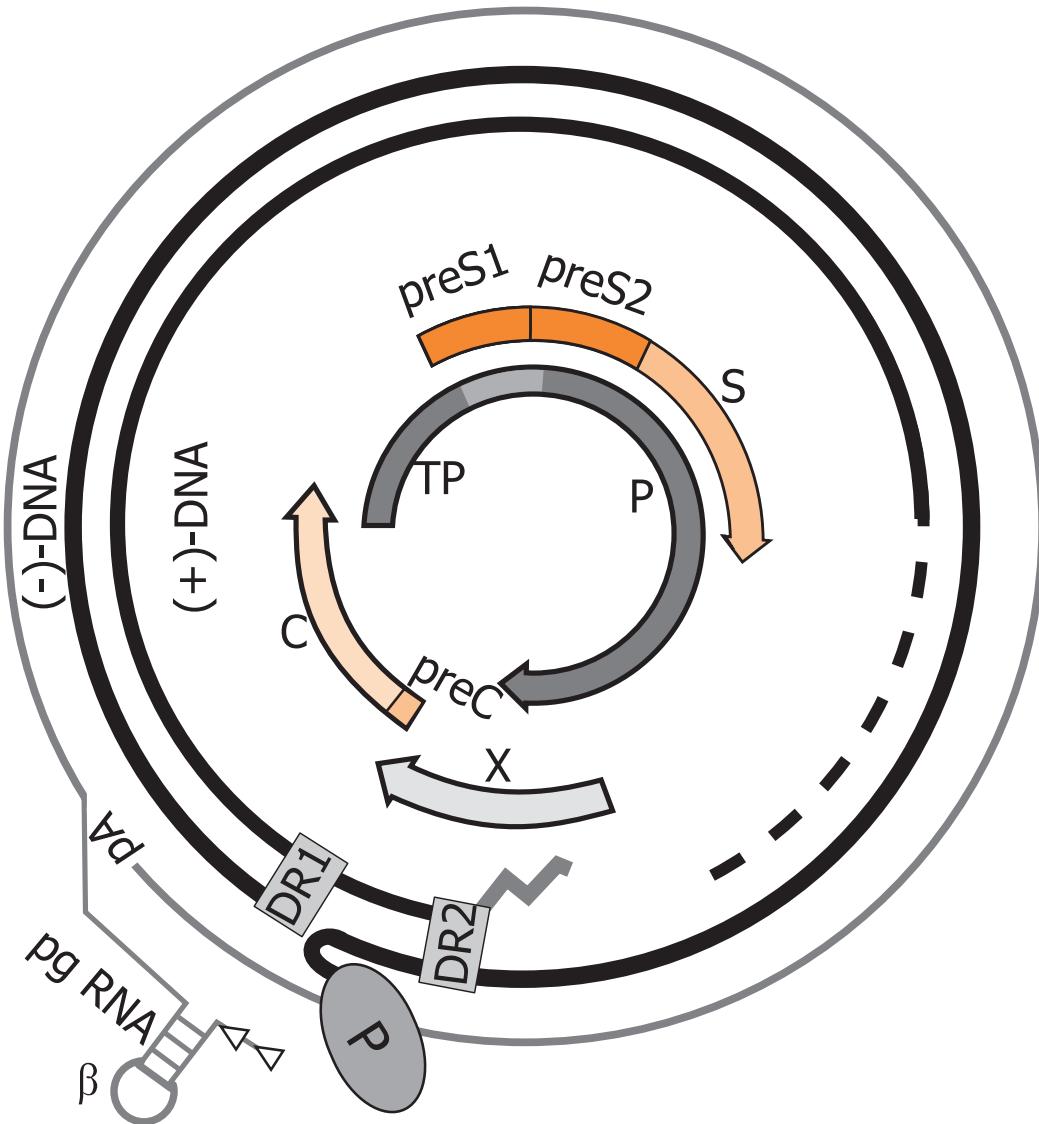
HBV is primarily an hepatotropic virus, and hepatocytes are the only confirmed site of replication for all members of this virus family. Although the virus has been detected in other cells such as bile duct epithelial cells, peripheral blood mononuclear cells and cells in the pancreas and kidneys, the evidence for viral replication in these cells is controversial ([Seeger & Mason, 2000](#)).

### 1.1.6 Function of the gene products

#### (a) Surface proteins

The HBV surface protein: small (SHB), medium (MHB) and large (LHB), together with cellular lipid material, form the viral envelope ([Kann, 2002](#)).

Fig. 1.1 Transcriptional and translational map of HBV



The partially double-stranded, circular rc-DNA is indicated by thick black lines, with the polymerase (P) covalently linked to the 5'end of the (-)-DNA, and the RNA primer (zigzag line) at the 5'end of (+)-DNA. The dashed part symbolizes the heterogeneous lengths of the (+)-strands. DR1 and DR2 are the direct repeats. The outer circle symbolizes the terminally redundant pgRNA with ε close to the 5'end, and the poly-A tail at the 3'end. The precore mRNA is nearly identical, except it starts slightly upstream. The relative positions of the open reading frames for core (C), P, preS/S, and X are shown inside. TP, Terminal protein domain of P; pgRNA, pregenomic RNA

From Beck J, Nassal M, Hepatitis B virus replication, World J Gastroenterology, 2007; 13(1):48-64

SHB antigen which represents 85% of hepatitis B surface antigen (HBsAg), is highly immunogenic and provokes the host's immune response to HBV. Excess surface protein circulating in subviral particles is thought to dilute the host's immunological response to the virus.

LHB, in contrast to MHB, is essential for infection and viral morphogenesis. It represents 10–30% of the HBsAg of virions and filaments. LHB plays a role in viral entry into hepatocytes, although SHB may also be needed in this process ([Kann, 2002](#)).

(b) *Core protein and 'e' antigen*

Core protein (C) is the major structural component of the nucleocapsid. The preC/C ORF is transcribed into a precore/core fusion protein. During entry into the endoplasmic reticulum, 19 amino acids are cleaved from the N-terminal end of the precore protein by a signal peptidase. When transported into the Golgi compartment, additional amino acids are removed from the C-terminal end by intra-Golgi proteases to form HBe antigen. This antigen is secreted into the serum. The biological function of HBe remains unsolved ([Kann, 2002](#)).

(c) *Polymerase protein*

Polymerase (P) has four domains: a terminal domain, which serves as a protein primer for reverse transcription of pregenomic viral RNA; a spacer region without apparent function; the polymerase domain, which has reverse transcription activity; and the RNase H domain, which is responsible for the degradation of the RNA template during reverse transcription ([Kann, 2002](#)).

(d) *X protein*

The X protein (HBx) has been shown to be a promiscuous regulator of transcription that is essential for viral replication. Although not binding itself to DNA, it regulates transcription

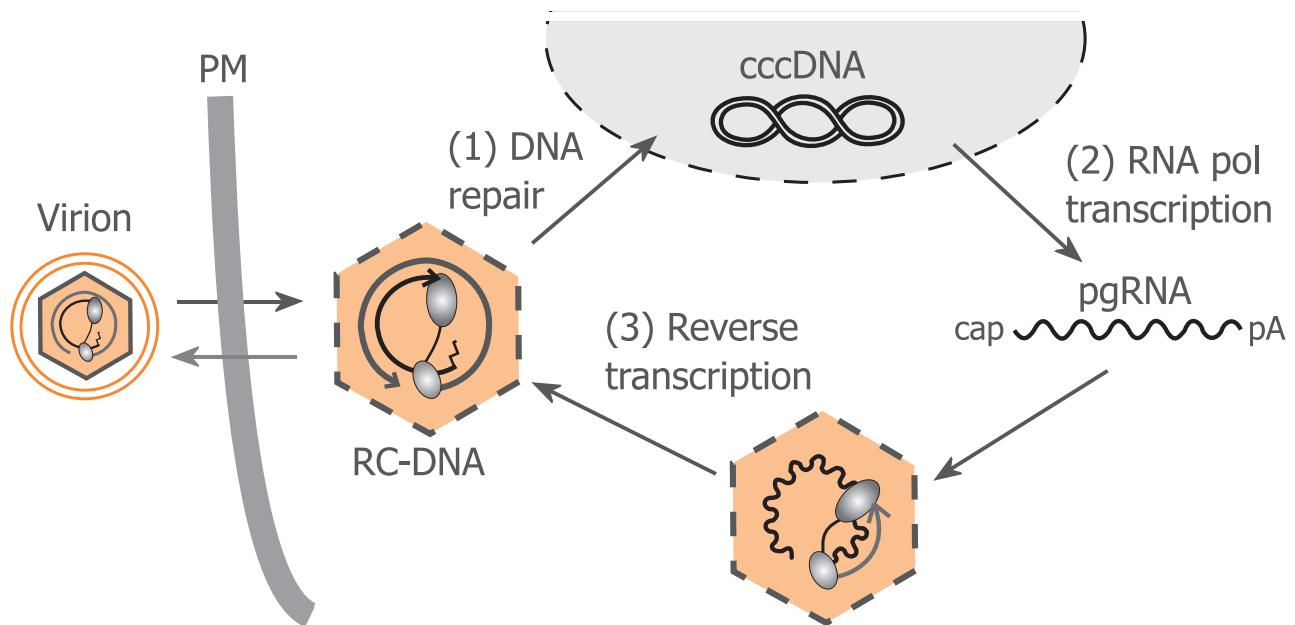
from HBV enhancers/promoters, and from the promoters of cellular genes, including oncogenes, cytokines, growth factors, and several genes involved in cell-cycle control and progression, DNA repair, apoptotic cell death, and cellular adhesion. HBx also forms complexes with several signal transduction proteins and regulators of cell growth and survival ([Murakami, 1999](#); [Feitelson et al., 2005](#); [Benhenda et al., 2009](#)). It is suspected to play a central role in HBV regulation and pathogenesis (see Section 4).

### 1.1.7 *Viral life cycle*

During both acute and persistent infection, high levels of infectious HBV particles (virions) circulate in the bloodstream, together with an excess of empty particles.

Hepatocytes, the major targets of the virus, are separated from the bloodstream by endothelial and Kupffer cells that line the sinusoids of the liver. Liver sinusoidal endothelial cells have long cytoplasmic components that contain fenestrations with a diameter of 50–100 nm. Virions are thought to pass through these fenestrations from the sinusoids of the liver to the space of Disse, which is immediately adjacent to the surface of the hepatocytes. Infectious virions bind by means of the PreS1 domain of LHBs (and perhaps by the envelope lipid) to specific, as yet unidentified, receptors on the hepatocyte surface ([Seeger & Mason, 2000](#); [Jilbert et al., 2002](#); [Beck & Nassal, 2007](#); [Kann et al., 2007](#)).

HBV then proceeds following a characteristic replication strategy shared by all the members of the hepadnaviridae family (Fig. 1.2; [Seeger & Mason, 2000](#); [Jilbert et al., 2002](#); [Beck & Nassal, 2007](#); [Kann et al., 2007](#)). The nucleocapsid is released into the cytoplasm and translocated by microtubules to the microtubule-organizing centre (MTOC) near the nucleus. How the nucleocapsid gets from the MTOC to the nucleus is not known at the time of writing. Access to the nucleus is gained through nuclear pores ([Kann](#)

**Fig. 1.2 Replication cycle of Hepanaviral genome**

Enveloped virions infect the cell, releasing rc-DNA containing nucleocapsids into the cytoplasm. rc-DNA is transported to the nucleus, and repaired to form cccDNA (1). Transcription of cccDNA by RNA polymerase II (2) produces, among other transcripts (not shown), pgRNA. pgRNA is encapsidated, together with P protein, and reverse transcribed inside the nucleocapsid (3). (+)-DNA synthesis from the (-)-DNA template generates new rc-DNA. New cycles lead to intracellular cccDNA amplification; alternatively, the rc-DNA containing nucleocapsids are enveloped and released as virions. PM, plasma membrane; pgRNA, pregenomic RNA; cccDNA, covalently closed circular DNA; rc-DNA, relaxed-circular DNA; P, viral polymerase.

From Beck J, Nassal M, Hepatitis B virus replication, World J Gastroenterology, 2007; 13(1):48-64

[et al., 2007](#)), and may be mediated by polymerase or heat shock proteins. The exact stage and mechanism by which the viral genome is released from the nucleocapsid is not currently known. In the nucleus, rc-DNA is converted into cccDNA, the key template in HBV replication. The steps in achieving this conversion are uncertain, but they include completion of the positive DNA strand by polymerase; removal of the covalently linked polymerase, the 5'capped oligonucleotide primer (and the terminal redundancy), and ligation of the 5' and 3'ends of the positive and negative DNA strands.

Several genomic and subgenomic RNAs are transcribed by cellular RNA polymerase II using cccDNA as the transcriptional template. Of these, the polyadenylated pregenomic RNA, with a length corresponding to the entire genome length plus a terminal redundancy of

120 nucleotides, is selectively packaged into nucleocapsids. It is then reverse-transcribed by the co-packaged polymerase into new rc-DNA genomes. Following encapsidation of the pregenomic RNA-polymerase complex, polymerase initiates negative-strand DNA synthesis by reverse transcription ([Jilbert et al., 2002](#)).

The synthesis of polymerase and core proteins is accomplished by two translation events ([Jilbert et al., 2002](#)). The mechanism that allows translation from the downstream polymerase protein start codon is not yet known, but may include a direct internal ribosomal entry-site-like binding of the ribosomal subunits at, or near to, the polymerase start codon, ‘leaky’ scanning of the ribosomes that allow passage to the downstream start codon, or the presence of a ‘minicistron’ upstream of the polymerase ORF that is translated ([Jilbert et al., 2002](#)).

## 1.2 Epidemiology of infection

HBV is one of the most common infectious viruses worldwide. It is estimated that more than two billion people are infected. Approximately 360 million of these are chronically infected ([Lee, 1997](#); [Chen et al., 2007a](#); [Dienstag, 2008](#)). Approximately one million people die each year from HBV-related chronic liver disease, including liver cirrhosis and hepatocellular carcinoma (HCC) ([Mahoney, 1999](#)). HCC is one of the most common cancers in the world, and chronic HBV infection is responsible for 50–90% of HCC in high-risk areas ([Chen et al., 1997](#)).

### 1.2.1 Prevalence, geographic distribution

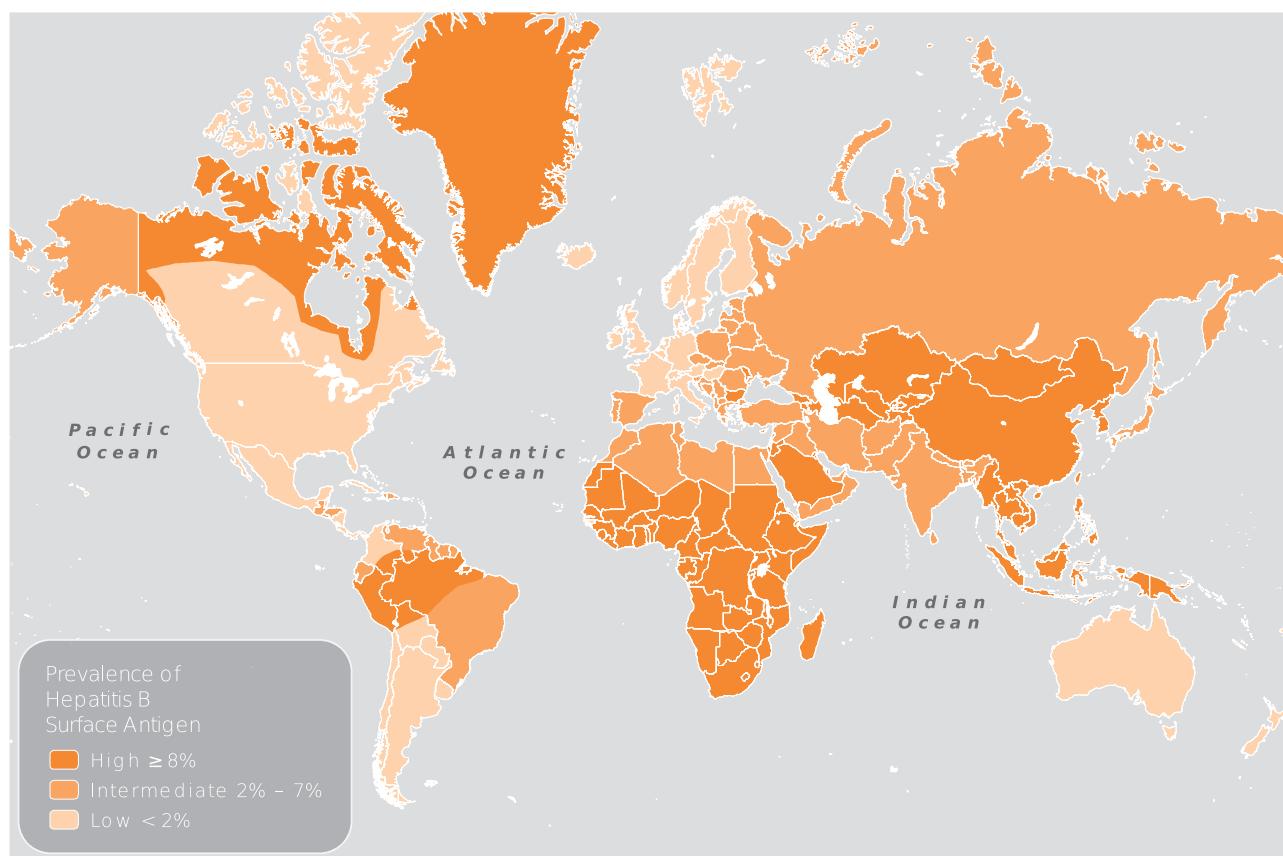
There is a wide variation of HBV infection in the world as shown in Fig. 1.3 ([Custer et al., 2004](#); [CDC, 2012](#)). Approximately 45% of the world population lives in areas where chronic HBV infection is highly endemic (>8% of the population are HBsAg-positive); 43% live in areas where endemicity is intermediate (2–7% HBsAg-positive); and 12% live in areas where endemicity is low (<2% HBsAg-positive). The prevalence of chronic HBV infection is lowest in North America, Northern and Western Europe, Australia and New Zealand; intermediate in Japan, the Middle East, Eastern and Southern Europe and parts of South America; and highest in sub-Saharan Africa, the Amazon Basin, the People’s Republic of China, the Republic of Korea, Taiwan (China), and several other countries in South-east Asia ([Chen et al., 2000](#); [Custer et al., 2004](#)).

The worldwide variation in the endemicity of HBV infection is influenced primarily by the predominant age at which infection occurs and the modes of transmission by which it occurs. In areas of high endemicity, the lifetime risk of HBV infection is more than 60%, and most infections are acquired from perinatal and child-to-child transmission, when the risk of

developing chronic infection is greatest. In these areas, acute hepatitis B is uncommon because most perinatal and early childhood infections are asymptomatic. However, rates of liver cancer and cirrhosis in adults are very high. Chronic carriage is thought to result from vertical transmission in China, Taiwan (China), and the Republic of Korea ([Chen et al., 2000](#)). Of note, HBV infection in newborns is less common in Africa. A lower prevalence of HBeAg positivity has been observed in mothers from sub-Saharan Africa compared with mothers in Asia. Child-to-child horizontal transmission accounts for high hepatitis B infection in this region of Africa.

In areas where endemicity is intermediate, mixed patterns of transmission exist, including infant, early childhood, and adult transmission. In low endemicity areas, most HBV infections occur in adolescents and young adults with relatively well defined high-risk groups, including injection drug users, homosexual males, health care workers, and patients who require regular blood transfusion or haemodialysis. In countries where adult horizontal transmission patterns are the principal transmission routes, the incidence of HBV infection is highest in adults ([Custer et al., 2004](#)).

HBV is a prototype member of the hepadnavirus family. Currently, eight genotypes of HBV (A through H) have been identified on the basis of greater than 8% nucleotide divergence over the whole genome ([Devesa et al., 2004](#)). Genotype A is prevalent in Europe, Africa, and North America. Genotype B is prevalent in Taiwan (China), China, Thailand, South-east Asia, and genotype C is prevalent in China, Japan, the Republic of Korea, and South-east Asia. Genotype D is predominant in India, Mediterranean areas, and the Middle East region. Genotype E is limited to West Africa. Genotypes F and G are mostly found in Central and South America. Genotype H has been observed in Mexico and Central America (see [Table 1.1](#)).

**Fig. 1.3 Prevalence of chronic infection with hepatitis B virus, 2006**

Source: [CDC \(2012\)](http://wwwnc.cdc.gov/travel/yellowbook/2012/chapter-3-infectious-diseases-related-to-travel/hepatitis-b.htm). Available at: <http://wwwnc.cdc.gov/travel/yellowbook/2012/chapter-3-infectious-diseases-related-to-travel/hepatitis-b.htm>

Compared with patients infected with the HBV genotype B, those infected with genotype C have a significantly lower rate of spontaneous HBeAg seroconversion ([Furusyo et al., 2002](#); [Kao et al., 2004](#)), a higher histological activity index of necroinflammation or fibrosis score ([Lindh et al., 1999](#); [Chan et al., 2002](#); [Kobayashi et al., 2002](#); [Lee et al., 2003a](#)), and a higher risk of developing acute exacerbations ([Chu et al., 2002](#); [Kao et al., 2004](#)), reactivation of HBV ([Chu & Liaw, 2005, 2007](#)), end-stage liver disease ([Kao et al., 2000](#); [Chan et al., 2003](#); [Chu & Liaw, 2005](#)), and HCC ([Yu et al., 2005](#); [Yang et al., 2008](#)).

### 1.2.2 Transmission and risk factors for infection

HBV is highly contagious and is transmitted by percutaneous and permucosal exposure to infected blood and other body fluids (i.e. semen and vaginal fluid). The highest concentrations of the virus occur in blood and wound secretions ([WHO, 2001](#)). Moderate concentrations of HBV are found in semen and vaginal fluid, and lower concentrations occur in saliva. HBV is not spread by air, food, or water. Common modes of transmission include mother-to-infant, child-to-child, unsafe injection practices and blood transfusions, and sexual contact. HBV may be

**Table 1.1 Global distribution of HBV genotypes**

Region/Country	Authors, Year	Study subjects	HBV genotypes
Asia/Taiwan, China	<a href="#">Kao et al. (2000)</a>	100 Asymptomatic carriers & 170 patients with histologically verified chronic liver disease and HCC	A:4%, <b>B:53%</b> , C:32%, D:5%, F:5%, unclassified:1%
Asia/Taiwan, China	<a href="#">Liu et al. (2002)</a>	122 Patients with chronic HBV	A:< 1%, <b>B:57%</b> , C:39%, F:4%
Asia/Taiwan, China	<a href="#">Lee et al. (2003a)</a>	265 Patients with chronic HBV infection	A:1%, <b>B:60%</b> , C:34%, D:2.5%, unclassified:2.5%
Asia/Taiwan, China	<a href="#">Yu et al. (2005)</a>	154 HCC cases and 316 matched controls	Among control group: <b>B:82%</b> , C:15%
Asia/Taiwan, China	<a href="#">Yang et al. (2008)</a>	2762 HBsAg carriers	<b>B:64%</b> , C:32%, B+C:4%
Asia/Japan	<a href="#">Usuda et al. (1999)</a>	514 HBsAg-positive blood donors	A:5%, B:38%, <b>C:55%</b> , D:0.4%, F:0.6%
Asia/Japan	<a href="#">Orito et al. (2001)</a>	720 Patients with chronic HBV infection	A:2%, B:12%, <b>C:85%</b> , D:0.4%, mixed type:1%
Asia/Japan	<a href="#">Kobayashi et al. (2002)</a>	1077 Patients with chronic hepatitis B	A:2%, B:9%, <b>C:88%</b> , D:0.2%, F:0.2%, unclassified:0.6%
Asia/China	<a href="#">Ding et al. (2001)</a>	97 Asymptomatic HBV carriers, 46 chronic hepatitis, 37 liver cirrhosis and 44 HCC patients in Shanghai	A:1%, B:17%, <b>C:81%</b>
Asia/China	<a href="#">Zeng et al. (2005)</a>	1096 Chronic HBV carriers from nine provinces in China	B: 41%, <b>C:53%</b> , A and D: rare
Asia/China	<a href="#">Zhu et al. (2008)</a>	101 HBeAg(-) patients in Hong Kong Special Administrative Region, Shanghai, Beijing	B:36%, <b>C:64%</b>
Asia/Hong Kong Special Administrative Region	<a href="#">Yuen et al. (2004)</a>	776 Asymptomatic HBsAg carriers	B: 33%, <b>C:63%</b> , mixed type:4%
Asia/Republic of Korea	<a href="#">Kim &amp; Song (2003)</a>	65 Patients with chronic HBV infection	<b>C:100%</b>
Asia/Republic of Korea	<a href="#">Song et al. (2005)</a>	200 Patients with chronic HBV infection	<b>C:100%</b>
Asia/Republic of Korea	<a href="#">Kim et al. (2007)</a>	209 Patients with chronic HBV infection (107 in Seoul and 102 in Jeju)	<b>C2 (100%)</b>
Asia/Thailand	<a href="#">Sugauchi et al. (2002)</a>	107 Hepatitis B carriers	B:25%, <b>C:72%</b> , D:3%
Asia/Thailand	<a href="#">Tangkijvanich et al. (2005)</a>	93 Asymptomatic carriers, 103 chronic hepatitis patients, 60 cirrhosis patients, 76 HCC patients	B:21%, <b>C:73%</b>
Asia/Thailand	<a href="#">Jutavijittum et al. (2006)</a>	216 HBsAg-positive voluntary blood donors	A:0.5%, B:7%, <b>C:89%</b> ; B+C:2%
Asia/Thailand	<a href="#">Suwannakarn et al. (2008)</a>	147 Asymptomatic HBsAg and HBV DNA carriers	A:1%, B:12%, <b>C:87%</b>
Asia/Philippines	<a href="#">Sakamoto et al. (2006)</a>	32 Chronic hepatitis patients, 37 cirrhosis patients, 31 HCC patients	<b>A:51%</b> , B:22%, C:27%
Asia/Viet Nam	<a href="#">Toan et al. (2006)</a>	375 HBV-infected (289 symptomatic, 29 on haemodialysis, 86 asymptomatic)	A:18%, B:10%, <b>C:25%</b> , D:20%, E:4%, F:2%, G:5%
Asia/India	<a href="#">Thakur et al. (2002)</a>	130 Patients with chronic HBV infection	A:46%, <b>D:48%</b> , A+D:6%
Asia/India	<a href="#">Gandhe et al. (2003)</a>	19 Asymptomatic carriers 30 chronic hepatitis B patients 8 acute hepatitis B patients 5 fulminant hepatitis B patients	<b>D:92%</b>

**Table 1.1 (continued)**

Region/Country	Authors, Year	Study subjects	HBV genotypes
Asia/India	<a href="#">Vivekanandan et al. (2004)</a>	122 Chronic hepatitis B patients and 67 blood donors	A:18%, C:12%, <b>D:57%</b> among chronic hepatitis B patients A:12%, C:0%, <b>D:76%</b> among blood donors
Asia/India	<a href="#">Thippavazzula et al. (2006)</a>	85 Chronic hepatitis B patients	A:15%, B:2%, <b>D:82%</b>
Mediterranean/Turkey	<a href="#">Yalcin et al. (2004)</a>	32 Chronic hepatitis B patients and 12 HBsAg carriers	<b>D:100%</b>
Mediterranean/Turkey	<a href="#">Bozdayi et al. (2004)</a>	41 Chronic hepatitis B patients	<b>D:100%</b>
Mediterranean/Turkey	<a href="#">Sunbul &amp; Leblebicioglu (2005)</a>	88 Chronic hepatitis B patients	<b>D:89%</b>
Africa/Nigeria	<a href="#">Odemuyiwa et al. (2001)</a>	20 New isolates of HBV	<b>E:100%</b>
Africa/West Africa	<a href="#">Mulders et al. (2004)</a>	105 Strains from 12 locations in West Africa	<b>E:91%</b>
Africa	<a href="#">Kramvis &amp; Kew (2007)</a>	Literature review	A: predominantly in southern, eastern and central Africa D: predominantly in northern Africa E: predominantly in western Africa
America/USA	<a href="#">Chu et al. (2003)</a>	694 Chronic hepatitis B patients	<b>A:35%, B:22%, C:31%, D:10%, E:0.4%, F:0.6%, G: 1%</b>
America/Central	<a href="#">Arauz-Ruiz et al. (1997)</a>	90 Strains from 5 different countries in Central America (Guatemala, El Salvador, Honduras, Nicaragua and Costa Rica)	A:14%, C:1%, D:6%, <b>F:79%</b>
America/Mexican	<a href="#">Sánchez et al. (2007)</a>	42 Chronic or acute hepatitis B patients	A:5%, D:21%, <b>H:74%</b>
Europe	<a href="#">Schaefer (2007b)</a>	Literature review	A: prevalent in northern Europe D: prevalent in Mediterranean countries and eastern Europe
Australian	<a href="#">Sugauchi et al. (2001)</a>	5 Australian Aborigines	C:40%, <b>D:60%</b>
Australian	<a href="#">Alestig et al. (2001)</a>	5 Australian Aborigines	<b>C:100%</b>
World	<a href="#">Lindh et al. (1997)</a>	187 HBeAg-positive chronic carriers	northern Europeans: <b>A:60%</b> , D:31% southern Europeans and Middle Easterners: <b>D:96%</b> Africans: <b>A:53%</b> , D:27%, E:20% East Asians: A:14%, <b>B:43%, C:43%</b>
World	<a href="#">Westland et al. (2003)</a>	694 Chronic hepatitis B patients in clinical trial centres	Asian/Oceanic centres: B:2%, <b>C:46%</b> North American centres: A:34%, <b>C:40%</b> Mediterranean centres: A: 14%, <b>D:83%</b> European centres: <b>A:40%, D:35%</b>

HBV, hepatitis B virus; HCC, hepatocellular carcinoma; HBeAg, hepatitis B 'e' antigen; HbsAg, hepatitis B surface antigen  
Compiled by the Working Group

detected in serum 30–60 days following infection, and may persist for widely variable periods of time.

Perinatal transmission from HBsAg-positive mothers to their newborn infants (vertical) or transmission from one child to another (horizontal) is a major source of HBV infections in many countries where chronic HBV infection is highly endemic ([WHO, 2001](#)). Perinatal transmission usually happens at the time of birth; in-utero transmission is relatively rare, accounting for less than 2% of perinatal infections in most studies. There is no evidence that HBV can be spread by breastfeeding ([Beasley et al., 1975](#)). The risk of perinatal transmission depends on the HBeAg serostatus of the mother. The risk of HBV infection approximately ranges from 70–90% for HBeAg-positive mothers to 5–20% for HBeAg-negative mothers ([Okada et al., 1976](#); [Beasley et al., 1977](#)). The spread of HBV from child to child usually happens in household settings but may also occur in child daycare centres and schools ([WHO, 2001](#)). The most probable pathways of child-to-child spread involve contact of skin sores, small breaks in the skin, or mucous membranes with blood or skin sore secretions ([Margolis et al., 1997](#)). HBV may also spread because of contact with saliva through bites or other breaks in the skin, and as a consequence of the pre-mastication of food ([MacQuarrie et al., 1974](#); [Scott et al., 1980](#); [Beasley & Hwang, 1983](#); [Williams et al., 1997](#)). The virus may spread from inanimate objects such as shared towels or toothbrushes, because it can survive for at least 7 days outside the body, and can be found in high titres on objects, even in the absence of visible blood ([Petersen et al., 1976](#); [Bond et al., 1981](#); [Martinson et al., 1998](#)). Among Gambian children aged 6 months to 5 years, a significant association was observed between HBV infection and the presence of bedbugs in each child's bed ([Vall Mayans et al., 1990](#)). But controlling bedbugs by insecticide spraying of the child's dwelling did not have

any effect on HBV infection ([Vall Mayans et al., 1994](#)).

Unsafe injection practices such as the re-use of a syringe or needle from patient to patient without sterilization are a common source of transmission of HBV in many developing countries ([Kane et al., 1999](#); [Simonsen et al., 1999](#)). In addition, unsatisfactory infection control practices, including the re-use of contaminated equipment for medical, cosmetic or dental procedures, failure to use appropriate disinfection and sterilization practices for equipment and environmental surfaces, and improper use of multidose medication vials, can also result in the transmission of HBV. Blood transfusion is also a common source of HBV transmission in countries where the blood supply is not screened for HBsAg. In addition, the injection of illicit drugs using shared needles is a common mode of HBV transmission in many developed countries.

HBV is efficiently transmitted by sexual contact, which accounts for a high proportion of new infections among adolescents and adults in countries with low and intermediate endemicity of chronic HBV infection ([Alter & Margolis, 1990](#)). Risk factors for sexual transmission include multiple sexual partners, prostitution, and lack of protection in sexual activity (e.g. the use of condoms). In countries where HBV infection is highly endemic, sexual transmission does not account for a high percentage of cases because most persons have been infected since childhood.

### 1.2.3 Persistence, latency, and natural history of infection

Persons infected with HBV have both short-term and long-term outcomes. On becoming infected, a person can have either a symptomatic disease (i.e. acute hepatitis B), or an asymptomatic infection with no signs or symptoms of disease. In persons with acute hepatitis B, the incubation period after becoming infected is usually

3–4 months, with a range of 6 weeks to 6 months. Symptoms and signs of disease usually last for several weeks. About 1–2% of persons with acute hepatitis B die from fulminant hepatitis. Both symptomatically and asymptomatically infected persons may either recover from the infection and develop lifelong immunity, or develop a chronic infection that usually lasts throughout life. Persons affected with chronic infection often do not become sick from their infection for decades after becoming infected. However, about 25% of those who become chronically infected during childhood and 15% of those who acquire chronic infection at older ages develop either HCC or cirrhosis.

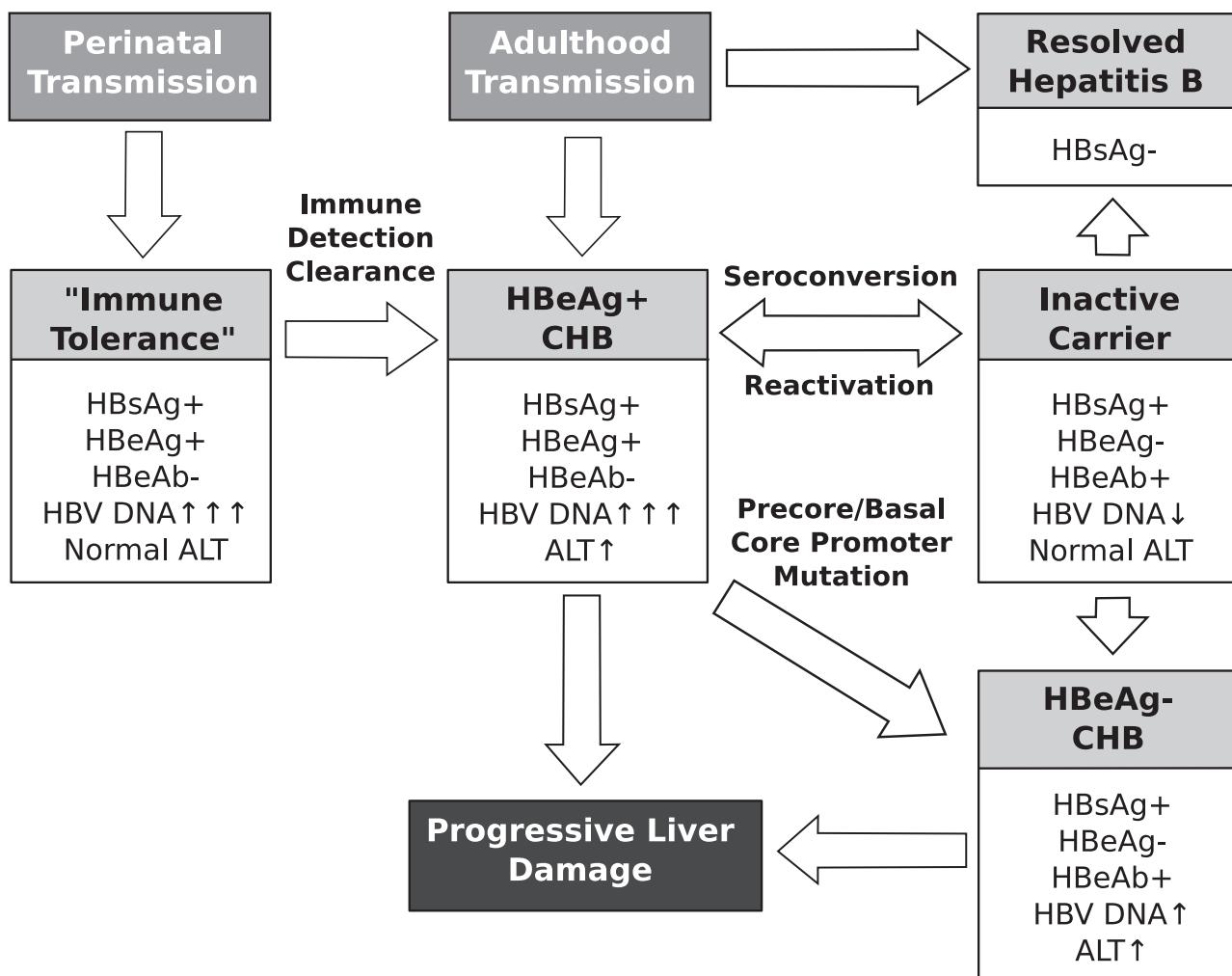
The age at which a person becomes infected with HBV is the main factor determining the risk of developing chronic infection. Among children who are under 5 years of age when they become infected, fewer than 10% are symptomatic. However, 80–90% of those infected infants and 30–50% of children infected between 1–4 years of age develop a chronic infection. In contrast, 30–50% of adults are symptomatic when first infected but only 2–5% of adults develop a chronic infection. Most of the disease burden associated with HBV infection is in persons who develop the chronic condition.

Thus, the natural course of chronic HBV infection is highly variable at an individual level but also varies with age of infection. The classical description of the natural history of chronic HBV infection is shown in Fig. 1.4 ([Chen et al., 2007a; Dienstag, 2008](#)). Early life/perinatal infection is characterized by a period of ‘immune tolerance’ where the host co-exists with the virus without apparent injury to the host. This period of immune tolerance is characterized by detectable circulating HBsAg, HBeAg, the absence of anti-HBe antibody, high levels of circulating HBV DNA and normal serum alanine aminotransferase (ALT). This immune tolerance may last for years generally without evidence of liver injury.

Following the immune tolerance phase, infected patients progress through a phase of immune detection/clearance where the host immune system tries to clear infected hepatocytes resulting in hepatic inflammation, elevation of serum ALT, and reduction of the circulating HBV DNA level. The immune clearance phase is highly variable in duration and frequency but a prolonged phase or recurrent episodes of acute liver inflammation may result in repeated cycles of injury and regeneration, resulting in necroinflammation/fibrosis and an increased risk of progression to cirrhosis and HCC. In some cases, conversion to anti-HBe-seropositive status follows the immune clearance phase. The progression of chronic hepatitis B to a state of detectable liver injury represents the start of the disease state, which is characterized by the presence of HBsAg and HBeAg in serum (HBeAg-positive chronic hepatitis B), moderate-to-high levels of circulating HBV DNA, elevation of serum ALT, and the absence of anti-HBe antibody. In some cases, where seroconversion to anti-HBe-seropositive status is associated with ongoing viral replication, there is detectable anti-HBe antibody (HBeAg-negative chronic hepatitis B). In these HBeAg-negative–anti-HBe-positive hepatitis B cases, the HBV DNA level in serum is usually lower than in HBeAg-positive chronic hepatitis B ([Chen et al., 2007a](#)).

Finally, a proportion of infected persons will be able to inactivate the infection and go into the ‘non-replicative phase’ of chronic HBV infection or what is sometimes referred to as the ‘inactive carrier state’. This phase is characterized by the continued presence of HBsAg in serum, the absence of HbeAg, and the presence of anti-HBe antibody, low levels of serum HBV DNA, and normal serum ALT. The patients in the inactive carrier state do not usually progress to liver injury. This may in part be dependent on the events that occurred during the immune clearance phase and the presence or absence of pre-existing liver fibrosis. Most of adult infections

Fig. 1.4 Natural history of chronic hepatitis B virus infection



Courtesy of WR Kim MD, Mayo Clinic, Rochester Minnesota

CHB: Chronic hepatitis B; HBsAg: hepatitis B surface antigen; HBeAg: hepatitis Be antigen; HBeAb: anti-HBe antigen; ALT: alanine aminotransferase; HBV DNA: hepatitis B viral DNA

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resolve spontaneously, and the few patients (approximately 5%) who do not clear the infection progress directly to the chronic infection phase, and do not experience an immune tolerance phase ([Chen et al., 2007a](#)).

A specific group of patients who are seronegative on HBsAg but seropositive on HBV DNA has been identified, and their infection defined

as occult hepatitis B ([Hu, 2002](#); [Torbenson & Thomas, 2002](#); [Chen, 2005](#)). Although occult hepatitis B has long been documented ([Hoofnagle et al., 1978](#)), it was difficult to investigate it before the availability of HBV polymerase chain reaction (PCR). The molecular and immunological mechanisms underlying occult hepatitis B still remain incompletely elucidated. Several

hypotheses have been proposed for the occurrence of occult HBV infection. They include the mutation of HBV surface, core and X genes, the integration of HBV DNA into host genomes, the HBV infection of peripheral blood mononuclear cells, the formation of the circulating immune complex containing HBV, the altered host's immune response to HBV, and the superinfection and interference of HBV by other viruses. Persons with occult HBV infection may transmit HBV through transfusion, haemodialysis, and organ transplantation. Occult HBV infection may contribute to the acute exacerbation of co-existing chronic hepatitis B and even fulminant hepatitis, and to the development of HCC. It also affects disease progression and treatment response of chronic hepatitis C ([Hu, 2002](#); [Torbenson & Thomas, 2002](#)).

There is a wide variation in the prevalence of occult hepatitis B among various patient groups, blood and organ donors, and healthy controls. The prevalence of seropositivity of HBV DNA in HBsAg-seronegative subjects is in the range of 0–10% among those without liver disease, 11–19% in patients affected with chronic hepatitis, and 12–61% in HCC patients ([Bréchot et al., 2001](#); [Chen, 2005](#)).

#### 1.2.4 Vaccination and viral treatment

Both vaccine and antiviral treatments are available for the control of HBV infection. The HBV vaccination programme has reduced the perinatal and horizontal transmission of HBVs and the prevalence of HBsAg in many countries including Taiwan (China) ([Tsen et al., 1991](#); [Hsu et al., 1999](#); [Ni et al., 2001, 2007](#); [Lin et al., 2002, 2003](#); [Chien et al., 2006](#); [Lu et al., 2006](#); [Su et al., 2007, 2008](#)), Saudi Arabia ([Al-Faleh et al., 1999](#); [Madani, 2007](#)), southern Italy ([Da Villa et al., 1998](#)), and Senegal and The Gambia ([Vildósola, 2000](#)). HBV vaccination has been shown to result in a dramatic decrease in the number of HBV infections among health care workers ([Mahoney](#)

[et al., 1997](#)).

It has been well documented that national HBV vaccination programmes have reduced the mortality in childhood fulminant hepatitis ([Kao et al., 2001](#); [Chien et al., 2006](#)), and HCC incidence ([Chang et al., 1997, 2000, 2005](#); [Chien et al., 2006](#)).

Alpha-interferon and nucleotide/nucleoside analogues have been used to treat patients affected by chronic hepatitis B. Randomized controlled trials have shown the efficacy of anti-viral treatment to improve the histological grade and to reduce the risk of liver cirrhosis and HCC ([Dienstag, 2008](#)).

## 2. Cancer in Humans

This section reviews the epidemiological data published since the previous *IARC Monograph* ([IARC, 1994](#)). The current review only focuses on cohort and case-control studies with the exception of those descriptive studies which may reflect the effect of hepatitis B vaccination. Many of these studies have not focused primarily on hepatitis B, but on other factors that potentially interact with hepatitis B in causing HCC.

### 2.1 Hepatocellular carcinoma

The previous *IARC Monographs* concluded that chronic HBV infection was associated with an increased risk of HCC in humans. The conclusion was based primarily on 15 cohort studies and several dozens of case-control studies conducted mostly in Asia and Africa, and some in Europe and North America. In most studies, chronic infection with HBV was determined by the presence of HBsAg positivity in serum. In all cohort studies reviewed, the relative risks ranged from 5.3–148. The majority of the case-control studies examined also showed a strong association. The odds ratios varied between 5–30, although the quality of some case-control studies was variable. This association did not appear to be confounded

by the presence of aflatoxin, infection with HCV, cigarette smoking or alcohol drinking. The evaluation of an association between the risk of HCC and the presence of other serological markers for HBV infection, such as antibody to hepatitis B core antigen (anti-HBc), and antibody to hepatitis B surface antigen (anti-HBs), was inconclusive due to the variability in the methods of determination, and the reporting of results.

### 2.1.1 Cohort studies

Table 2.1 (available at <http://monographs.iarc.fr/ENG/Monographs/vol100B/100B-02-Table2.1.pdf>) summarizes 12 cohort studies, published since the last *IARC Monograph*, that evaluate the risk of HCC among individuals who were infected with HBV. Of these, the majority of studies ( $n = 7$ ) were conducted in Asia ([Chang et al., 1994](#); [Lu et al., 1998](#); [Evans et al., 2002](#); [Yang et al., 2002](#); [Wang et al., 2003a](#); [Tanaka et al., 2004](#); [Gwack et al., 2007](#)), followed by two studies in Europe ([Crook et al., 2003](#); [Ribes et al., 2006](#)) and one study each from Africa ([Evans et al., 1998](#)), America ([Nomura et al., 1996](#)), and Australia ([Amin et al., 2006](#)). Just as the locations of the studies spread across the globe, the study populations and their size, length of follow-up, and study methodologies vary widely.

In these studies, the cohorts consisted of general populations of both genders ([Chang et al., 1994](#); [Nomura et al., 1996](#); [Lu et al., 1998](#); [Evans et al., 2002](#); [Yang et al., 2002](#); [Wang et al., 2003a](#); [Gwack et al., 2007](#)), Army recruits ([Evans et al., 1998](#)), blood donors ([Crook et al., 2003](#); [Tanaka et al., 2004](#); [Ribes et al., 2006](#)), and newly infected people notified to the Australian State Health Department ([Amin et al., 2006](#)). The average length of follow-up was as short as 4 years ([Amin et al., 2006](#)), and as long as 22 years ([Crook et al., 2003](#)). Most studies used an HBsAg-seronegative cohort as a comparison group; a few studies used the general population as a reference cohort ([Amin et al., 2006](#); [Crook et al., 2003](#)).

Some studies collected information on several other known and potential risk factors for HCC and entered them into a stratified or a multivariate analysis ([Chang et al., 1994](#); [Evans et al., 2002](#); [Yang et al., 2002](#); [Wang et al., 2003a](#); [Gwack et al., 2007](#)). In these studies, the risk of HCC was still significantly associated with chronic HBV infection with adjustment for the presence of anti-HCV, cigarette smoking, alcohol drinking, or serum glucose level.

In the cohort studies reviewed, the relative risks ranged from 9.6 (95%CI: 6.0–15.2) ([Yang et al., 2002](#)) to as high as 74 (95%CI: 45–121) ([Tanaka et al., 2004](#)). The relative risk was found to be even higher, as high as 161 (95%CI: 46–557), if an individual was co-infected with HCV ([Tanaka et al., 2004](#)).

A second group of cohort studies included the individuals who had pre-existing liver disease. As pointed out in the previous *IARC Monograph* ([IARC, 1994](#)), these studies are difficult to interpret because the causes of liver disease other than HBV infection may also be associated with an increased risk for HCC, leading to an attenuation of the estimated relative risk associated with HBV ([Benvegnù et al., 1994, 2001, 2004](#); [Zoli et al., 1996](#); [Tsai et al., 1997](#); [Yu et al., 1997a](#); [del Olmo et al., 1998](#); [Chiaramonte et al., 1999](#); [Di Marco et al., 1999](#); [Yamanaka et al., 2001](#); [Sangiovanni et al., 2004](#); [Ikeda et al., 2005](#); [Mahmood et al., 2005](#)).

### 2.1.2 Case-control studies

Many case-control studies have been published on the relationship between HCC and HBV infection since the previous *IARC Monograph*. The primary purpose of many of these studies was not to examine HBV infection, but to assess the effect of co-infection by HBV and HCV along with other potential risk factors for HCC. These studies are summarized in Table 2.2 (available at <http://monographs.iarc.fr/ENG/Monographs/vol100B/100B-02-Table2.2.pdf>).

In most studies, tests for HBV markers were performed once and “carriers” were defined as those positive for serum HBsAg at that time. Crude relative risks, as measured by odds ratio and 95% confidence intervals, were calculated by the Working Group when they were not provided by the authors, and wherever the data reported in the original papers allowed it. Studies of clinical series (typically, patients with liver disease) in which cases of HCC were a subgroup but in which there was no specifically defined control group were not included.

As with the cohort studies described above, most of the 31 case-control studies presented in Table 2.2 (on-line) were conducted in either Asia ( $n = 14$ ) or Europe ( $n = 9$ ), followed by seven in Africa, and one in the USA. The results from the case-control studies continue to demonstrate a significant association between HBV infection and the risk of HCC in humans. The adjusted odds ratios for HBsAg seropositivity ranged from 1.5–87.4. Eleven studies reported a more than 20-fold increased risk of HCC ([Pyong et al., 1994](#); [Park et al., 1995](#); [Shin et al., 1996](#); [Sun et al., 1996](#); [Tsai et al., 1996a](#); [Kew et al., 1997](#); [Zhang et al., 1998](#); [Chiesa et al., 2000](#); [Kuper et al., 2000](#); [Franceschi et al., 2006a](#); [Kumar et al., 2007](#)). The wide range in reported odds ratios from these studies is likely to be explained by the differences in the underlying prevalence of HBV in the communities studied, the numbers of cases and controls studied, the duration of infection, and the type of controls selected for a study.

Potential confounding by other risk factors for HCC, particularly infection with HCV, was addressed in many of these studies. HBV was found to be an independent risk factor for HCC in the presence of other known and potential risk factors such as HCV infection, alcohol drinking, cigarette smoking, and/or diabetes.

### (a) Occult hepatitis B infection

All of the epidemiological studies described above and in the previous *IARC Monograph* consider HBsAg seropositivity as a measure of persistent infection with HBV. However, the development of highly sensitive methods for the detection of HBV DNA has made it clear that there are individuals who are viraemic or who have integrated HBV DNA in hepatic tissue, and are negative for HBsAg ([Bréchot et al., 2001](#)). Several studies have now reported on the presence of occult HBV associated with HCC from Asia as well as regions with a low prevalence of typical chronic hepatitis B infection.

[Yu et al. \(1997c\)](#) presented a re-analysis of a case-control study included in the previous *IARC Monograph*. This was a study of 111 cases of histologically confirmed HCC and 128 controls, all non-Asian, living in Los Angeles County in the USA. They found a 4.7-fold (95%CI: 2.2–9.4) increased risk of HCC among individuals with evidence of a previous HBV infection but who were negative for HBsAg and HBV DNA. In a study of 19 HCC cases conducted at Johns Hopkins Hospital in the USA, it was found that three had HBV DNA present in the liver tissue despite being negative for HBsAg in serum ([Kannangai et al., 2004](#)). [Squadrito et al. \(2006\)](#) followed a cohort of 134 patients in Italy who had chronic hepatitis but were negative for HBsAg. The analysis of liver biopsy tissue established the presence of HBV DNA in 53 of these subjects. During a median 84-month follow-up, nine new HCC cases were observed, of which eight occurred in the group with occult HBV infection ( $P = 0.002$ ).

#### 2.1.3 Intervention studies

Vaccination to prevent hepatitis B infection and antiviral treatment of persistent HBV have both been evaluated in relation to their effect on HCC incidence. A decrease in the rate of HCC after vaccination against or treatment

for hepatitis B infection would provide further evidence that HBV is a cause of HCC.

A nationwide hepatitis B vaccination programme was introduced in Taiwan, China in July 1984. The subsequent age-specific incidence of HCC has been studied through the Taiwan (China) National Cancer Registry. In the initial report, the incidence of HCC in children aged 6–14 years declined from 0.70/100000 children during 1981–86 to 0.57 during 1986–90, and to 0.36 during 1990–94 ([Chang et al., 1997](#)). Subsequently, it was reported that the decline was primarily in boys born after 1984 whereas the decrease observed in girls was non-significant ([Chang et al., 2000](#)). The main problems preventing the eradication of HCC among children were vaccine failure, and a failure to receive hepatitis B immune globulin at birth ([Chang et al., 2005](#)). A randomized study of hepatitis B vaccination of children to prevent HCC has been in progress since the mid-80s in Qidong, China ([Sun et al., 1991](#)), and The Gambia ([The Gambia Hepatitis Study Group, 1987](#); [Viviani et al., 2008](#)). The results are expected in 2015–20 for the Chinese study, and 2017 for the Gambian study.

The treatment of persistent HBV infection with antivirals has been shown to reduce viral load and disease progression. [Liaw et al. \(2004\)](#) conducted a randomized placebo-controlled trial of lamivudine alone for 30 months in Asian patients with chronic hepatitis B and advanced liver disease. They reported that HCC developed in 7.4% of 215 subjects in the placebo group, and 3.9% of 436 in the lamivudine group (hazard ratio, 0.49; 95%CI: 0.25–0.99).

#### 2.1.4 HBV/HCV co-infection

No single study has sufficient numbers of co-infected individuals without clinically evident liver disease to provide a reliable estimate of the risk associated with dual infection with HBV and HCV. Two meta-analyses of studies have been carried out to address this difficulty. [Donato et al. \(1998\)](#) searched the literature published

between 1993–97 for appropriate studies using healthy carriers from cohort studies or healthy controls without chronic liver disease in case-control studies. Studies were only included if they used HBsAg and anti-HCV or HCV RNA for serological markers for HBV and HCV infection, respectively. No cohort studies were suitable to be included in the meta-analysis. A total of 32 case-control studies were included providing 4560 cases and 6988 controls. The summary odds ratio for being HBsAg-positive but anti-HCV/HCV RNA-negative was 20.4 (95%CI: 18.0–23.2), and for HBsAg-positive and anti-HCV/HCV RNA-positive, 135 (95%CI: 79.7–242). The odds ratio for HBsAg-negative and anti-HCV/HCV RNA-positive was 23.6 (20.0–28.1). Significant heterogeneity was found between studies that could not be explained by the generation of the HCV test, geographic area, or type of controls used. However, the results remained consistent in showing that the risk of concurrent infection with HBV and HCV for HCC was more than a sum of the risk from each, but less than a multiplicative product of the two.

In the second meta-analysis, [Shi et al. \(2005\)](#) restricted studies to those conducted in China. They searched for all studies between 1979–2004 that used appropriate serological markers of chronic viral infections: HBsAg for HBV and anti-HCV or HCV RNA for HCV infection. They only included studies that compared HCC cases with a control group without chronic liver disease. A total of 32 case-control studies, including 3201 cases and 4005 controls, met the inclusion criteria. Again, there was marked heterogeneity between studies that could not be explained by geographic area or type of control. The summary odds ratio for those HBsAg-positive and anti-HCV/HCV RNA-negative was 15.6 (95%CI: 11.5–21.3), and for HBsAg-positive and anti-HCV/HCV RNA-positive, 35.7 (95%CI: 26.2–48.5). Because the odds ratio for HCV infection alone was 8.1 (95%CI: 5.0–13.0), the result again indicates that the combined effect of

HBV and HCV infections in causing HCC lies between additive and multiplicative.

### 2.1.5 Hepatitis B viral factors

Viral factors have been shown to influence the risk of HCC in several cohort studies. In particular, HBe antigenaemia (as a surrogate for high viral load) and a high level of HBV DNA markedly increase the subsequent risk of HCC ([Yu et al., 2005](#); [Chen et al., 2006a, b](#); [Iloeje et al., 2007](#); [Chan et al., 2008](#)).

The viral genotype also appears to modify the risk of HCC. Some of this data is difficult to interpret because the subjects of the study had chronic liver disease at recruitment, and because of the global variation in viral genotypes. However, [Kew et al. \(2005\)](#), in a case-control study of liver cancer, found evidence for an association with genotype A among the Bantu-speaking people of South Africa. A total of 111 individuals with HCC were compared to an equal number of age- and sex-matched asymptomatic chronic carriers of HBV without HCC who were recruited after screening from factories in the Gauteng region of the country. Both cases and controls tested positive for the presence of HBsAg. Among cases, 96 (86.5%) were positive for genotype A compared to 76 (68.5%) of controls, resulting in a relative risk of 4.5 (95%CI: 1.9–10.9). The majority of other remaining subjects were infected with genotype D (8.1% of cases and 23.4% of controls). There was no genotype F detected in these populations.

[Livingston et al. \(2007\)](#), in a cohort study of Alaskan native people, examined the viral genotype in 47 patients with HCC and in 1129 subjects without HCC. Genotype F was found in 68% of cases and in 18% of non-HCC subjects. In addition, the median age at diagnosis of HCC was lower for patients with genotype F than patients with other genotypes (22.5 years versus 60 years). In the non-HCC population, 58% had genotype D, 13% genotype A, 7% genotype C, and 4% genotype B. This illustrates the marked

differences in genotype prevalence between two countries.

[Yang et al. \(2008\)](#) studied the incidence of HCC by genotype in a community-based cohort in Taiwan, China, where the prevalent viral genotypes are B and C. In a multivariable analysis controlling for age, sex, smoking, alcohol and viral load, the relative risk of HCC for genotype C was 1.8 (95%CI: 1.2–2.6) compared to genotype B.

Several studies have also identified an increased risk of HCC associated with mutation in the core promoter sequence of the virus (e.g. [Kao et al., 2003](#); [Chen et al., 2007b, c](#); [Chou et al., 2008](#); [Yang et al., 2008](#)).

### 2.1.6 Factors modifying the risk of HCC associated with hepatitis B

#### (a) Aflatoxin

Aflatoxin was last reviewed by IARC in Volume 82 ([IARC, 2002](#)). It was concluded that a role of aflatoxin in liver cancer etiology, especially among individuals who are carriers of HBsAg, is supported by the overall body of evidence. A key study that examined aflatoxin as a factor in modifying the risk of HCC associated with HBV was a nested case-control study conducted by [Qian et al. \(1994\)](#). The odds ratio associated with urinary aflatoxin biomarkers was 3.4 (95%CI: 1.1–10), and for HBsAg positivity alone 7.3 (95%CI: 2.2–24.4). However, when these two risk factors were positive, the odds ratio was 59 (95%CI: 17–212), suggesting multiplicative effect modification. Nonetheless, the previous Working Group commented that “the interpretation of studies was hampered by the difficulties in properly assessing an individual’s lifetime exposure to aflatoxins and the difficulties in disentangling the effects of aflatoxins from those of hepatitis infections.” Since then, a handful of studies have been published.

[Wu et al. \(2007, 2008\)](#) reported results from two different nested case-control studies based

on the same community-based Cancer Screen Program cohort in Taiwan, China. In one, they examined urinary 15-F<sub>2t</sub>-isoprostane as an indicator of oxidative stress, and showed that it was correlated with urinary aflatoxin-albumin adduct levels. They found that higher levels of this marker increased the risk of HCC particularly in HBsAg-positive subjects. In comparison to those with low urinary 15-F<sub>2t</sub>-isoprostane and without HBV infection, those with chronic HBV infection and 15-F<sub>2t</sub>-isoprostane above mean level had an odds ratio of 19.0 (95%CI: 6.7–54.2). In the second study, the association between exposure to polycyclic aromatic hydrocarbons (PAHs) and the risk of HCC was examined. The levels of PAH-albumin adducts were associated with HCC, and appeared to modify the effect of aflatoxin and HBV infection.

[Kirk et al. \(2005a, b\)](#) has explored the effects of high dietary exposure to aflatoxins on the risk of HCC in a case-control study in The Gambia, where HBV infection is highly endemic. In the first study, mutations of the *TP53* gene at codon 249 (a mutation associated with aflatoxin exposure) were measured in the plasma of HCC patients and of healthy subjects. The risk of HCC was found to be elevated in those HBsAg-positive (OR, 10.0; 95%CI: 5.2–19.6), in those 249(ser)-positive alone (OR, 13.2; 95%CI: 5.0–35.0), and when both markers were present (OR, 399; 95%CI: 48.6–3270). In the second study, human DNA was analysed for genetic polymorphisms in aflatoxin-metabolizing – and hence activating (*GSTM1*, *GSTT1*, *HYL1\*2*) – and DNA-repair (*XRCC1*) enzymes. Statistically significant associations were found for the null *GSTM1* genotype (OR, 2.45; 95%CI: 1.21–4.95), and also for the combined metabolizing enzyme genotypes. The HCC risk was most prominent among the individuals with the highest groundnut consumption (OR, 4.67; 95%CI: 1.45–15.1). These data suggest susceptibility to HCC can be altered by aflatoxin, but do not clearly demonstrate an interaction of aflatoxin with HBV in carcinogenesis.

### (b) Alcohol

The association between alcohol consumption and the risk of HCC has been reviewed recently by IARC in Volume 96 ([IARC, 2010](#)). The assessment was made difficult by the fact that signs and symptoms of cirrhosis often preceded the cancer, which may have led to a modification of alcohol intake. Thus, in general, any interaction between alcohol and HBV infection is best addressed in cohort studies.

Of the cohort studies reported in that volume, [Chang et al. \(1994\)](#) found no effect of alcohol consumption on risk of HCC; therefore it was dropped from the final multivariable model. In the cohort study of 11893 men, [Yang et al. \(2002\)](#) found that while alcohol consumption was associated with HCC (RR, 1.5; 95%CI: 1.0–2.3), when individuals who were positive for HBsAg were stratified according to alcohol use status, the relative risk for HCC was 11.4 (95%CI: 5.0–26.3) for men who drank alcohol, and 9.7 (95%CI: 5.6–16.9) for men who did not drink alcohol.

In [Evans et al. \(2002\)](#), a relative risk of 0.9 (95%CI: 0.8–1.0) was found for alcohol consumption of more than three drinks per week in men in a multivariable model with no interaction with HBsAg-positivity. In women, alcohol consumption had a relative risk of 0.6 (95%CI: 0.3–1.2) in the multivariable model, and again, no interaction with HBsAg positivity was shown. The studies of blood donors by [Crook et al. \(2003\)](#) and [Tanaka et al. \(2004\)](#) did not have information on alcohol consumption. A recent cohort study in the Republic of Korea specifically assessed the independent effect and an interaction of alcohol intake and HBV infection on the risk of mortality from HCC ([Jee et al., 2004](#)). A total of 1283112 men and women free of cancer at baseline were assessed and followed up from 1993–2002. During this time, 3807 deaths from HCC were observed. Heavy alcohol consumption in men was associated with a relative risk for HCC of 1.5 (95%CI: 1.2–2.0), but there was

no interaction between alcohol drinking and HBsAg positivity.

It is worth noting that these cohort findings of no interaction from Asia are contrary to the findings of many case-control studies. For example, [Donato et al. \(1997\)](#) found a positive interaction between self-reported history of heavy alcohol consumption and HBV infection. The relative risk for joint exposures (RR, 64.7; 95%CI: 20–210) was greater than the sum of the relative risks for HBsAg-positivity (RR, 9.1; 95%CI: 3.7–22.5), and for alcohol intake alone (RR, 4.2; 95%CI: 2.4–7.4).

### (c) Smoking

The cohort studies that examined smoking as a cofactor found modest elevations of the relative risk for HCC, with no evidence of interaction with HBsAg-positivity. [Chang et al. \(1994\)](#) found a small but statistically not significant increased risk of development of HCC associated with cigarette smoking (RR, 1.22; 95%CI: 0.55–2.71). The cohort study of [Yang et al. \(2002\)](#) found that when HBV-infected men (HBsAg-positive and HBeAg-positive) were stratified by their smoking status, the relative risk for HCC was higher among smokers (RR, 76.9; 95%CI: 39.4–150.3) than non-smokers (RR, 67.0; 95%CI: 26.1–171.7). Although cigarette smoking was associated with an increased risk of HCC (RR, 1.5; 95%CI: 1.0–2.2), no interaction with HBV infection was apparent. [Evans et al. \(2002\)](#) found that smoking in men was not associated with an increased risk of HCC, whereas in women, smoking showed a dose-response trend with increasing cigarette consumption: 1–5 cigarettes/day, relative risk 1.5 (95%CI: 0.4–6.3); 6–10/day, 2.0 (95%CI: 0.6–6.5); > 10/day, 4.2 (95%CI: 1.3–13.8). [Jee et al. \(2004\)](#) also found that cigarette smoking was associated with an increased relative risk for HCC mortality. However the increase was in male smokers (RR, 1.4; 95%CI: 1.3–1.6), but not in women (RR, 1.1; 95%CI: 0.8–1.7). No interaction was found in either sex with HBsAg positivity.

### (d) Metabolic factors

Two of the cohort studies have reported on the effects of obesity (body mass index [BMI]  $\geq 30 \text{ kg/m}^2$ ) and diabetes on the risk of HCC. [Chen et al. \(2008\)](#) found that obesity was associated with a 4-fold risk of HCC (RR, 4.13; 95%CI: 1.38–12.4) in those who were anti-HCV-positive but the association was not significant in HBsAg-positive subjects (RR, 1.36; 95%CI: 0.64–2.89).

Diabetes was associated with an increased risk in those positive for HBsAg (RR, 2.27; 95%CI: 1.1–4.7) as well as those positive for anti-HCV (RR, 3.25; 95%CI: 1.20–8.85). In comparison to the referent group of individuals with no chronic HBV and HCV infections, no diabetes, and low BMI ( $< 30 \text{ kg/m}^2$ ), for individuals with chronic HBV and HCV infections, diabetes, and obesity (BMI  $\geq 30 \text{ kg/m}^2$ ), the relative risk was as high as 264.7 (95%CI: 35.2–1993). [Yu et al. \(2008\)](#) reported that excess weight increased the risk for HCC among HBsAg-positive men in Taiwan, China. In comparison to men with a normal weight, overweight men (BMI, 25– $< 30 \text{ kg/m}^2$ ) had a relative risk for HCC of 1.48 (95%CI: 1.04–2.12), and obese men (BMI  $\geq 30 \text{ kg/m}^2$ ), 1.96 (95%CI: 0.7–5.4).

### (e) Human genetics

There have been several studies of human genetic polymorphisms and their effect on the risk of HCC among HBV carriers, e.g. [Yu et al. \(2003\)](#). However, there are, as yet, no consistent findings.

## 2.2 Cancers other than HCC

### 2.2.1 Cholangiocarcinoma

In the previous IARC Monograph ([IARC, 1994](#)), two case-control studies were reported showing no association between HBV and cholangiocarcinoma. Since then, many studies have examined this issue further, and these are summarized in Table 2.3 (available at

<http://monographs.iarc.fr/ENG/Monographs/vol100B/100B-02-Table2.3.pdf>).

In all case-control studies, the carrier status for HBV was determined by the presence of HBsAg in serum. The risk for cholangiocarcinoma was increased in association with HBsAg seropositivity, with estimates of odds ratios ranging from 1.3–8.9. Potential confounding by HCV, liver fluke infection, gallstones, alcohol consumption and cirrhosis appear to have been excluded in studies in which those factors were evaluated. The odds ratios for three studies were statistically significant, whereas odds ratios for four studies were not.

### 2.2.2 Non-Hodgkin lymphoma

#### (a) Cohort studies

Five cohort studies were carried out in countries where the prevalence of HBV carrier status is low, and the transmission patterns differ from that of Asia and Africa. Three were conducted in Europe ([Crook et al., 2003](#); [Franceschi et al., 2006b](#); [Ribes et al., 2006](#)), and one study each from the USA ([Ulcickas Yood et al., 2007](#)), and Australia ([Amin et al., 2006](#)). In all five cohorts, the HBV carrier status of individuals was determined by the presence of HBsAg in serum. In recognition of the possibility of confounding by HIV infection, three of five studies addressed the potential effect of co-infection by multivariate analysis or by excluding HIV-infected individuals from the study ([Amin et al., 2006](#); [Franceschi et al., 2006b](#); [Ulcickas Yood et al., 2007](#)). The remaining two studies ([Crook et al., 2003](#); [Ribes et al., 2006](#)) could not evaluate the potential confounding effect of HIV infection because no HIV-infected individuals were found in the HBsAg-negative group, or this information was not collected. These two studies of blood donors present higher standardized mortality ratios (SMRs) among HBsAg-seropositive individuals: 3.2 (95%CI: 1.2–6.9), and 3.5 (95%CI: 1.7–6.2). The estimates of relative risks among

the three studies that controlled for HIV infection was lower than the above two studies, and ranged from 0.62 (95%CI: 0.32–1.20) to 2.8 (95%CI: 1.2–6.8).

See Table 2.4 available at <http://monographs.iarc.fr/ENG/Monographs/vol100B/100B-02-Table2.4.pdf>.

#### (b) Case-control studies

Of the nine case-control studies, of variable quality, that reported on the relationship between HBV infection and risk of non-Hodgkin lymphoma, seven studies found a positive association with the odds ratios for HBsAg-seropositivity among non-Hodgkin lymphoma cases varying from 1.8 (95%CI: 1.1–3.1) to 4.1 (95%CI: 1.2–14.4). Potential confounding by HCV and HIV was addressed by exclusion or adjustment during the analysis in those studies that evaluated these factors. A concern was raised that because HBV infection can be reactivated in 14–50% of patients undergoing chemotherapy for non-Hodgkin lymphoma ([Coiffier, 2006](#)), some of the positive association observed in case-control studies may be artefactual ([Anderson et al., 2008](#)). Only one study provided information sufficient to discern when the viral marker screening was performed ([Kim et al., 2002](#)). In this study, laboratory tests for HBsAg, anti-HCV, and anti-HIV were performed on admission or during the first visit to the outpatient clinic, before any treatment including cancer chemotherapy was administered. The HBsAg carrier status was specifically associated with B-cell non-Hodgkin lymphoma (OR, 4.6; 95%CI: 2.0–10.3), but not with T-cell non-Hodgkin lymphoma (OR, 1.0; 95%CI: 0.2–4.5). The odds ratio was larger against non-cancer controls than other cancer controls (4.6 versus 2.4).

#### (c) Other

There is very limited information available to evaluate the relationship between HBV infection and the risk of extra-hepatic cancer other than

non-Hodgkin lymphoma. A few studies were specifically conducted for this purpose.

#### (i) *Cancer of the pancreas*

[Hassan et al. \(2008\)](#) hypothesized that due to the anatomical proximity of the liver to the pancreas, and because the two organs share common blood vessels and ducts, the pancreas may be another potential target organ for hepatitis viruses. The fact that HBsAg was detected in pure pancreatic juice and bile supports the hypothesis. They compared 476 histologically confirmed cases of pancreatic cancer to 879 age-, sex-, and race-matched healthy controls who were genetically unrelated companions of patients at the same cancer centre in Texas, USA. Serum samples were tested for HBsAg, anti-HBc, and anti-HBs. No cases and only one control were positive for HBsAg. However, past exposure to HBV (anti-HBc-positive) with evidence for HBV recovery or immunity (anti-HBs-positive) was significantly associated with an increased risk of pancreatic cancer (OR, 2.3; 95%CI: 1.2–4.3). Past exposure to HBV without evidence of recovery (anti-HBc-positive/anti-HBs-negative) was associated with a greater risk (OR, 4.0; 95%CI: 1.4–11.1). These odds ratios were adjusted for age, sex, race, state of residency, educational level, smoking, diabetes, alcohol, and family history of cancer.

[Berrington de Gonzalez et al. \(2008\)](#) reported on pancreatic cancer and the HBsAg status of 631172 men and women who participated in the Korean Cancer Prevention Study ([Lee et al., 2004](#)). HBsAg status was not associated with pancreatic cancer risk (RR, 1.13; 95%CI: 0.84–1.52). The interpretation of the result was somewhat limited because the information on HBsAg was only available for 32% of the cohort.

#### (ii) *Hodgkin disease*

One case-control study of Hodgkin disease was available for evaluation. [Dal Maso et al. \(2004\)](#) studied 62 histologically confirmed incident Hodgkin disease cases, and 504 control

patients. The prevalence of HBsAg in the cases was 1.9% (one HBsAg-positive), and 0.9% in controls (four HBsAg-positive), resulting in an adjusted odds ratio of 1.8 (95%CI: 0.1–21.5).

One cohort study of HBV infected individuals listed Hodgkin disease as one of the outcomes, with a standardized incidence ratio (SIR) of 0.8 (95%CI: 0.3–2.1) ([Amin et al., 2006](#)).

## 3. Cancer in Experimental Animals

In this volume, the Working Group decided not to include a separate section on “Cancer in Experimental Animals” in the *Monographs* on viruses but rather to include description of such studies under Section 4 (below). The reasoning for this decision is explained in the General Remarks.

## 4. Other Relevant Data

### 4.1 Introduction

At a molecular level, the genesis of HBV-induced HCC is a complex, multifaceted, and multistep process with the essential components being a series of genetic or epigenetic changes in the genes that govern cell proliferation and cell death. The precise roles of the virus and the molecular mechanisms involved in hepatocarcinogenesis, how they interact, and the sequence in which they occur in the pathogenesis of HCC remain elusive. However, the available evidence supports the notion that the development of the tumour is the result of a combination of host responses to the presence of the virus and molecular mechanisms that are directly or indirectly induced by the virus.

HBV is a non-cytopathic virus and the hepatic inflammation and injury that occur in acute and chronic hepatitis and cirrhosis are attributed to the immune responses of the host to the presence

of the virus, especially those of class-1-restricted cytotoxic T lymphocytes. A large proportion of HBV-induced HCCs occurs in association with cirrhosis or, less often, chronic hepatitis, suggesting that the underlying chronic necro-inflammatory hepatic disease frequently provides a mitogenic and possibly also a mutagenic environment in which virus-induced genetic changes can lead to hepatocarcinogenesis ([Chisari, 2000](#); [Arbuthnot & Kew, 2001](#)). However, the proportion of patients developing HCC following pre-existing cirrhosis seems to vary in different parts of the world. In particular, in regions of high exposure to aflatoxin, the proportion of patients with pre-existing cirrhosis may be significantly lower than in regions in which aflatoxin is not a risk factor ([Brechot et al., 2010](#)).

A recent study compared the transcriptome-genotype-phenotype of more than 50 HCCs, and could identify specific patterns for HBV-associated HCCs ([Boyault et al., 2007](#)).

## 4.2 Chronic necro-inflammatory hepatic disease in hepatocarcinogenesis

HBV-induced chronic necro-inflammatory hepatic disease (cirrhosis and chronic hepatitis) is characterized by continuous or intermittent necrosis of hepatocytes, followed by regenerative proliferation. Its central role in hepatocarcinogenesis is supported by the observation that the lifetime risk for developing HCC in chronic HBV carriers with cirrhosis is higher than that in carriers without cirrhosis ([Arbuthnot & Kew, 2001](#)).

Hepatocytes are normally in a quiescent state with an extremely low turnover rate, but they react to the loss of liver cells with an extraordinarily vigorous proliferative response. This response is tightly controlled and lasts only until the initial number of hepatocytes is restored; it does not normally lead to cancer ([Fausto, 1997](#),

[1999](#); [Overturf et al., 1997](#)). Existing quiescent hepatocytes are responsible for this regenerative cell proliferation, and only uncommonly do hepatic progenitor (oval) cells directly give rise to tumour cells, although it is possible that hepatocytes originating from these cells are at higher risk for oncogenesis than other hepatocytes ([Fausto, 1997, 1999](#)).

The proliferation of hepatocytes is regulated by several factors, including nuclear factor- $\kappa$ -B (NF- $\kappa$ B), transforming growth factor- $\alpha$  (TGF- $\alpha$ ), insulin-like growth factor-2 (IGF-2), and hepatocyte growth factor (HGF) ([Grisham, 2001](#)). Transcriptional activation of these factors by mediators, such as tumour necrosis factor- $\alpha$  (TNF- $\alpha$ ), chemokines, and interleukins released during the inflammatory process regulates proliferation, and has an anti-apoptotic effect through the upregulation of the anti-apoptotic target gene *BCL2* ([Grisham, 2001](#)).

With sustained proliferation, at some point and for reasons as yet poorly understood, the regulation of proliferation may become unrestrained, which is an essential step in hepatocarcinogenesis, complicating chronic necro-inflammatory hepatic disease. Unrestrained hepatocyte proliferation, in association with the accumulation over time of several genetic and epigenetic changes, results in the formation of hyperplastic nodules that may progress to dysplastic nodules, and finally to HCC ([Fausto, 1999](#)).

By increasing the hepatocyte turnover rate, chronic necro-inflammatory hepatic disease:

- enhances the risk of a cell acquiring critical mutations,
- leads to reactivation of telomerase, and
- may also provide an opportunity for other selective growth advantage of cells to become manifest.

Concurrently with these oncogenic mechanisms, distortion of the lobular architecture of the liver by fibrosis, and nodular regeneration of hepatocytes in cirrhosis modify normal cell-to-cell and cell-to-extracellular matrix interactions,

which may contribute to the loss of cell-growth control, senescence, and apoptosis ([Davis & Kresina, 1996](#)).

#### **4.2.1 Virus-induced chronic necro-inflammatory hepatic disease**

One way in which mutations can arise is by the generation of reactive oxygen species and/or reactive nitrogen species that induce oxidative/nitrosative stress and DNA damage ([Trush & Kensler, 1991](#); [Bartsch & Nair, 2006](#)). Putative mechanisms of free-radical-induced hepatocyte damage and malignant transformation are the mutagenic properties of the free radicals and their effect on lipid peroxidation ([Cheeseman, 1993](#); [Bartsch & Nair, 2006](#)). In addition to DNA modifications caused directly by reactive oxygen species and reactive nitrogen species, DNA bases can be modified by lipid peroxidation products such as *trans*-4-hydroxy-2-nonenal (HNE), 4-hydroperoxy-2-nonenal (HPNE), and malondialdehyde (MDA) to form various exocyclic adducts, including malondialdehyde-deoxyguanine (M<sub>1</sub>dG), and etheno- and propano-DNA adducts ([Bartsch & Nair, 2006](#)).

Oxidative stress and upregulation of inducible nitric oxide synthase (iNOS) has been demonstrated in chronic viral hepatitis ([Loguercio & Federico, 2003](#)). A massive increase (up to 90-fold) in the 1,N<sup>6</sup>-ethenodeoxyadenosine (εdA) concentration in urine was detected in HBV-infected patients with chronic hepatitis and liver cirrhosis ([Bartsch & Nair, 2006](#)). εdA could arise from HBV-induced chronic inflammation, overproducing reactive oxygen species, reactive nitrogen species, and DNA-reactive lipid-peroxidation-derived aldehydes such as HNE (see Fig. 4.1).

#### **4.2.2 Reactivation of telomerase**

During the progression of chronic hepatitis to cirrhosis, progressive shortening of telomeres occurs as a consequence of multiple cycles of cell injury, death, and regeneration, and results in the premature senescence of hepatocytes. Telomere-shortening beyond a critical length causes a proliferative block, which becomes manifest as chromosomal instability, end-to-end fusion, and cell death. Hepatocarcinogenesis is characterized by the evolution of clones of hepatocytes with increased telomerase expression and an immortalized phenotype ([Farazi et al., 2003](#)).

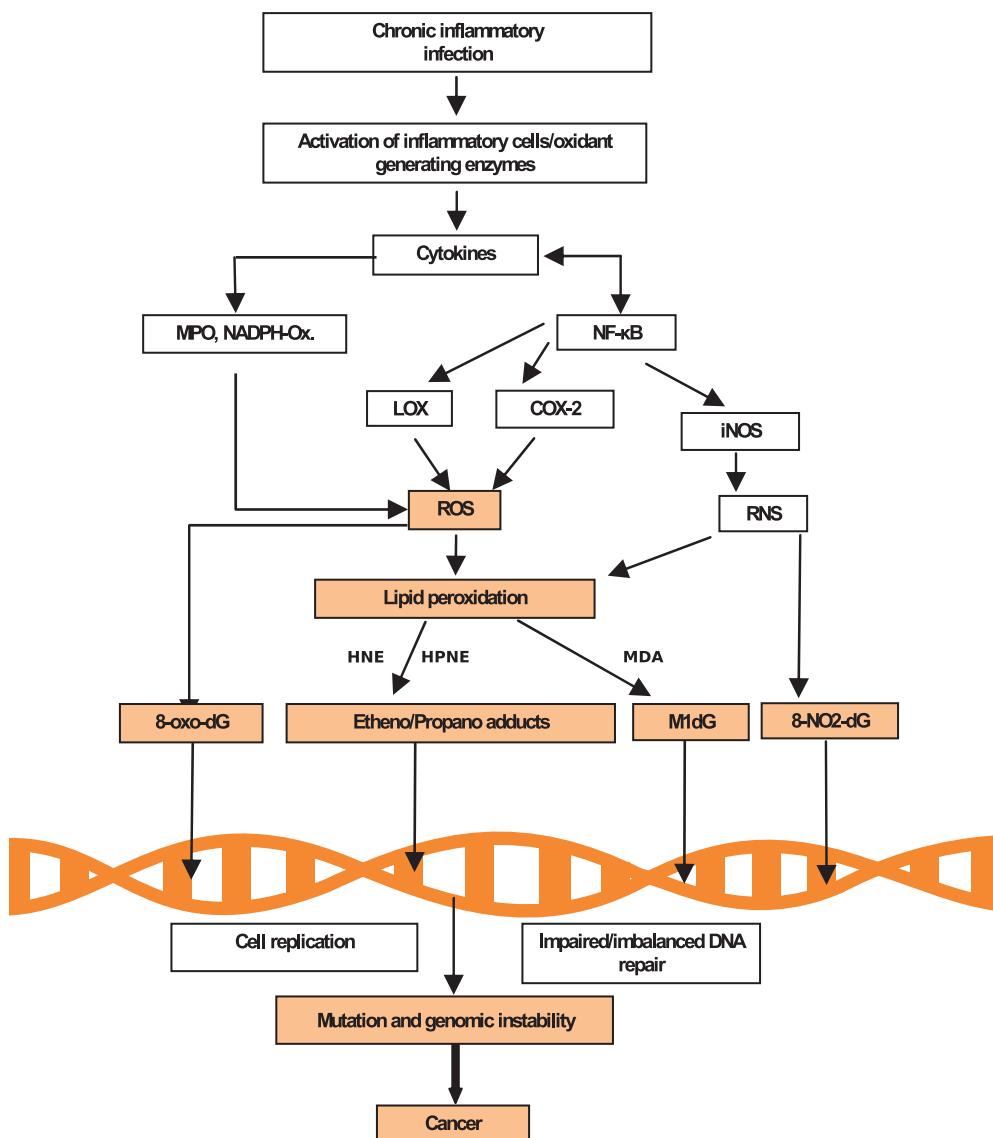
### **4.3 Direct mechanisms of hepatocarcinogenesis**

HBV may also play a direct role in HCC via two major mechanisms. The first mechanism is the integration and mutation of the viral genome into the host cellular DNA, which may result in the altered expression of important cellular genes. The second one is the expression of HBV proteins, which may have a direct effect on cellular functions and in the promotion of malignant transformation ([Brechot et al., 2010](#)).

#### **4.3.1 Role of the integration of HBV DNA into the host genome**

Although insertion of hepadnaviral DNA into host DNA is not a requirement for viral replication, HBV genome integrations have been reported in over 85–90% of HBV-related HCCs ([Bonilla Guerrero & Roberts, 2005](#)). Integration occurs as a result of a recombination event and takes place at one or, far more often, multiple sites ([Matsubara & Tokino, 1990](#); [Rogler & Chisari, 1992](#); [Robinson, 1994](#)). Integration is an early event and selective clonal amplification of hepatocytes with unique integration patterns is thought to occur during progression to malignancy ([Minami et al., 2005](#)). The integrant may be a single linear sequence of the viral genome

**Fig. 4.1 Illustration explaining how chronic infection and inflammatory processes can lead to deregulation of cellular homeostasis and carcinogenesis**



ROS, reactive oxygen species; RNS, reactive nitrogen species; iNOS, inducible nitric oxide synthase; COX-2, cyclooxygenase-2; NADPH-Ox, NADPH oxidase; MPO, myeloperoxidase; LOX, lipoxygenase; HNE, trans-4-hydroxy-2-nonenal; HPNE, 4-hydroperoxy-2-nonenal; MDA, malondialdehyde; M<sub>1</sub>dG, malondialdehyde-deoxyguanine; 8-oxo-dG, 8-oxo-7,8-dihydro-2'-deoxyguanosine; 8-NO<sub>2</sub>-dG, 8-nitroguanine  
With kind permission from Springer Science+Business Media: Langenbecks Arch Surg, Chronic inflammation and oxidative stress in the genesis and perpetuation of cancer: role of lipid peroxidation, DNA damage, and repair, 391, 2006, 499–510, Bartsch H, Nair J, Fig. 1.

(almost always with nucleotides missing from one or both ends), but more often comprises rearranged fragments of viral DNA. Complete and intact viral genomic DNA has been found only rarely in integrants. No two insertions are alike. Linear viral DNA is the preferential form used as an integration substrate ([Yang & Summers, 1999](#)).

The exact mechanisms of hepadnaviral DNA integration into cellular DNA are not known, but the evidence available suggests that integration preferentially occurs at sites of double-strand DNA breaks in the cellular DNA ([Bill & Summers, 2004](#)). The frequent cell divisions and DNA strand breaks that occur in chronic hepatitis and cirrhosis create opportunities for HBV DNA to be integrated into chromosomal DNA ([Dandri et al., 2002](#)). In addition, the existence of preferred topoisomerase-1 cleavage motifs in the vicinity of the DR1 and DR2 sites, may predispose the insertion of HBV DNA into cellular DNA ([Wang & Rogler, 1991](#)).

There are several ways in which integrated hepadnaviral DNA may contribute to hepatocarcinogenesis. The insertion of HBV DNA into cellular DNA in human HCC does not occur at specific sites; however, several reports have found integration events near cellular oncogenes or other genes involved in cellular growth ([Paterlini-Bréchot et al., 2003](#); [Bonilla Guerrero & Roberts, 2005](#); [Minami et al., 2005](#)). Among the cellular genes with HBV integration events are retinoic acid receptor  $\beta$ , cyclin A2, mevalonate kinase, mcm8, neurotropic tyrosin receptor kinase 2 (NTRK2), IL1R-associated kinase 2 (IRAK2), p42 mitogen-activated protein kinase 1 (p42 MAPK1), telomerase, and others ([Dejean et al., 1986](#); [Wang et al., 1990](#); [Paterlini-Bréchot et al., 2003](#); [Bonilla Guerrero & Roberts, 2005](#); [Minami et al., 2005](#)). These studies suggest that the presence of an integrated HBV genome could lead to the inappropriate activation of targeted cellular genes, and in these cases, provide a mechanism for HBV-mediated carcinogenesis.

In the case of woodchuck hepatitis virus (WHV), the integration of the viral genome in or near c-MYC or N-MYC proto-oncogenes in 50% of infected animals considerably enhances the transcriptional activity of the corresponding cellular promoters ([Fourel et al., 1990](#); [Tennant et al., 2004](#)). This observation provides strong evidence for a direct role of WHV in hepatocarcinogenesis. However, given that no human cellular gene appears to be targeted with a similar frequency by HBV, it is currently unclear to what extent this mechanism applies to human liver cancer.

Integration of HBV DNA into cellular DNA may also induce structural changes in the flanking DNA sequences. These are highly varied, include small and large deletions, translocations, duplications, or amplification of chromosomal sequences ([Matsubara & Tokino, 1990](#); [Takada et al., 1990](#); [Buendia, 2000](#); [Laurent-Puig et al., 2001](#)). Such changes occur more frequently in HBV-related HCC than in HCC attributable to other causes ([Laurent-Puig et al., 2001](#)).

#### *4.3.2 Role of HBV proteins*

Transcriptional activation by HBV proteins of cellular genes distant from the site of integration (transactivation) that influence cellular proliferation and differentiation or apoptosis is a more frequent, and probably more important, mechanism of inherent hepatocarcinogenesis ([Buendia, 2000](#)). This effect is mediated through signal transduction pathways. Two HBV proteins, HBx and PreS/S (when 3' truncated during or after integration), have been shown to have indirect transactivating capability, and have been implicated in the development of HCC by this means ([Murakami, 1999](#); [Feitelson, 2006](#)).

(a) *Hepatitis B virus X protein (HBx)*

The smallest HBV protein (16.5 kDa), HBx, expressed both in the cytoplasm and the nucleus, is essential for viral replication ([Murakami, 1999](#); [Feitelson, 2006](#)).

Because the gene is close to the preferred integration sites of HBV, it is the region of the genome most often included in integrants ([Paterlini et al., 1995](#)), and a selective accumulation of HBx gene transcripts has been reported in HBV-related HCC. Antibodies against HBx have been demonstrated in the sera of chronic carriers, which confirms the expression of the viral protein ([Pál et al., 2006](#)).

Integrated HBx, even when truncated and mutated, may still retain some of its functions. Alternatively, mutation and/or truncation may activate specific functions of HBx ([Schlüter et al., 1994](#)). Evidence that HBx is contributing to HCC comes in part from knockdown experiments in which reduction in the levels of HBx leads to growth suppression ([Chan & Ng, 2006](#); [Cheng et al., 2007](#)). In addition, no HCC related to avian hepadnaviruses, which are devoid of the X ORF, have been reported ([Murakami, 1999](#)).

HBx does not contain any structural motifs that indicate a capacity to bind DNA directly, and functions through protein-to-protein interaction. HBx activates transcription from various HBV promoters, other viral promoters, and from the promoters of a large number of cellular genes including oncogenes, cytokines, growth factors, and several genes involved in cell-cycle control and progression, DNA repair, apoptotic cell death, cellular adhesion, and angiogenesis ([Murakami, 1999](#); [Feitelson, 2006](#); [Table 4.1](#)).

A wide variety of cis-elements have been shown to be responsive to HBx which includes binding sites for AP-1, AP-2, NF-κB, SRF, c/EBP, Ets, ATF1, and CREB ([Table 4.1](#)).

Transcriptional regulation by HBx may occur through direct interaction with transcription factors in the nucleus, like shown for ATF-2,

CREB, Oct-1, p53, bZIP, and other components of the basal transcription machinery ([Table 4.2](#)).

HBx transactivation may also occur by modulating cell-signalling pathways within the cytoplasm ([Table 4.3](#)). NF-κB-signalling that mediates cellular stress responses that control the expression of several acute-phase response proteins, cytokines, and adhesion molecules is among the pathways modulated by HBx though the activation of “Mitogen Activated Protein Kinase” (MAPK) pathways ([Benhenda et al., 2009](#)).

HBx has also been shown to inhibit the activity of some serine protease inhibitors and components of the proteasome complex, specifically PSMA7 ([Zhang et al., 2000](#)), and might thus modulate the turnover of certain cellular proteins involved in transcription or regulation of cell-cycle progression, or both.

Among the cellular proteins whose functions are known to be perturbed by HBx protein is the tumour-suppressor p53. The p53 protein maintains chromosomal integrity by arresting the cell cycle in G<sub>1</sub>, regulating the DNA damage control responses, and regulating the induction of apoptosis and/or senescence ([Shimamura & Fisher, 1996](#)). *In vitro* HBx expression studies have shown that HBx protein binds to specific sequences in the C-terminal end of p53, preventing its entry into the nucleus, and abrogating its sequence-specific DNA-binding and transcriptional activity ([Elmore et al., 1997](#); [Takada et al., 1997](#)).

“Phosphatase and Tensin homology deleted on chromosome 10” (PTEN) is another important tumour-suppressor which has been shown to be a transcriptional target of p53 ([Stambolic et al., 2001](#)). Inactivation of both p53 and PTEN proteins by HBx protein results in increased levels of hypoxia-induced factor-1α (HF1-α) and vascular endothelial growth factor (VEGF), both of which are important for the survival and neovascularization of early-stage tumours ([Huang & Kontos, 2002](#)).

**Table 4.1 Some targets of transactivation by HBx**

Gene product	Binding factor involved
Interleukin-8	NF-IL6 and NF-κB-like
HLA-DR	nd
ICAM-1	nd
EGF receptor	
Alpha-fetoprotein	c/EBP site
MDR	
TGFα	<sup>a</sup> AP-2
TGFβ	Egr1 (Ets family) HBx interaction with Egr1
Interleukin-6	NF-κB
TNFα	Proximal promoter
C-FOS	nd
C-JUN	AP-1
C-MYC	nd
TBP	nd

<sup>a</sup> reference [Kim & Rho \(2002\)](#)

nd, not determined

Adapted from [Murakami \(1999\)](#)

HBx protein may impair DNA-repair mechanisms by several means. Besides its potential inhibition of p53-dependent DNA repair, HBx can repress the transcription of two components of the repair factor TFIIH – XPB and XPD ([Jaitovich-Groisman et al., 2001](#)). The protein also directly interferes with DNA repair by forming a complex with the DNA-repair protein, HBx-associated protein (XAP-1), which normally binds to damaged DNA in the first step of nucleotide excision repair ([Becker et al., 1998](#)). Recent evidence shows that HBx binds the UV-damaged DNA binding protein 1 (DDB1), a protein involved in DNA repair and cell-cycle regulation. This interaction leads to interference with S-phase progression and induces lagging chromosomes during mitosis. Consequently, HBx may exert deleterious activities in dividing, but not quiescent, hepatoma cells ([Martin-Lluesma et al., 2008](#)).

In addition to its inhibitory effects on p53-induced apoptosis, HBx protein inhibits caspase-3-dependent apoptosis ([Gottlob et al., 1998](#)). Conversely, HBx may also sensitize cells

to programmed cell death induced by TNF-α, an effect mediated by prolonged stimulation by N-MYC transcription and the stress-mediated MAPK pathway ([Su & Schneider, 1997](#)).

#### (b) Hepatitis B virus 3' truncated PreS/S proteins

Like the *HBx* gene, the *PreS/S* gene is frequently included in HBV DNA integrants in patients with HCC. When 3' truncated during or after integration, the gene has transactivating properties and might contribute to oncogenesis ([Schlüter et al., 1994](#)). The truncated medium surface protein is exclusively cytoplasmic in location and has pleotropic effects on gene transcription. Its transactivating effects are mediated by modulating protein kinase C (PKC) signal transduction and interaction with several transcription factors such as NF-κB and AP-1 ([Lauer et al., 1994](#); [Hildt et al., 1996](#)). Potentially oncogenic transcriptional effects include the stimulation of promoter sequences of c-MYC, c-FOS, and c-HA-RAS oncogenes, and the inflammation-associated cytokine, IL-6 ([Kekulé et al., 1990](#); [Meyer et al., 1992](#); [Lauer et al., 1994](#)). Mutated

**Table 4.2 Some HBx-interacting cellular proteins**

Protein	Function
<i>Transcription factors</i>	
bZip family	
ATF, CREB/ATF-2	
ATF3, NF-IL6	
Chop10, ICER II $\gamma$	
Oct1	
Egr1	
P53	Tumour suppressor Transcription factor
<i>General transcription factors</i>	
TBP	TATA binding
RPB5	A common subunit of pol I, II & III
TFIIB	Initiation factor
<i>DNA repair</i>	
TFIIE	Complex necessary for initiation and elongation
ERCC2	
ERCC3	
UVDDDB1 (XAP1)	DNA repair
<i>Protease subunit</i>	
XAPC7	Proteasome $\alpha$ -subunit
<i>Other pX-binding proteins</i>	
XAP2	AhR ligand-binding subunit ARA9 family of BREF-2
XAP3	PKC Binding protein
XIP	Novel, ubiquitous
P55sen	In family of EGF-like proteins

Adapted from [Murakami \(1999\)](#)

PreS protein may induce endoplasmic reticulum stress ([Wang et al., 2003b](#)), which in turn stimulates the expression of cyclooxygenase-2 through activation of NF- $\kappa$ B and P38-MAPK ([Hung et al., 2004](#)). In transgenic mice that overproduce in hepatocytes the large envelope protein of HBV, this protein accumulated in the endoplasmic reticulum. This led to cytopathic effects that contributed to a progressive disease culminating in liver cancer ([Chisari et al., 1989](#)). The relevance of this animal model to HBV-associated HCC in human remains unclear.

#### 4.4 Epigenetic mechanisms

Methylation of CpG islands of tumour-related genes is an early and frequent event in the multi-step process of hepatocarcinogenesis, with an increasing number of tumour-suppressor genes being affected by epigenetic silencing ([Lee et al., 2003b](#); [Oh et al., 2007](#)). There is evidence that genome-wide methylation patterns may vary according to HCC etiology ([Hernandez-Vargas et al., 2010](#)). Deregulated expression of DNA methyltransferases by HBx may contribute to the epigenetic modulation of cellular genes involved in cell cycle ([Kanai et al., 1997](#); [Lee et al., 2003b](#); [Oh et al., 2007](#); [Park et al., 2007](#); [Su et al., 2007a](#)).

**Table 4.3 Some reported interactions of the HBx viral protein with major cellular signal-transduction pathways**

Signalling pathways	Reported HBx interactions
p53/PTEN	- binds to and inactivates p53 - blocks PTEN expression via binding p53
pRB	- promotes hyperphosphorylation (inactivation) of pRB - promotes pRB expression
p21 <sup>WAF1/CIP1</sup>	- suppresses <i>p21</i> promoter via binding to wild type p53 or p53 <sup>sen</sup>
MYC	- stimulates the <i>c-MYC</i> promoter
RAS/RAF/MAPK	- stimulates RAS/RAF/MAPK signalling
E-Cadherin	- stimulates methylation of the <i>E-cadherin</i> promoter
IGFR1	- upregulates IGFR1 - inactivates p53
TGFβ1	- upregulates TGFβ1 and TGFβ1 signalling resulting in loss of sensitivity of cells to TGFβ1.
JAK/STAT	- activates JAK

Adapted from [Feitelson \(2006\)](#)

## 4.5 Other major risk factors in hepatocarcinogenesis

### 4.5.1 HCV infection

Dual infection with HBV and HCV is common, and is associated with more severe chronic hepatic parenchymal disease and an increased frequency and a younger age of development of HCC than occurs with either virus alone ([Kaklamani et al., 1991](#); [Kew, 2006](#)). Understanding the nature of the synergistic interaction between the two viruses in hepatocarcinogenesis will have to wait until a clearer understanding of the mechanisms involved in HCC induced by either virus alone is attained. Moreover, the replicative dominance of one virus over the other and the effect that this may have on the progression of liver disease and the development of HCC remains a matter of debate ([Zarski et al., 1998](#); [Kew, 2006](#)). Nevertheless, several possible mechanisms for the synergistic hepatocarcinogenic interaction between the two viruses are suggested by currently available information, and the major players appear to be HBx protein, HCV core, and NS5a proteins.

In Africa and Asia, chronic infection with HBV that gives rise to HCC is predominantly acquired very early in life, whereas chronic HCV infection in industrialized countries is largely acquired much later in life. It is likely that HCV infection is superimposed on a long-standing HBV infection in the great majority of patients with dual infection in Asia and Africa; whereas in developed countries, it is probable that the two infections are obtained either at the same time or within a relatively short interval. These differences may influence the mechanisms involved in hepatocarcinogenesis in patients co-infected with HBV and HCV ([Kew, 2006](#)).

Dual infection with HBV and HCV results in a higher incidence of cirrhosis than with either virus alone ([Tsai et al., 1996b](#)), so the possible mechanisms implicated in malignant transformations complicating chronic necro-inflammatory hepatic disease are even more likely to be applicable with co-infection. Apart from the importance of hepatocyte necrosis and regeneration in generating oxidative damage, the HCV core and NS5a proteins have been reported to directly generate reactive oxygen species ([Gong et al., 2001](#); [Okuda et al., 2002](#)).

HBx and HCV core proteins can additively repress transcription of the *p21* gene ([Han et al., 2002](#)). Because the tumour-suppressor protein p21 is a universal inhibitor of cyclin-CDK complexes and proliferating cell nuclear antigen (PCNA) and hence DNA replication by inducing cell-cycle arrest at the G1-S checkpoint, the combined repression of *p21* by HBx and HCV core proteins may result in an additive growth stimulation of hepatocytes ([Han et al., 2002](#)).

A specific mutation, T1936C, has been reported in the proximal core region of HBV that may be involved in the accelerated progression to HCC in co-infected patients ([De Mitri et al., 2006](#)).

The relevance of potential interactions between the two viruses in human HCC is supported by the recent report by [Rodríguez-Iñigo et al. \(2005\)](#), who demonstrated by in-situ hybridization that HCV and HBV can coexist in the same hepatocyte in liver biopsy samples from patients with chronic HCV infection with occult HBV infection.

#### 4.5.2 Aflatoxin B<sub>1</sub>

Early evidence of hepatocarcinogenic synergism between hepadnavirus infection and dietary exposure to the fungal toxin, aflatoxin B<sub>1</sub> (AFB<sub>1</sub>) was provided by experiments in transgenic HBV-mice and in woodchucks infected with WHV. This evidence was confirmed in ecological studies wherein the majority of which, the increased risk was multiplicative (reviewed in [Kew, 2003](#); [Gouas et al., 2009](#); [Wild & Montesano, 2009](#)).

Several mechanisms have been suggested to explain the synergism. The first is that the cytochrome P450s that convert the AFB<sub>1</sub> parent molecule to the highly reactive AFB<sub>1</sub>-8,9-exo-epoxide may be induced by either chronic hepatitis caused by HBV infection or the presence of the virus itself ([Kirby et al., 1994](#)). A recent study shows that this epoxide forms preferential

adducts in DNA at G bases located in sequence in a similar context to the one of codon 249 in TP53 ([Besaratinia et al., 2009](#)).

Another way in which hepatocytes may be sensitized to the carcinogenic effects of AFB<sub>1</sub> is by the decreased activity of the phase II detoxification enzymes, glutathione-S-transferase (GST) and EPHX ([McGlynn et al., 1995](#)). In human liver, GST activity is lower in the presence of HBV DNA ([Zhou et al., 1997](#)). This suggests that the ability of hepatocytes to detoxify chemical carcinogens may be compromised in HBV-infected individuals.

The accelerated hepatocyte proliferation caused by HBV-induced chronic necro-inflammatory hepatic disease increases the likelihood of AFB<sub>1</sub>-induced mutations (including 249<sup>ser</sup> TP53 mutation) being formed, and the subsequent clonal expansion of hepatocytes containing these mutations ([Kew, 2003](#)). It also results in the generation of reactive oxygen and nitrogen intermediates, which also induce these mutations ([Kew, 2003](#)). AFB<sub>1</sub>-DNA adducts, which are normally repaired by the nucleotide excision repair pathway, may persist because of the interference of HBx protein with this pathway ([Jia et al., 1999](#)).

### 4.6 Role of HBV in other cancers

#### 4.6.1 B-cell lymphoma

At the time of writing, no mechanisms are known that might explain the noted limited association between HBV and B-cell lymphoma.

#### 4.6.2 Cholangiocarcinoma

At the time of writing, no mechanisms are known that might explain the noted limited association between HBV and cholangiocarcinoma.

## 4.7 Synthesis

There is strong evidence to support an indirect role for HBV in hepatocarcinogenesis resulting from chronic necro-inflammatory hepatic disease (cirrhosis), as well as moderate evidence for a direct role largely associated with HBx.

## 5. Evaluation

There is *sufficient evidence* in humans for the carcinogenicity of chronic infection with HBV. Chronic infection with HBV causes hepatocellular carcinoma. Also, positive associations have been observed between chronic infection with HBV and cholangiocarcinoma and non-Hodgkin lymphoma.

Chronic infection with HBV is *carcinogenic to humans (Group 1)*.

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# HEPATITIS C VIRUS

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The hepatitis C virus was considered by a previous IARC Working Group in 1993 ([IARC, 1994](#)). Since that time, new data have become available, these have been incorporated in the *Monograph*, and taken into consideration in the present evaluation.

## 1. Exposure Data

### 1.1 Taxonomy, structure, and biology

#### 1.1.1 Taxonomy

In the 1970s and 1980s, serological tests developed for hepatitis A and B viruses (HAV, HBV) indicated that most transfusion-associated hepatitis was not caused by either HAV or HBV, and were therefore named non-A, non-B hepatitis (NANBH). After detection of the first NANBH-specific clone, the entire viral genome of the now termed hepatitis C virus (HCV) was sequenced, and based on its structural and functional organization, HCV was classified into the family of the *Flaviviridae*, where it forms its own genus '*hepacivirus*' ([Choo et al., 1989](#); [Kuo et al., 1989](#)).

At least six major viral genotypes (1 to 6) have been identified ([Simmonds et al., 2005](#)). Viral genotypes have specific geographic distribution. The determination of the viral genotype is important for transmission studies, and has major therapeutic implications. Patients infected with genotype 1 have a significantly lower rate of response to antiviral therapy compared to other genotypes ([Manns et al., 2007](#)).

Genotypes display differences in nucleotide sequences below 30–35%, and within a genotype genomes are allocated into different subtypes if their sequences differ by over 20–25%. Furthermore, viral isolate(s) present in an infected individual can mutate into quasi-species ([Simmonds et al., 2005](#); [Xu et al., 2008](#)).

#### 1.1.2 Structure of the virion

In line with other members of the *Flaviviridae*, HCV consists of an enveloped nucleocapsid that assembles intracellularly in close conjunction with membranes derived from the endoplasmic reticulum ([Moradpour et al., 2007](#)). Released HCV particles are about 40–70 nm in diameter, but the morphology of particles detected in the serum of patients may be heterogeneous due to the association with immunoglobulins or very low density lipoproteins (vLDL) ([André et al., 2005](#)).

#### 1.1.3 Structure of the viral genome

The HCV genome is a single-stranded, positive sense RNA of approximately 9.6 kb, and is represented in Fig. 1.1. It contains short non-coding regions (NCRs) at each end that encompass the coding sequence of a polyprotein. The 5' NCR, a well conserved 341nt sequence element which

forms into a complex secondary structure, is needed for efficient RNA replication and drives cap-independent translation of a single large open reading frame (ORF) that encodes approximately 3000 residues ([Moradpour et al., 2007](#)). The HCV 3' NCR consists of a short variable domain of about 40nt and a polyuridine/polypyrimidine tract, followed by a highly conserved domain of 98nt that is essential for RNA replication ([Tanaka et al., 1995](#)). The N-terminal region of the polyprotein encodes the structural proteins including the nucleocapsid protein (core) and two envelope glycoproteins (E1 and E2) that form the viral particle, followed by several non-structural proteins, designated as NS2 to NS5B (Fig. 1.1). The C-terminal regions of the core and envelope proteins contain signal sequences, and are cleaved in the endoplasmic reticulum by host signal peptidase and signal peptide peptidase ([Yasui et al., 1998](#); [McLauchlan et al., 2002](#); [Okamoto et al., 2004](#)). Alternative cleavage sites at the C-terminal of E2 yield the viroporin p7. The NS2/NS3 junction is cleaved in *cis* by metalloproteinase activity. The remaining cleavages are carried out by the NS3 serine protease, which requires NS4A as a cofactor. Besides participating in polyprotein processing, NS2 plays important roles in viral assembly ([Moradpour et al., 2007](#); [Jirasko et al., 2008](#)). The non-structural genes NS3, NS4A, NS4B, NS5A and NS5B have diverse functions (see Section 4), and are all required for RNA replication ([Lindenbach et al., 2007](#); [Moradpour et al., 2007](#)). NS5B, the viral replicase, lacks proofreading activity. This lack of proofreading in the context of a very high viral production rate, which has been estimated to be as much as  $10^{12}$  virions per day in infected patients, is thought to be responsible for the high genetic variability of HCV ([Moradpour et al., 2007](#)). Thus, HCV has been classified into six genotypes and several subtypes based on sequence similarities. In addition, the HCV genome is prone to acquiring mutations that

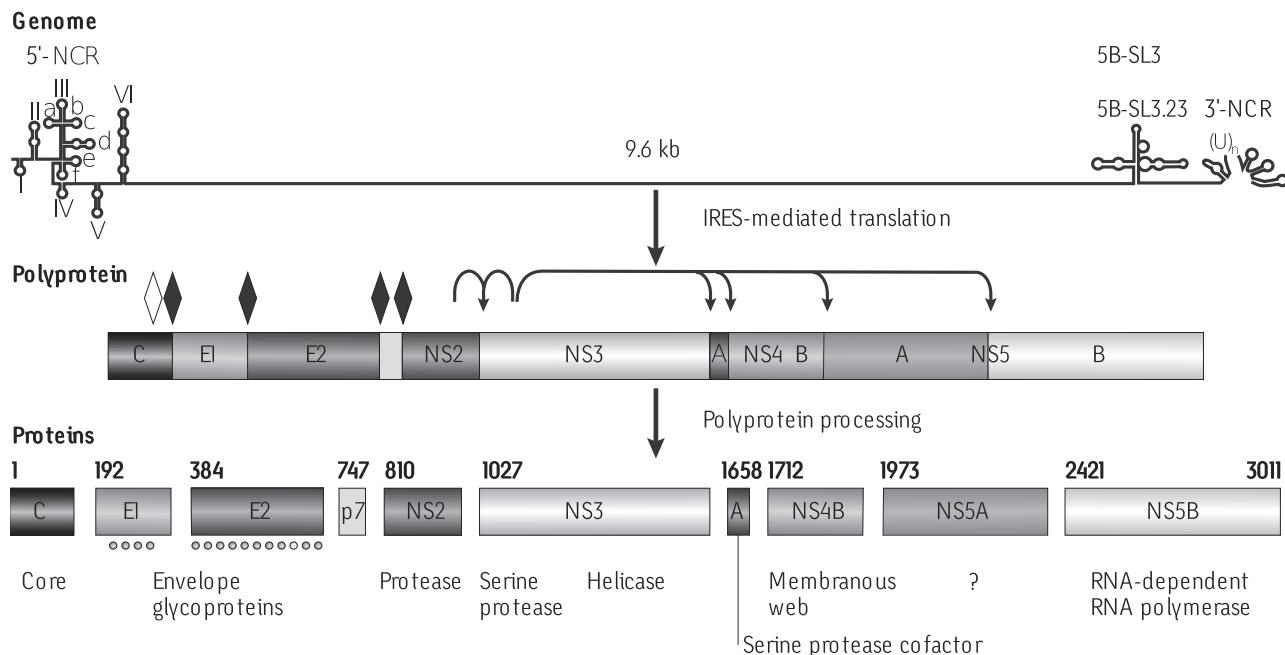
lead to the presence of quasi-species in infected patients ([Xu et al., 2008](#)).

#### 1.1.4 Host range

HCV is hepatotropic, and only man and higher primates such as chimpanzees have been, until recently, receptive to HCV infection and disease. Later, it was shown that the marmoset as well as tupaia, which are members of the tree shrew genus, are also susceptible to HCV infection ([Shimizu et al., 1998](#); [Sung et al., 2003](#); [Pachiadakis et al., 2005](#)).

#### 1.1.5 Tissue target

While HCV RNA has been unequivocally detected in the hepatocytes of liver biopsies of chronically infected patients and chimpanzees, the HCV genome has also been suggested to replicate in cells of lymphoid origin and dendritic cells ([Shimizu et al., 1998](#); [Sung et al., 2003](#); [Pachiadakis et al., 2005](#)), but this observation remains to be substantiated. Whether the tropism of HCV is limited to hepatocytes at the level of cell entry and/or replication via the association of HCV with lipoproteins and/or by other factors remains unclear. Expression patterns of all cell entry factors isolated to date are either ubiquitous or not unique to hepatocytes. This includes the lipoprotein receptors scavenger receptor B1 (SR-B1), and the low-density lipoprotein receptor (LDLr), which may mediate indirect uptake of HCV via HCV-associated lipoproteins ([Moradpour et al., 2007](#)). The replication of HCV seems mostly restricted to hepatocytes, and even though HCV replicons have been shown to amplify in cell lines of non-hepatic origin, the levels of replication reported are significantly lower than those observed in cell lines of hepatic origin ([Zhu et al., 2003](#); [Ali et al., 2004](#); [Chang et al., 2006](#)).

**Fig. 1.1 The HCV genome structure, viral polyprotein expression and processing into viral proteins**

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### 1.1.6 Life cycle, replication, and regulation of gene expression

Virus-binding to the cell surface and cell entry may involve the LDLr, glycosaminoglycans, SR-B1, the tetraspanin CD81, and the tight junction factors Claudin-1 and Occludin ([Evans et al., 2007](#); [Lindenbach et al., 2007](#); [Moradpour et al., 2007](#); [Ploss et al., 2009](#)). The tight junction factors are thought to act at late stages of cell entry, and their involvement in HCV cell entry suggests that the state of polarization of hepatocytes is likely to be important for the cell entry process ([Evans et al., 2007](#)). Internalization occurs via clathrin-coated vesicles, and their acidification induces the fusion machinery of the HCV glycoproteins. Little is known about the uncoating process and the initial events that allow the assembly of replication complexes, IRES-mediated replication,

polyprotein processing, and virion assembly. RNA replication occurs in membrane-like webs that are formed at the endoplasmic reticulum. The assembly and secretion process is thought to occur in tight relation with the vLDL biosynthesis machinery, which may explain the possible association of secreted HCV particles with vLDL ([André et al., 2005](#); [Nielsen et al., 2006](#); [Miyanari et al., 2007](#)).

### 1.1.7 Diagnosis of HCV infection

The diagnosis of HCV infection relies on laboratory tests which include: 1) anti-HCV antibody detection assays relying on third-generation enzyme-linked immunosorbent assays (ELISAs) whose sensitivity and specificity have been demonstrated; 2) viral genome detection assays mainly relying on real time Polymerase

Chain Reaction (PCR) technologies allowing the quantification of the viral genome; 3) genotyping assays to determine viral genotypes that allow the prediction of treatment outcome, and the determination of treatment duration ([Zoulim, 2006](#)).

The detection of anti-HCV antibodies in plasma or serum is based on the use of third-generation enzyme immunoassays (EIAs) that detect antibodies directed against various HCV epitopes. Recombinant antigens are used to capture circulating anti-HCV antibodies onto the wells of microtitre plates, microbeads, or specific holders adapted to closed automated devices. The specificity of third-generation EIAs for anti-HCV is greater than 99% and has been improved by the addition of viral epitopes that are recognized by antibodies present in the serum of infected patients ([Colin et al., 2001](#)). Their sensitivity is more difficult to determine, given the lack of a gold standard method, but it is excellent in HCV-infected immunocompetent patients. EIAs can be fully automated, and are well adapted to large volume testing. Immunoblot tests are nowadays clinically obsolete given the good performance of third-generation anti-HCV EIAs ([Colin et al., 2001](#)).

## 1.2 Epidemiology of infection

### 1.2.1 Prevalence and geographic distribution

The estimated prevalence of HCV infection worldwide is 2.2%. Region-specific estimates range from < 1.0% in northern Europe to > 3% in northern Africa (Fig. 1.2; [Alter, 2007](#)). High prevalences of HCV ( $\geq 10\%$ ) were found in some areas of Italy and Japan and, most notably, in Egypt (15–20%) following mass injection treatment for schistosomiasis. More recently, high HCV prevalences have been reported in some Asian areas, notably in Pakistan ([Ahmad, 2004](#)), and the People's Republic of China ([Zhang et al., 2005](#)). Within Europe, the highest prevalence

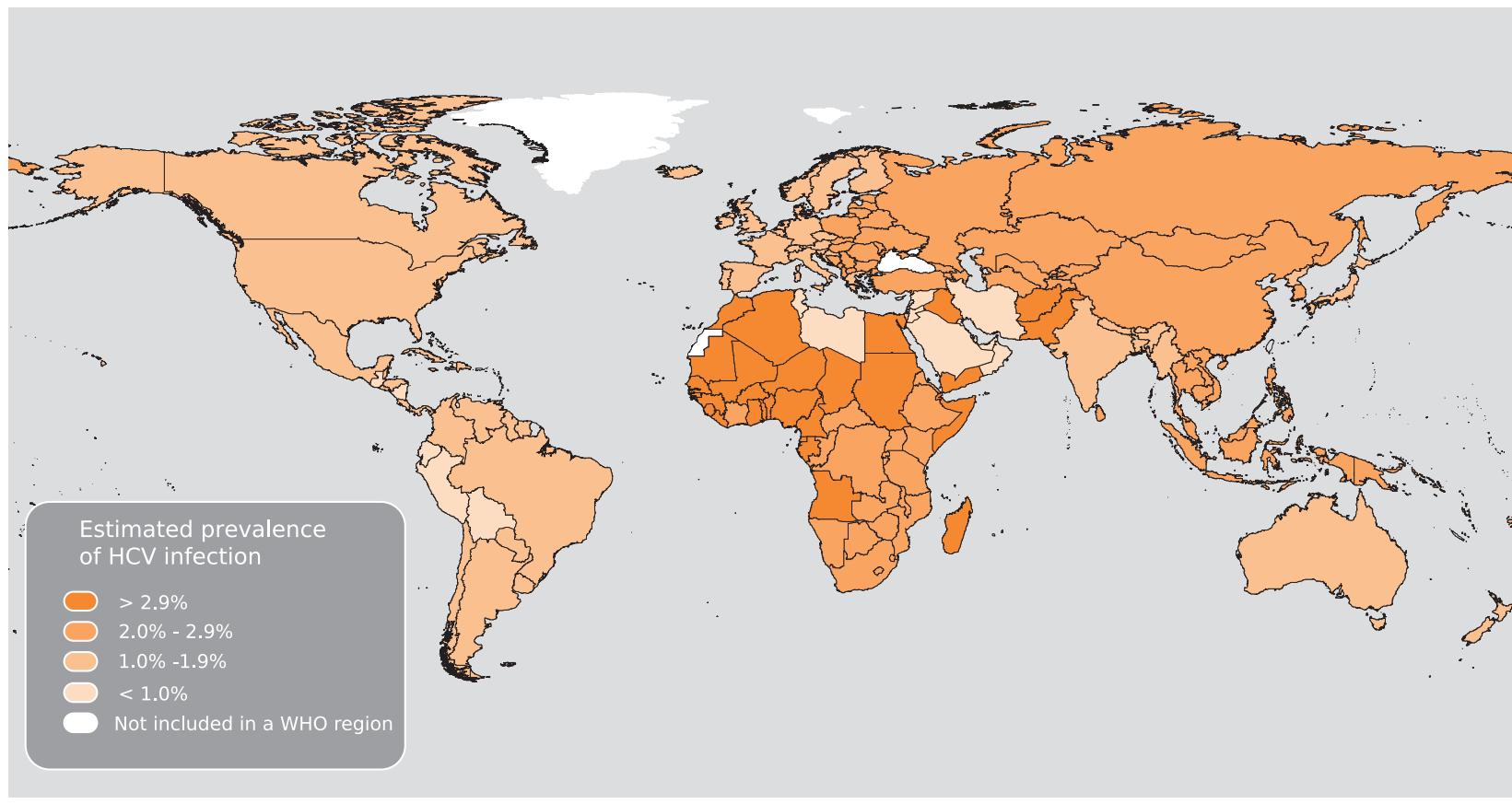
rate of HCV infection was reported in southern Italy where 7.5% of the general population and up to 23.2% of those aged 65 years old or older who were randomly selected were HCV-infected ([Fusco et al., 2008](#)). The lowest prevalence (0.01–0.1%) was reported for the United Kingdom and Scandinavia.

### 1.2.2 Transmission and risk factors for infection

HCV can be transmitted by transfusion of blood and blood products, transplantation of solid organs from infected donors, injection drug abuse, unsafe therapeutic injections, and occupational exposure to blood (primarily contaminated needles) ([Alter, 2007](#)). Transfusion-associated HCV infection was an important source of infection before HCV testing of blood donors was introduced in the early 1990s. Since then, transfusion-associated HCV infection has been virtually eliminated in those countries where routine HCV-testing has been implemented (Safe Injection Global Network (SIGN), 2001). Iatrogenic HCV transmission through unsafe (therapeutic) injections has sustained substantial epidemics of the infection in Japan, Italy in the 1940s and Egypt in the 1950s, and it is currently very frequent in low-resource countries ([Ahmad, 2004](#); [Raza et al., 2007](#)) where disposable needles tend to be re-used, and injections tend to be preferred to the oral route for the administration of common treatments. It has been estimated that approximately 2 million HCV infections are caused annually by contaminated health-care-related injections. Injection drug use accounts for most of the newly acquired infections in developed countries. The incidence rate among new drug injectors has been observed to range from 9 to 30% per 100 person-years ([Des Jarlais et al., 2003](#)).

HCV is less efficiently transmitted by occupational, perinatal and high-risk sexual exposures compared to those involving large or repeated percutaneous exposures to blood ([Alter, 2007](#)).

**Fig. 1.2 Estimated prevalence of HCV infection by region**



Adapted from [Alter \(2007\)](#), data source World Health Organization

The rate of transmission after an accidental needle-stick injury involving HCV-positive blood ranges from 0–10% ([Hernandez et al., 1992](#); [Mitsui et al., 1992](#)). The rate of perinatal HCV transmission is 4–7% and occurs only when HCV RNA is detectable in the maternal serum at delivery. There has been no difference in the rate of HCV transmission between vaginal delivery, caesarian section or breastfeeding. However, co-infection with HIV increases the rate of transmission 4–5-fold. The extent to which HCV is transmitted by sexual activity is very controversial ([Alter, 2007](#)). Although case-control studies of acute hepatitis C have identified sex with an infected partner or with multiple partners as independent risk factors for acquiring the disease, in long-term monogamous relationships with a partner with chronic HCV infection, there was little evidence for sexual transmission of HCV ([Clarke & Kulasegaram, 2006](#)).

Currently the data are too scant to determine whether cosmetic procedures (e.g. tattooing, body piercing) or intranasal illicit drug use significantly contribute to the overall HCV transmission ([Alter, 2002](#); [Hwang et al., 2006](#)).

### *1.2.3 Persistence, latency, and natural history of infection*

Persistence of HCV infection occurs in the majority of HCV-infected individuals. HCV infection is often asymptomatic. Indeed, acute HCV infection, whether symptomatic or not, resolves spontaneously only in 10–40% of cases ([Poynard et al., 2003](#); [Afdhal, 2004](#)). Persistent HCV infection is characterized by the persistence of elevated aminotransferase levels and HCV RNA in serum. Serological distinction of chronic carriers is difficult. Chronically HCV-infected patients are in general asymptomatic, and some report nonspecific symptoms such as fatigue or abdominal discomfort. Approximately 15–27% of chronically infected patients are estimated to develop cirrhosis. The time to progression to

severe liver disease is highly variable. Factors that accelerate clinical progression include being of masculin gender, older at the age of infection, alcohol intake, and co-infection with HIV and/or HBV ([Lauer & Walker, 2001](#); [Perz et al., 2006](#); [Alter, 2007](#)).

### *1.2.4 Vaccination and viral treatment*

To date, an active or passive vaccination against HCV is not yet available. The main factor that hampers the development of an efficient vaccine is the considerable genetic heterogeneity of this positively-stranded RNA virus. However, better understanding of the natural immunity to HCV and the proof of vaccine efficacy in the chimpanzee challenge model allows some optimism about the development of an at least partly effective vaccine against this heterogeneous pathogen ([Houghton & Abrignani, 2005](#)).

In view of the fact that the natural course of chronic HCV infection is variable and that the currently established antiviral combination therapy with a pegylated interferon (PEG-IFN) and ribavirin (RBV) has numerous side-effects, the decision to treat or not to treat must be determined on an individual basis ([Poynard et al., 2003](#); [Afdhal, 2004](#); [Deutsch & Hadziyannis, 2008](#)). The main goal of antiviral therapy is a sustained virological response, defined as undetectable HCV RNA in serum 24 weeks after the end of treatment, determined with the most sensitive PCR technique. Treatment, regimens, and responsiveness vary depending of the HCV genotypes.

In patients infected with genotype 1 or 4, HCV eradication rates range between 45–52%. In contrast, in patients infected with HCV genotype 2 or 3, antiviral therapy results in HCV eradication in 75–90% of cases. Currently, several novel antiviral agents are being evaluated in individual studies, e.g. NS3–4A protease inhibitors, RNA-dependent RNA polymerase inhibitors, and different immune therapies ([Manns et al., 2007](#)).

## 2. Cancer in Humans

This section focuses on cohort and case-control studies published since the last *IARC Monograph* ([IARC, 1994](#)). Only those studies that used second- or third-generation assays to determine HCV antibody status are examined. If a measure of relative risk (RR) was not provided by the authors, the Working Group calculated a crude relative risk and 95% confidence intervals (CI). Only those studies in which a relative risk could be specifically estimated were included. Studies that focused specifically on the effect of the interaction of HCV and another factor (e.g. HBV) on the occurrence of hepatocellular carcinoma (HCC) are addressed in the relevant subsection.

### 2.1 Hepatocellular carcinoma

In the previous *IARC Monograph* ([IARC, 1994](#)), data on the relationship between infection with HCV, as indicated by the presence of antibodies to HCV (anti-HCV), were examined in three cohort studies and over 20 case-control studies. Seropositivity for HCV antibodies was measured by either first- or second-generation tests. An increased risk for HCC was apparent in all three cohort studies. Among the case-control studies, odds ratio (OR) estimates ranging from 1.3–134 were observed in 17 studies in which first-generation tests were used, and were statistically significant in 15 of the studies. In six case-control studies that used second-generation assays, the estimated odds ratios ranged from 1.1–52, and were significant in three studies. In the few case-control studies in which the analysis took into account possible confounding of the effects of HCV by other risk factors for HCC, such as smoking and alcohol consumption, the association with HCV was not materially altered.

#### 2.1.1 Cohort studies

Table 2.1 (available at <http://monographs.iarc.fr/ENG/Monographs/vol100B/100B-03-Table2.1.pdf>) provides a detailed summary of the cohort studies that investigated the association of HCV with the development of HCC, by geographic area. Cohort studies of patients with chronic liver disease/cirrhosis were excluded, due to the difficulties in interpreting the findings from such studies; this exclusion is analogous to that for case-control studies involving control groups comprising chronic liver disease patients.

Of the eight cohort studies considered, six were conducted in Asia and one each in the Americas and Australia. Of note, the cohort study conducted in Australia ([Amin et al., 2006](#)) involved persons included in the New South Wales Notifiable Diseases Database. An association between HCV seropositivity and HCC was observed in each of the eight cohort studies, with relative risks ranging from 2.5–88. The effect estimate was statistically significant in all but one study ([Yuan et al., 1995](#)). Potential confounding by risk factors for HCC, particularly infection with HBV, was not addressed in four of the studies ([Yuan et al., 1995](#); [Boschi-Pinto et al., 2000](#); [Mori et al., 2000](#); [Guiltinan et al., 2008](#)). The wide range in the relative risks reported most likely reflects variations across the study populations in the underlying prevalence of HCV, as well as in the duration of HCV infection. Only the study by [Mori et al. \(2000\)](#) examined the effect of anti-HCV titre and reported a stronger association for a high anti-HCV titre (RR, 40.4) than for a low antibody titre (RR, 3.4).

#### 2.1.2 Case-control studies

Details of the 18 case-control studies summarized can be found in Table 2.2 (available at <http://monographs.iarc.fr/ENG/Monographs/vol100B/100B-03-Table2.2.pdf>). A few studies were excluded based on the following criteria: less

than 20 HCC cases were included in the study; the control group was comprised of patients with chronic liver disease; or, information related to the comparability of case and control subjects with respect to age and sex was not provided.

Most of the case-control studies were performed either in Europe ( $n = 7$ ) or Asia ( $n = 6$ ). The results from all 18 studies, both hospital-based and population-based, support a carcinogenic role of HCV in the development of HCC. The adjusted odds ratios for anti-HCV seropositivity ranged from 2.8–170; eight studies reported a more than 20-fold increased risk of HCC ([Park et al., 1995](#); [Shin et al., 1996](#); [Tanaka et al., 1996](#); [Tsai et al., 1996](#); [De Vita et al., 1998](#); [Tagger et al., 1999](#); [Kuper et al., 2000](#); [Yuan et al., 2004](#)). Potential confounding by risk factors for HCC, particularly infection with HBV, was addressed in half of these studies (see Table 2.2 on-line). As with the cohort studies, the wide range in the observed odds ratios across the case-control studies is likely to be related to the underlying prevalence of HCV, and duration of infection. In those case-control studies in which the presence of anti-HCV and HCV RNA was determined, the observed association was stronger for positivity to both markers than for anti-HCV alone ([Kew et al., 1997](#); [Tagger et al., 1999](#); [Gelatti et al., 2005](#); [Franceschi et al., 2006a](#)).

### 2.1.3 Meta-analyses

Two meta-analyses have examined the interactive effect of HBV and HCV infections on the occurrence of HCC (see the *Monograph* on HBV in this volume) based on case-control studies (including case-control studies nested within cohorts). In both analyses, estimates of the overall association between HCV and HCC were also provided. [Donato et al. \(1998\)](#) calculated a summary odds ratio for 21 studies, published between 1992–97, using second-generation anti-HCV or HCV RNA assays. The overall odds ratio for HCV was 8.2 (95%CI: 6.7–9.9). The estimate

was higher for studies in areas where HBV infection is at low-to-intermediate endemicity and where HCV infection is predominant among HCC cases (Japan and Mediterranean countries; OR, 16.8; 95%CI: 11.9–24.1) than for studies in areas where HBV infection is highly endemic (sub-Saharan and southern Africa, Taiwan (China), China, Republic of Korea, Viet Nam; OR, 6.2; 95%CI: 4.9–7.8). The summary odds ratio was also slightly higher for studies with community controls (OR, 9.0; 95%CI: 7.0–11.6) than for those with hospital controls (OR, 6.8; 95%CI: 5.1–9.1). In a similar analysis of 32 case-control studies published in the Chinese literature, [Shi et al. \(2005\)](#) reported a summary odds ratio of 4.6 (95%CI: 3.6–5.9) for anti-HCV/HCV RNA positivity and HCC. The calculated odds ratio was somewhat larger in higher HCC incidence areas (OR, 5.3; 95%CI: 3.8–7.4) than in lower incidence areas (OR, 3.8; 95%CI: 2.8–5.2). There was little difference in the estimates based on studies with community controls or with hospital controls (OR, 4.7; 95%CI: 3.6–6.1 and OR, 4.4; 95%CI: 2.9–6.6, respectively).

### 2.1.4 HCV genotype

Table 2.3 (available at <http://monographs.iarc.fr/ENG/Monographs/vol100B/100B-03-Table2.3.pdf>) summarizes the results from four case-control studies and one community-based cohort study in which the effect of HCV genotype on HCC was examined. Two case-control studies in Japan reported significant increased odds ratios for HCV genotype 1b, compared to other genotypes, among persons infected with HCV ([Tanaka et al., 1996](#), [1998a](#)). A weaker crude association between Group 1 (1a, 1b) infection and HCC incidence was obtained from the nested case-control study of HCC conducted within the community-based Town C HCV Study ([Suruki et al., 2006](#)). The Brescia HCC study examined the HCV genotype in the HCV RNA-positive HCC cases and non-cancer patient controls

([Tagger et al., 1999](#)), and reported a larger effect size for genotype 1b infection (OR, 34.2) than for genotype 2 infection (OR, 14.4), relative to anti-HCV seronegatives. A comparison of HCV genotypes 1b to 2 yielded an adjusted odds ratio of 2.9 (95%CI: 0.9–10) ([Donato et al., 1997](#)). In contrast, an additional hospital-based study in Italy observed increased risks (27-fold) of HCC for both genotype 1 and genotype 2 infections, compared to anti-HCV seronegatives ([Franceschi et al., 2006a](#)).

Seven cohort studies ([Bruno et al., 1997, 2007](#); [Naoumov et al., 1997](#); [Niederau et al., 1998](#); [Tanaka et al., 1998b](#); [Ikeda et al., 2002](#); [Imazeki et al., 2003a](#); [Obika et al., 2008](#)) and two case-control studies ([Hatzakis et al., 1996](#); [Silini et al., 1996](#)) have examined the association between HCV genotype and HCC among patients with HCV-related liver disease. However, uncertainty about HCV treatment in these patients as well as potential bias related to their identification and inclusion as study subjects make the interpretation of the findings from such studies difficult.

### *2.1.5 Cofactors modifying the risk of HCV-associated HCC*

A brief summary of the findings from studies that examined potential modifiers of the effect of HCV on the development of HCC is provided below and in Table 2.4 (available at <http://monographs.iarc.fr/ENG/Monographs/vol100B/100B-03-Table2.4.pdf>). Those studies that evaluated the interaction of the factor with a combined HCV and/or HBV infection status were not considered.

#### *(a) Heavy alcohol consumption*

Alcohol consumption has been classified by IARC as a human carcinogen, with a role in the etiology of liver cancer ([IARC, 1988, 2010](#)). A positive interaction, on the additive scale, between HCV and habitual alcohol drinking was observed in a community-based cohort study

in Japan ([Mori et al., 2000](#)); of note, only crude relative risks could be calculated for this study. [Sun et al. \(2003\)](#) also investigated the potential synergy of HCV infection with habitual alcohol drinking in their cohort study in Taiwan, China ([Sun et al., 2003](#); [Wang et al., 2003](#)), and no interaction was observed in this low (20% prevalence) alcohol consumption population. The synergy index obtained suggested that the combined effect of HCV and habitual alcohol drinking was not more than the additive effect of either factor alone. A positive, more than additive, interaction between HCV and heavy alcohol consumption was reported in the hospital-based Brescia HCC case-control study in Italy ([Donato et al., 2002](#)). However, because alcohol-induced cirrhosis may alter alcohol consumption before the development of HCC, the evaluation of alcohol in case-control studies can be problematic.

#### *(b) Smoking*

IARC has also identified tobacco smoking as a liver carcinogen ([IARC, 2004](#)). In the cohort studies in Japan ([Mori et al., 2000](#)) and Taiwan, China ([Sun et al., 2003](#)), a more than additive effect of anti-HCV seropositivity and cigarette smoking on HCC incidence was suggested; as noted in the alcohol subsection above, no adjustment for potential confounders was performed in the Japanese study.

#### *(c) Diabetes mellitus*

A meta-analysis of 26 studies (13 case-control studies and 13 cohort studies) published through February 2005 indicated that diabetes mellitus appears to be an independent risk factor for HCC ([El-Serag et al., 2006](#)). However, no clear evidence with respect to a possible interaction between diabetes and HCV infection on the risk of HCC was found based on a Surveillance, Epidemiology and End Results(SEER)-Medicare database case-control study of HCC in the United States of America ([Davila et al., 2005](#)),

and a community-based cohort study in Taiwan, China ([Lai et al., 2006](#)).

(d) *Betel quid chewing*

Two studies in Taiwan, China, examined the interaction between HCV and habitual betel quid chewing ([Sun et al., 2003](#); [Tsai et al., 2001](#)). The study by [Sun et al. \(2003\)](#) resulted in a synergy index of 4.2, suggestive of a greater than additive increased risk of HCC related to the combined effect of HCV infection and betel quid chewing than of either factor alone. The interaction appeared to be weaker in the study by [Tsai et al. \(2001\)](#), with a synergy index of 1.66; the reported odds ratios were not adjusted for potential confounders.

(e) *Human T-lymphotropic virus type 1 infection*

A more than additive effect of human T-lymphotropic virus type 1 (HTLV-1) co-infection on the association of HCV with liver cancer death was observed (RR, 21.9 for HCV-positive/HTLV-1-positive) in the Miyazaki Cohort Study of an HTLV-1 endemic population in Japan ([Boschi-Pinto et al., 2000](#)).

(f) *Radiation exposure*

The Radiation Effects Research Foundation Life Span Study by [Sharp et al. \(2003\)](#) reported a greater than multiplicative interaction for the combined effect of HCV infection and radiation exposure in both the second (OR, 55.1) and third (OR, 28.7) tertiles.

(g) *Schistosoma infection*

A case-control study conducted at the National Cancer Institute in Egypt investigated the interaction between infection with HCV and infection with *Schistosoma mansoni* among the subjects negative for HBsAg ([Hassan et al., 2001](#)); however, no meaningful interaction was observed.

(h) *Helicobacter pylori infection*

It has been hypothesized that *Helicobacter* species could play a role in the development of HCC ([Pellicano et al., 2008](#)). Several cross-sectional studies have detected the presence of *Helicobacter* in HCV-infected HCC cases ([Ponzetto et al., 2000](#); [Pellicano et al., 2004](#); [Rocha et al., 2005](#)).

## 2.1.6 Co-infection HCV/HBV

See the *Monograph* on HBV in this volume.

## 2.2 Cancers other than hepatocellular carcinoma

### 2.2.1 Biliary tract/gallbladder

(a) *Cohort studies*

The cohort study in Australia ([Amin et al., 2006](#)), included in Table 2.1 (on-line), also investigated the effect of monoinfection with HCV on the incidence of gallbladder cancer; however, no increased rate of the malignancy was observed (standardized incidence ratio [SIR], 0.5; 95%CI: 0.1–2.0; based on 2 newly reported cases).

(b) *Case-control studies*

Table 2.5 (available at <http://monographs.iarc.fr/ENG/Monographs/vol100B/100B-03-Table2.5.pdf>) summarizes the seven case-control studies that have examined the association of HCV with cholangiocarcinoma and biliary tract cancers, five of which were performed in Asian countries (Republic of Korea, Japan, and China).

In the Republic of Korea, a hospital-based study of 203 HCC cases (summarized in Table 2.2 on-line) also included 41 cases of cholangiocarcinoma, without distinction of site, and observed a positive association (OR, 3.9; 95%CI: 0.9–17.1) of anti-HCV seropositivity with that malignancy ([Shin et al., 1996](#)). Five case-control studies provided results with respect to the association

between anti-HCV seropositivity and intrahepatic cholangiocarcinoma. Statistically significant odds ratios were observed in three of those studies ([Donato et al., 2001](#); [Yamamoto et al., 2004](#); [Shaib et al., 2007](#)), with a more than 5-fold increased risk of intrahepatic cholangiocarcinoma related to HCV. The largest study ([Lee et al., 2008](#)), which included 622 cases in the Republic of Korea, did not find any association between anti-HCV positivity and intrahepatic cholangiocarcinoma (OR, 1.0; 95%CI: 0.5–1.9); of note, the reported odds ratio was not adjusted for any potential confounders. One study also included cases of extrahepatic cholangiocarcinoma ([Shaib et al., 2007](#)), but the association between anti-HCV and this subtype was weaker, and not statistically significant. Not presented in Table 2.5 (on-line) is a case-control study based on the SEER cancer registry and Medicare claims data, which determined HCV infection status and case status using ICD-9 diagnostic codes ([Welzel et al., 2007](#)). In that study as well, the effect was more pronounced for intrahepatic cholangiocarcinoma (adjusted OR, 4.4; 95%CI: 1.4–14.0;  $n = 535$  cases) than for extrahepatic cholangiocarcinoma (adjusted OR, 1.5; 95%CI: 0.2–11.0;  $n = 549$  cases).

[Hsing et al. \(2008\)](#) conducted a case-control study of incident biliary tract cancers in Shanghai. An age-adjusted increased risk associated with anti-HCV seropositivity was not observed for cancer of either the gallbladder, the extrahepatic bile ducts, or the ampulla of Vater.

## 2.2.2 Lymphoid malignancies

### (a) Cohort studies

Table 2.6 (available at: <http://monographs.iarc.fr/ENG/Monographs/vol100B/100B-03-Table2.6.pdf>) includes detailed descriptions of seven cohort studies that examined the effect of HCV infection on the occurrence of lymphoid malignancies, by the HIV status of the subjects. In the five studies of HIV-negative subjects,

three found an approximately 2-fold excess risk of either non-Hodgkin lymphoma generally or B-cell non-Hodgkin lymphoma, specifically among individuals who were infected with HCV ([Ohsawa et al., 1999](#); [Duberg et al., 2005](#); [Ulcickas Yood et al., 2007](#)). Due to the small number of cases that occurred in all three cohorts, the association was statistically significant only in the Swedish study of B-cell non-Hodgkin lymphoma ([Duberg et al., 2005](#)). The Swedish study also observed a similar, but statistically non-significant, effect of HCV infection on the development of the chronic lymphocytic leukaemia subtype as well as multiple myeloma. A nested case-control study within a cohort of parents and offspring in the USA did not detect HCV in any of the B-cell non-Hodgkin lymphoma, multiple myeloma, or Hodgkin disease cases or the matched controls ([Rabkin et al., 2002](#)). A cohort study in Australia also reported no association of HCV with non-Hodgkin lymphoma, nor with subtypes such as follicular lymphoma, diffuse non-Hodgkin lymphoma, and T-cell non-Hodgkin lymphoma ([Amin et al., 2006](#)); in addition, an increased risk was not found for either multiple myeloma or Hodgkin disease. Not included in Table 2.6 is a cohort study from the USA, which used the Department of Veterans Affairs administrative patient databases with HCV status, and the occurrence of lymphoid malignancies based on ICD-9 CM diagnostic codes ([Giordano et al., 2007](#)). The adjusted relative risks were 1.28 (95%CI: 1.12–1.45) for non-Hodgkin lymphoma ( $n = 1359$  cases), 0.89 (95%CI: 0.68–1.15) for chronic lymphocytic leukaemia ( $n = 412$  cases), 0.95 (95%CI: 0.76–1.19) for multiple myeloma ( $n = 526$  cases), and 0.97 (95%CI: 0.74–1.27) for Hodgkin disease ( $n = 360$  cases).

For both cohort studies of HIV-positive subjects, a null association was observed between anti-HCV seropositivity and non-Hodgkin lymphoma ([Waters et al., 2005](#); [Franceschi et al., 2006b](#)).

### (b) Case-control studies

This section summarizes the case-control studies included in the meta-analyses by [Matsuo et al. \(2004\)](#) and [Dal Maso & Franceschi \(2006\)](#). Other case-control studies were considered if: at least 20 cases of lymphoma were investigated in the study; the control group was not comprised of patients with other haematological malignancies and/or lymphoproliferative diseases; and, information related to the comparability of case and control subjects with respect to age and sex was provided.

Results related to a particular non-Hodgkin lymphoma subtype are included when data were provided for  $\geq 20$  cases. In addition to B-cell non-Hodgkin lymphoma, the subtypes evaluated were T-cell non-Hodgkin lymphoma, diffuse large B-cell lymphoma (DLBCL), follicular lymphoma, marginal zone lymphoma, and chronic lymphocytic leukaemia/small lymphocytic lymphoma (CLL/SLL).

A large number of case-control studies have examined the association between HCV infection and non-Hodgkin lymphoma. The detailed description of these studies and their findings, by geographic area, are provided in Table 2.7 (available at <http://monographs.iarc.fr/ENG/Monographs/vol100B/100B-03-Table2.7.pdf>). Most studies were conducted in Europe ( $n = 20$ ), followed by the Americas ( $n = 8$ ), and Asia ( $n = 7$ ); one study each was performed in Africa and Australia. B-cell non-Hodgkin lymphoma specifically and/or non-Hodgkin lymphoma generally was the primary malignancy of interest in all 37 case-control studies. Approximately 80% of the studies reported at least a 2-fold increased risk of non-Hodgkin lymphoma or a B-cell non-Hodgkin lymphoma with HCV seropositivity. The association was evident in case-control studies conducted across all geographic areas (except for the study in Australia), and using different sources of control groups (i.e. hospital-based, population-based,

or blood donors). Odds ratios greater than 5.0 were observed in 13 studies: nine studies were from Europe ([Ferri et al., 1994](#); [Mazzaro et al., 1996](#); [Musto et al., 1996](#); [De Rosa et al., 1997](#); [De Vita et al., 1998](#); [Paydas et al., 1999](#); [Vallisa et al., 1999](#); [Zucca et al., 2000](#); [Yenice et al., 2003](#)), and four studies were from elsewhere ([Zuckerman et al., 1997](#); [Harakati et al., 2000](#); [Kuniyoshi et al., 2001](#); [Chindamo et al., 2002](#)). An additional 16 case-control studies reported a 2–4-fold excess risk of either non-Hodgkin lymphoma or B-cell non-Hodgkin lymphoma associated with HCV infection; the effect was statistically significant in more than half ([Silvestri et al., 1996](#); [Mizorogi et al., 2000](#); [Montella et al., 2001b](#); [Imai et al., 2002](#); [Mele et al., 2003](#); [Cowgill et al., 2004](#); [Engels et al., 2004](#); [Iwata et al., 2004](#); [Talamini et al., 2004](#); [Schöllkopf et al., 2008](#); [Spinelli et al., 2008](#)). The remaining eight case-control studies with odds ratios less than 2 did not demonstrate a statistically significant effect of HCV on the occurrence of non-Hodgkin lymphoma or B-cell non-Hodgkin lymphoma ([Kaya et al., 2002](#); [Avilés et al., 2003](#); [Morgensztern et al., 2004](#); [de Sanjose et al., 2004](#); [Sève et al., 2004](#); [Vajdic et al., 2006](#); [Sonmez et al., 2007](#); [Park et al., 2008](#)). Not included in Table 2.7 is the SMAHRT (SEER-Medicare Assessment of Hepatopoietic Malignancy Risk Traits) study, a case-control study of haematopoietic malignancies based on the use of the SEER-Medicare data in the USA ([Anderson et al., 2008](#)). Cancer diagnosis as well as HCV status was obtained from the ICD codes included in the two databases. The adjusted odds ratio for the association of HCV with non-Hodgkin lymphoma overall ( $n = 33940$  cases) was 1.35 (95%CI: 1.1–1.7).

The findings of two pooled analyses are also included in Table 2.7. One pooled study reported an overall odds ratio of 1.5 (95%CI: 0.95–2.2) for B-cell lymphoma, based on 1465 cases from five European countries ([Nieters et al., 2006](#)). The other pooled study found a similar association (OR, 1.8; 95%CI: 1.4–2.3) for all non-Hodgkin

lymphoma, using 4784 cases from seven studies involving centres in the USA, Canada, Australia, and Europe ([de Sanjose et al., 2008](#)). The latter analysis included the results from five previously published studies ([Engels et al., 2004](#); [Morton et al., 2004](#); [Talamini et al., 2004](#); [Nieters et al., 2006](#); [Vajdic et al., 2006](#)).

In two case-control studies of non-Hodgkin lymphoma, a slightly stronger association was found for low-grade B-cell non-Hodgkin lymphoma, in contrast to intermediate/high-grade disease ([Engels et al., 2004](#); [Talamini et al., 2004](#)). Based on an *a priori* hypothesis that HCV would be related to non-Hodgkin lymphoma at the potential target organ of infection, an early study in Italy examined the effect of HCV on liver/salivary gland non-Hodgkin lymphoma, and reported a marked elevation in risk (OR, 51.5) ([De Vita et al., 1998](#)).

With respect to other subtypes of non-Hodgkin lymphoma, an effect of HCV infection has less consistently been observed. Moreover, when an association is suggested, the effect estimate usually is highly unstable, due to the small number of cases involved. From the results of the studies included in Table 2.7 (on-line), an association with HCV seems more evident for the DLBCL and CLL/SLL subtypes. For DLBCL, results were available from 14 case-control studies; 13 studies observed an association consistent with an effect of HCV seropositivity on DLBCL, which was significant for about half ([Silvestri et al., 1996](#); [Vallisa et al., 1999](#); [Zucca et al., 2000](#); [Chindamo et al., 2002](#); [Mele et al., 2003](#); [Talamini et al., 2004](#); [Spinelli et al., 2008](#)). Eight of the 12 studies that examined CLL/SLL reported an odds ratio of at least 2, with the association being statistically significant in three case-control studies ([Musto et al., 1996](#); [De Rosa et al., 1997](#); [Paydas et al., 1999](#)). For the fewer number of studies ( $n = 10$ ) that included separate odds ratios for follicular lymphoma and marginal zone lymphoma, slightly more than half reported an odds ratio greater than 2 for HCV seropositivity ([Silvestri](#)

[et al., 1996](#); [Vallisa et al., 1999](#); [Zucca et al., 2000](#); [Mele et al., 2003](#); [Engels et al., 2004](#); [Spinelli et al., 2008](#)). In contrast, the findings from almost all studies did not support a notable effect of HCV on T-cell non-Hodgkin lymphoma. The SMAHRT study reported the following odds ratios for non-Hodgkin lymphoma subtypes: 1.5 (95%CI: 1.05–2.2) for DLBCL ( $n = 10144$  cases); 2.2 (95%CI: 1.2–3.95) for marginal zone lymphoma ( $n = 1908$  cases); 1.9 (95%CI: 1.2–3.0) for follicular lymphoma ( $n = 4491$  cases); 1.1 (95%CI: 0.70–1.7) for chronic lymphocytic lymphoma ( $n = 10170$  cases); and 0.42 (95%CI: 0.10–1.7) for T-cell non-Hodgkin lymphoma ( $n = 1870$  cases) ([Anderson et al., 2008](#)). In a pooled analysis ([de Sanjose et al., 2008](#)), a statistically significant association was found for DLBCL (OR, 2.2; 95%CI: 1.7–3.0) and marginal zone lymphoma (OR, 2.5; 95%CI: 1.4–4.2). The other pooled study ([Nieters et al., 2006](#)) also reported a significant association for DLBCL (OR, 2.2; 95%CI: 1.2–3.9), but no association was reported for marginal zone lymphoma. The effect of HCV was weaker and not statistically significant for CLL/SLL and not apparent for follicular lymphoma or T-cell lymphoma in both pooled studies.

Of eight case-control studies that examined multiple myeloma (Table 2.7 on-line), four studies observed a statistically significant association between HCV seropositivity and the malignancy ([Musto et al., 1996](#); [De Rosa et al., 1997](#); [Paydas et al., 1999](#); [Montella et al., 2001a, b](#)). The pooled analysis by the EPILYMPH group ([Nieters et al., 2006](#)) did not report a notable association for multiple myeloma (OR, 1.4; 95%CI: 0.61–3.2).

More than ten of the case-control studies included in Table 2.7 (on-line) conducted separate analyses of the effect of HCV on the occurrence of Hodgkin disease. An increased risk was observed in eight case-control studies, although it was significant only in one study ([Paydas et al., 1999](#)). The US record-based study by [Anderson et al. \(2008\)](#) found an odds ratio of 1.2 (95%CI: 0.38–3.7) based on 1155 Hodgkin disease cases.

In the pooled analysis by [Nieters et al. \(2006\)](#), no effect of HCV on Hodgkin disease was found (OR, 0.97; 95%CI: 0.27–3.5).

#### (c) Meta-analyses

Several meta-analyses of the relationship between HCV and lymphoma have been published ([Gisbert et al., 2003](#); [Matsuo et al., 2004](#); [Negri et al., 2004](#); [Dal Maso & Franceschi, 2006](#)). [Matsuo et al. \(2004\)](#) performed a meta-analysis of 23 case-control studies (including nested case-control studies) published between January 1990 and August 2003. Studies were included if an odds ratio or a relative risk was calculated “by comparing the HCV-positive category to the negative category”, and non-cancer controls and a second- or third-generation HCV antibody test were used. A summary odds ratio of 5.7 (95%CI: 4.1–8.0) for non-Hodgkin lymphoma was obtained; the association was somewhat stronger for B-cell non-Hodgkin lymphoma (OR, 5.0; 95%CI: 3.6–7.1) than for T-cell non-Hodgkin lymphoma (OR, 2.5; 95%CI: 1.4–4.6). The variation was related to the use of blood donor controls (OR: 8.4 versus OR: 4.65 for non-blood donor controls), and to the year of publication (i.e. lower OR for the more recent publications). [The Working Group noted that the relative risks obtained in this meta-analysis might be inflated by underadjustment, particularly by age group.]

In their meta-analysis, [Dal Maso & Franceschi \(2006\)](#) examined 15 case-control studies and three cohort studies published up to July 2006. Studies were eligible for inclusion based on the following criteria: if the control group did not include patients with other lymphoproliferative diseases, if cases and controls were comparable with respect to age and sex or age and sex were taken into account in the analysis, if second- or third-generation anti-HCV assays were used, and if HIV-positive subjects were excluded. Only case-control studies with  $\geq 100$  cases were included in the overall analyses of non-Hodgkin

lymphoma; findings regarding specific non-Hodgkin lymphoma subtypes and other lymphoid malignancies were shown when there were  $\geq 20$  cases for that subtype. The sex- and age-adjusted meta-relative risk for all non-Hodgkin lymphoma was 2.5 (95%CI: 2.1–3.0), and was similar for case-control (RR, 2.5; 95%CI: 2.1–3.1) and cohort (RR, 2.0; 95%CI: 1.8–2.2) studies. The effect of HCV on non-Hodgkin lymphoma was estimated to be higher in those studies with a higher HCV prevalence ( $\geq 5\%$ ) among control subjects (RR, 3.0; 95%CI: 2.4–3.75) than those studies with a lower prevalence (RR, 1.9; 95%CI: 1.8–2.05). The results were relatively consistent by geographic area (RR, 2.7 in southern Europe; RR, 2.6 in the USA; RR, 2.1 in the Republic of Korea/Japan; RR, 2.3 in other areas) and year of publication (RR, 2.9 before 2003; RR, 2.2 during 2003 and after). However, the association appeared to be somewhat weaker in studies using population-based control subjects (RR, 1.9) than in those using hospital-based controls (RR, 2.7). With respect to non-Hodgkin lymphoma subtypes, the relative risks obtained were: 2.65 (95%CI: 1.9–3.7) for DLBCL; 2.7 (95%CI: 2.2–3.4) for follicular lymphoma; 3.4 (95%CI: 2.4–4.9) for marginal zone; 1.65 (95%CI: 1.35–2.0) for CLL/SLL; and 1.5 (95%CI: 1.13–2.05) for T-cell non-Hodgkin lymphoma. In addition, a summary relative risk was estimated for multiple myeloma (RR, 1.6; 95%CI: 0.69–3.6) as well as for Hodgkin disease (RR, 1.5; 95%CI: 1.0–2.1). Of note, the meta-analysis did not include the results from two recently conducted, large case-control studies of lymphoid malignancies: the nationwide study of the Danish and Swedish populations by [Schöllkopf et al. \(2008\)](#), and the pooled InterLymph study by [de Sanjosé et al. \(2008\)](#).

#### (d) Treatment of HCV infection in patients with lymphoma

In a striking finding, [Hermine et al. \(2002\)](#) reported remission in seven of nine patients with splenic lymphoma with villous lymphocytes who

were infected with HCV after they were treated with interferon  $\alpha$ ; two more patients without a substantial antiviral response experienced remission after the addition of ribavirin to interferon  $\alpha$ . None of six HCV-negative patients with splenic lymphoma with villous lymphocytes responded to interferon treatment. A systematic review by [Gisbert et al. \(2005\)](#) reported on the findings of 16 case reports and case series in which a total of 65 HCV-infected patients with lymphoproliferative disorders were treated with interferon  $\alpha$  (with and without ribavirin). Of the 65 patients in those studies, complete remission of the disorder was achieved in 75% (95%CI: 64–84) of the cases. [The Working Group noted that one case series contributed almost a third of the patients analysed ( $n = 20$ ); however, the reference was to an abstract, for which a full paper has not been published.]

### 2.2.3 Other cancers

#### (a) Leukaemias

In the large cohort study of US veterans who were infected with HCV, a diagnosis of HCV was not related to an increased rate of acute lymphocytic leukaemia (RR, 0.75), chronic myeloid leukaemia (RR, 0.84), or acute non-lymphocytic leukaemia (RR, 1.04) ([Giordano et al., 2007](#)).

Table 2.8 (available at <http://monographs.iarc.fr/ENG/Monographs/vol100B/100B-03-Table2.8.pdf>) summarizes the four case-control studies that investigated the effect of HCV infection on the development of leukaemia. No study reported statistically significant associations between anti-HCV seropositivity and acute myeloid leukaemia, acutelymphocytic leukaemia, or chronic myeloid leukaemia. However, [Bianco et al. \(2004\)](#) suggested that HCV infection might be associated with both acute myeloid leukaemia and chronic myeloid leukaemia in the Italian study. In Japan, [Murashige et al. \(2005\)](#) reported no association of anti-HCV seropositivity with the risk of myeloid malignancy.

#### (b) Cancer of the thyroid

The three cohort studies in Sweden ([Duberg et al., 2005](#)), Australia ([Amin et al., 2006](#)), and the USA ([Giordano et al., 2007](#)) examined the incidence of thyroid cancers among people who were infected with HCV. A non-significant increased risk was found based on five thyroid cancer cases in the Swedish cohort (SIR, 1.55; 95%CI: 0.50–3.6); whereas a significant decreased risk was reported based on nine cases in the Australian cohort (SIR, 0.3; 95%CI: 0.2–0.7), and 46 cases in the US cohort (HR, 0.72; 95%CI: 0.52–0.99). A case-control study of a group of cancers in Italy ([Montella et al., 2001a](#)) observed a significant association between HCV infection and this malignancy (OR, 2.8; 95%CI: 1.2–6.3).

## 3. Cancer in Experimental Animals

In this Volume, the Working Group decided not to include a separate section on “Cancer in Experimental Animals” in the *Monographs* on viruses but rather to include description of such studies under Section 4 (below). The reasoning for this decision is explained in the General Remarks.

## 4. Other Relevant Data

The sharp increase of HCC cases over the last several decades in industrialized countries (western Europe, North America, and Japan) has been attributed to an expansion of chronic HCV infections, and is frequently linked with liver cirrhosis. Most cohort studies and clinical experience have shown that liver fibrosis resulting from long-lasting chronic inflammation is likely to be an important predisposing factor of HCC development. The same clinical observations also suggest that ongoing liver regeneration resulting from chronic immune mediated hepatocyte

death is a likely factor contributing to the development of HCC ([Liang & Heller, 2004](#)). This aspect has been developed in the *Monograph* on HBV in this volume.

But the mechanisms underlying the progression of HCV infection to liver cancer, which often takes many decades, remain ill-defined. Transcriptomics and proteomics have helped to identify many genetic and epigenetic alterations associated with HCC clusters. However, the identified changes to gene expression patterns are very heterogeneous in tumour cells, raising the question as to whether yet unidentified, specific changes at early, preneoplastic stages trigger the transformation process, and what cell type could be at the origin of HCC ([Sell & Leffert, 2008](#)). HCV has an exclusively cytoplasmic life cycle ([Lindenbach et al., 2007](#); [Moradpour et al., 2007](#)), and therefore, HCV replication and potentially pro-oncogenic events are restricted to the cytoplasm. While HCV infection leads to chronic inflammation, steatosis, fibrosis and oxidative chromosomal DNA damage, several HCV proteins have been shown to have direct oncogenic effects, and to upregulate mitogenic processes ([Koike, 2007](#); [McGivern & Lemon, 2009](#)). The accumulation of DNA damage within a setting of restricted cell-cycle checkpoint controls is thought to compromise gene and chromosome stability, and to form the genomic basis for the malignant phenotype.

One of the major challenges with the study of the carcinogenic role of HCV is the difficulty to localize the HCV genome and viral proteins in both the liver of infected patients and liver tumours.

## 4.1 Biochemical properties of HCV proteins

See [Table 4.1](#).

### 4.1.1 The structural proteins

#### (a) Core protein

The core protein is the most conserved among the structural proteins. It is highly basic, rich in proline, and multimerizes. Interaction of the mature core with the 5' and 3' NCRs can, respectively, inhibit IRES function, and mediate genome dimerization ([Shimoike et al., 1999](#); [Cristofari et al., 2004](#)). The core protein is targeted to the cytoplasmic surface of the endoplasmic reticulum and lipid droplets ([Moradpour et al., 1996](#); [Barba et al., 1997](#)). Its interaction with lipid droplets may be related to the increased incidence of liver steatosis in patients with HCV, and in certain transgenic mice that overexpress HCV core ([Moriya et al., 1997a](#); [Barbaro et al., 1999](#)). It has also been shown that core can localize to mitochondrial outer membranes ([Schwer et al., 2004](#)).

#### (b) HCV glycoproteins

The HCV glycoproteins, E1 (30 kDa) and E2 (70 kDa), are type I transmembrane glycoproteins that associate into non-covalently attached heterodimers, and mediate HCV cell entry and membrane fusion ([Bartosch & Cosset, 2006](#); [Lavillette et al., 2006](#); [Lindenbach et al., 2007](#)).

### 4.1.2 The non-structural proteins

#### (a) p7

p7 is a small 7 kDa hydrophobic protein predicted to span the membrane twice, with endoplasmic-reticulum-lumenal N- and C-termini and a short, positively charged cytoplasmic loop. The localization of p7 is not yet clear, but overexpression of an epitope-tagged p7 has been localized to the endoplasmic reticulum and mitochondria ([Griffin et al., 2005](#)). The p7 protein is essential for virus assembly and release *in vitro* ([Steinmann et al., 2007](#)), and infectivity *in vivo* ([Sakai et al., 2003](#)).

**Table 4.1 HCV proteins, their role in the viral life cycle, and putative role in hepatocyte transformation**

HCV proteins	Role in viral life cycle	Potential role in cellular transformation (examples)
Core protein	Nucleocapsid assembly	Insulin resistance/steatosis Interference (direct or indirect) with p53, p73, pRb Interference with host cell signalling (NF-κB, Wnt/β-catenin pathway) Interference with TGF-β signalling Transcriptional activation of cellular genes Apoptosis
E1/E2 glycoprotein	Virus morphogenesis  Cell entry	Interference with the interferon-inducible protein kinase (PKR) activity
p7	Virus assembly, export and infectivity	
NS2	Polyprotein processing and Viral assembly	Inhibition of apoptosis
NS3 N-terminal domain	Serine protease activity	Interference with hepatocyte innate response
NS3 C-terminal domain	Helicase activity HCV genome replication	
NS4A	Co-factor of NS4B and NS5A	Induction of ER stress
NS4B	Formation of membranous web structures	
NS5A	Part of the replication complex	Inhibition of the interferon-inducible PKR Oxydative stress Activation of cell signalling pathways (STAT-3, NF-κB etc) Accumulation of β-catenin by indirect mechanism
NS5B	RNA-dependent RNA polymerase	

ER, endoplasmic reticulum; PKR, double-stranded RNA-activated protein kinase  
Compiled by the Working Group

### (b) NS2

NS2 is a 23 kDa membrane-spanning protein with a C-terminal cysteine protease activity that cleaves the NS2/3 junction ([Grakoui et al., 1993](#); [Lorenz et al., 2006](#)). NS2 is furthermore required for viral assembly. Its N-terminal domain may interact with the structural proteins and p7, whereas downstream sequences may interact with other NS proteins ([Lindenbach et al., 2007](#); [Jirasko et al., 2008](#)).

### (c) NS3

HCV NS3 is a 70 kDa multifunctional protein, containing an N-terminal serine protease domain and a C-terminal RNA helicase/NTPase domain that unwinds double-stranded nucleic

acids ([Lindenbach et al., 2007](#)). Although the precise role of the NS3 helicase is not yet known, helicase activity has been shown to be essential for HCV RNA replication and viral infectivity ([Lam & Frick, 2006](#)).

### (d) NS4A

NS4A is an 8 kDa protein with multiple functions in the virus life cycle. It is a cofactor that assists in the correct folding of the serine protease, and facilitates recognition of RNA substrates by the NS3 protease/helicase ([Pang et al., 2002](#)). NS4A can physically interact with NS4B and NS5A and uncleaved NS4B-5A to promote NS5A hyperphosphorylation ([Lindenbach et al., 2007](#); and references therein).

(e) NS4B

NS4B is a 27 kDa integral membrane protein containing four central transmembrane domains with yet unclear topology. Its expression is sufficient to induce the formation of ‘membraneous web’ structures that contain the membrane-bound replication complex ([Egger et al., 2002](#); [Gosert et al., 2003](#)), and it encodes a GTPase activity that seems to be critical for RNA replication ([Einav et al., 2004](#)).

(f) NS5A

NS5A is a 58 kDa phosphoprotein with an important yet unclear role in RNA replication ([Shimakami et al., 2004](#)). It localizes to active replication complexes ([Gosert et al., 2003](#); [Moradpour et al., 2004](#)), interacts with NS5B and inhibits its RNA polymerase activity ([Shirota et al., 2002](#); [Dimitrova et al., 2003](#)).

(g) NS5B

NS5B is a 68 kDa endoplasmic-reticulum-membrane-associated protein with RNA-dependent polymerase activity. Mutations that interfere with its membrane association destroy RNA replication. Intramolecular interactions as well as oligomerization of NS5B stimulate RNA synthesis, and the NS3 helicase enhances primed RNA synthesis activity in contrast to NS4B and NS5A, which inhibit RNA synthesis ([Lindenbach et al., 2007](#); and references therein). NS5B has been and remains a major target for the development of HCV-specific drugs; at the time of writing, drug research and development is focusing on cellular cofactors of NS5B, the cyclophilins. The function of NS5B has been shown to be upregulated by cyclophilin B, which in turn is regulated, and thus sensitive to the immunosuppressant ciclosporin A. Compounds belonging to this family are currently investigated for their antiviral efficacy ([Watashi et al., 2005](#); [Watashi & Shimotohno, 2007](#)).

## 4.2 Biological properties of HCV proteins

See [Table 4.1](#).

[The Working Group noted that besides the complex interactions among themselves, the viral proteins interact with a significant number of host factors and signalling pathways that may contribute to the pathological consequences of HCV infection. These interactions interfere with innate immunity and thus contribute to persistence of infection and inflammation; but they have also been described to modulate transcription, translation and post-translational events, to alter cell signalling, apoptosis, membrane physiology and trafficking. Furthermore, they can induce oxidative stress, genomic instability and possibly cellular transformation.

Many studies of the potential role of viral proteins in hepatocyte transformation have been performed in experimental models that are based on the overexpression of viral proteins after transient transfection of already transformed hepatocytes (such as HepG2 or Huh7 cells). These studies show the interaction of viral proteins with cellular partners that may be involved in cellular transformation. However, because the expression of these viral proteins has been difficult to demonstrate in liver tumours, a link between these *in vitro* observations and their *in vivo* relevance in infected humans still needs to be established. Because of the lack of relevant models for mechanistic studies of HCV-induced HCC, the results of the major molecular studies have been described below to provide an overview of the current hypotheses.]

Of the HCV gene products core, NS3, NS4B and NS5A have all been shown to exhibit transformation potential when transiently or stably expressed in tissue culture, or in the context of transgenic mice carrying the single viral proteins or an HCV polyprotein ([Sakamuro et al., 1995](#); [Ray et al., 1996](#); [Gale et al., 1999](#); [Park et al., 2000](#)).

However, many of the data below need to be substantiated in the context of a viral infection.

#### 4.2.1 The structural proteins

##### (a) Core protein

Core has been implied in changes of host cell signalling, transcriptional activation, apoptosis, lipid metabolism, and transformation. Among an impressive list of interactions with cellular factors, core has been shown to physically and functionally interact with p53 ([Ray et al., 1997](#); [Lu et al., 1999](#)), and p73 ([Alisi et al., 2003](#)), and to decrease the expression of pRb ([Cho et al., 2001](#)) tumour-suppressor proteins. For instance, it was shown that HCV core co-immunoprecipitates with p73 in HepG2 and SAOS-2 cells. This interaction results in the nuclear translocation of HCV core protein. In addition, the interaction with HCV core protein prevents p73- $\alpha$ , but not p73- $\beta$ -dependent cell growth arrest in a p53-dependent manner. The results suggested that HCV core protein may directly influence the various p73 functions, thus playing a role in HCV pathogenesis ([Alisi et al., 2003](#)).

Core also modulates the expression of the cyclin-dependent kinase (CDK) inhibitor p21/Waf in a p53-independent manner ([Kwun & Jang, 2003](#)). p21, a well known transcriptional target of p53, blocks activities of cyclin/CDK complexes involved in cell-cycle control and tumour formation.

Core induces activation of the Raf1/mitogen-activated protein kinase (MAPK) pathway ([Aoki et al., 2000](#); [Hayashi et al., 2000](#)), relieves cells from serum starvation and growth arrest, and favours cell proliferation.

Conflicting reports have shown both activation ([Ray et al., 2002](#)) and repression ([Joo et al., 2005](#)) of the NF- $\kappa$ B pathways by HCV core.

HCV core has been shown to activate the Wnt/ $\beta$ -catenin pathway, which is implicated in cell proliferation, DNA synthesis, and cell-cycle progression ([Fukutomi et al., 2005](#)).

Furthermore, core variants isolated from liver tumours, but not those isolated from adjacent non-tumourous liver, have been shown to interact with Smad3 and inhibit the TGF- $\beta$  pathway ([Pavio et al., 2005](#)). TGF- $\beta$ -signalling not only controls cell proliferation, differentiation and apoptosis but also stimulates liver regeneration and fibrogenesis through its actions on the extracellular matrix. TGF- $\beta$  levels are frequently increased in chronic HCV patients and correlate with the degree of fibrosis ([Nelson et al., 1997](#); [Marcellin et al., 2002](#)).

Finally, HCV core protein associates with cellular membranes ([Barba et al., 1997](#); [Moriya et al., 1997a](#)) and lipid vesicles ([Moriya et al., 1997a](#)), binds to apolipoprotein II, and reduces microsomal triglyceride transfer protein (MTP) activity ([Perlemuter et al., 2002](#)), leading to defects in the assembly and secretion of vLDL and steatosis, which in turn induces oxidative stress. The *in vivo* relevance of this interaction is supported by the development of steatosis ([Moriya et al., 1997b](#); [Perlemuter et al., 2002](#)) and liver cancer ([Moriya et al., 2001](#); [Lerat et al., 2002](#)) in transgenic mice expressing HCV core.

##### (b) E2

Overexpression of E2 inhibits eIF2 $\alpha$  phosphorylation by the dsRNA-activated protein kinase (PKR) or the endoplasmic-reticulum-stress signalling kinase PERK. E2 also physically interacts with PKR; the E2/PKR interaction may account for the intrinsic interferon's resistance of HCV genotypes 1a and 1b ([Taylor et al., 1999](#); [Pavio et al., 2003](#)).

#### 4.2.2 The non-structural proteins

##### (a) NS4A, NS4B or NS4A-4B

Overexpression of NS4A, NS4B, or NS4A-4B has been reported to induce an endoplasmic-reticulum- stress-mediated unfolded protein response, reduce endoplasmic-reticulum-to-Golgi traffic, inhibit protein synthesis, and to

cause cytopathic effects ([Lindenbach et al., 2007](#); and references therein).

(b) NS2

NS2 interacts with the cellular proapoptotic molecule CIDE-B and inhibits CIDE-B-induced apoptosis ([Erdtmann et al., 2003](#)). NS2 has also been shown to downregulate the transcription of several cellular and viral promoters in gene-reporter assays ([Dumoulin et al., 2003](#)).

(c) NS3-4A

NS3-4A serine protease has been reported to block the activation of the transcription factors IRF-3 and NF-κB, and to antagonize innate anti-viral defenses by interfering with the cytosolic RNA helicases, RIG-1- and MDA5-, and TLR3-mediated signal transduction ([Lindenbach et al., 2007](#)).

(d) NS5A

NS5A has multiple functions. It has been shown to interact with the geranylgeranylated cellular protein FBL2 ([Wang et al., 2005](#)), an F-box-motif-containing protein that is likely to be involved in targeting cellular proteins of yet unknown identity for ubiquitylation and degradation.

Several studies suggest that NS5A is also involved in the resistance to interferon treatment ([Lindenbach et al., 2007](#); and references therein), and one possible mechanism may be its ability to induce expression of the type I interferon antagonist IL-8 ([Polyak et al., 2001](#)). In addition, NS5A contains an ‘interferon sensitivity determining region’ (ISDR) that mediates inhibition of PKR, an activator of innate immunity; accumulation of mutations in this region is thought to correlate with treatment efficacy ([Enomoto et al., 1995, 1996](#)).

Overexpression of NS5A has been reported to induce several effects in cells, including oxidative stress; activation of signalling pathways,

including STAT-3, PI3K, and NF-κB ([Gong et al., 2001](#); [He et al., 2002](#); [Street et al., 2004](#)); and degradation of pRB ([Munakata et al., 2005](#)).

Other reported NS5A interaction partners include apolipoprotein A1, the major protein found on High Density Lipoprotein (HDL); the tumour suppressor, p53; Grb-2, an adaptor protein involved in mitogen signalling; SRCAP, an adenosine triphosphatase (ATPase) that activates cellular transcription; karyopherin β3, a protein involved in nuclear trafficking; Cdk1/2, cyclin-dependent and Fyn, Hck, Lck, and Lyn, Src-family kinases ([Lindenbach et al., 2007](#); and references therein).

More recently, it has been reported that NS5A-expression in the context of HCV polyprotein results in the inhibition of the transcription factor Forkhead as well as in the phosphorylation and inactivation of the GSK-3, leading to the accumulation of β-catenin and to the stimulation of β-catenin-dependent transcription ([Street et al., 2005](#)).

[The Working Group noted that, so far, the biological functions of the HCV proteins have been investigated *in vitro* or *in vivo* using transgenic mice constitutively expressing HCV proteins alone, in combination, or the entire polyprotein. Whether the results generated by these experimental approaches reflect the pathological consequences of an HCV infection *in vivo* remains to be addressed, and this issue will only be resolved with the establishment of immunocompetent mouse models or other more physiological cellular models that permit chronic and productive HCV replication.]

## 4.3 Experimental evidence for a role of HCV in malignant conversion

### 4.3.1 Role of HCV chronic infection in HCC development

Successful clearance of chronic HCV infection has been shown to reduce the overall liver-related mortality and the incidence of HCC providing further evidence for a causal role of HCV in this cancer ([Kasahara et al., 1998](#); [Serfaty et al., 1998](#); [Imazeki et al., 2003b](#)).

Although chronic HCV infection is one major risk factor for HCC, the mechanisms by which HCV induces HCC remain uncertain ([Levrero, 2006](#); [McGivern & Lemon, 2009](#)). Chronic endoplasmic reticulum stress and inflammatory responses and the associated oxidative stress and altered intracellular redox state lead to the accumulation of genomic damage. These are likely to contribute to and predispose infected cells to hepatocarcinogenesis, possibly via changes in MAPK signalling, that regulates both cell metabolism and growth ([Tardif et al., 2002](#); [Waris et al., 2007](#)). Markers of intracellular oxidative stress have indeed been reported to be increased in chronic HCV patients ([Shimoda et al., 1994](#); [Sumida et al., 2000](#)) as well as in HCV core transgenic mice ([Moriya et al., 1998](#); [Moriya et al., 2001](#)). However, in addition, direct interactions of the various HCV proteins with host factors correlate with changes in cellular signalling cascades that are involved in the regulation of cell metabolism and division. The expression of some HCV proteins seem to be sufficient to induce hepatocarcinogenesis, at least in some specific transgenic mice lineages such as transgenic C57BL/6 mice ([Lerat et al., 2002](#)); liver tumour development was shown to be associated with HCV-induced liver steatosis ([Lerat et al., 2002](#); [Moriya et al., 1998](#)).

Overall, because of the lack of an experimental model that replicates the viral life cycle and natural history of the disease, the current view is

that synergistic effects between the consequences of chronic inflammation and direct virus–host cell interactions are most likely. Such synergistic effects would also explain the long ‘multistep’ transformation process in human HCC, which is consistent with the long time lag with which cirrhosis and HCC manifest themselves upon chronic infection, and would explain the wide variety of genetic defects observed in individual HCCs ([Thorgeirsson & Grisham, 2002](#); [Levrero, 2006](#); [McGivern & Lemon, 2009](#)).

Prospective and retrospective cohort studies of patients with chronic HCV infection have demonstrated a role for the long duration of the disease in HCC development, and the link between HCC development and liver cirrhosis. These studies showed the sequential occurrence of advanced liver fibrosis followed by the development of HCC. The incidence of HCC development was estimated to be between 3–5%/year in cirrhotic patients ([Tsukuma et al., 1993](#); [Tong et al., 1995](#); [Fattovich et al., 1997](#)).

### 4.3.2 Role of HCV-induced steatosis, insulin resistance, and oxidative stress in HCC development

Pro-carcinogenic cofactors in chronic HCV infection are steatosis, oxidative stress and insulin resistance, suggesting many parallels with non-alcoholic fatty liver disease (NAFLD). In NAFLD, chronic excess of nutrients causes endoplasmic reticulum stress, and leads to an increase of hepatic fat (steatosis) and insulin resistance; a complex interplay between these factors can lead to chronic liver inflammation, apoptosis and fibrogenesis, which are thought to form the prelude to liver cirrhosis and cancer ([Hotamisligil, 2006](#)).

An increased prevalence of steatosis and insulin resistance in HCV patients is well established and has prognostic implications, as it is associated with faster progression of fibrosis and a poorer response to treatment. In HCV patients

infected with genotypes 1 and 2, steatosis presents in general with concomitant obesity or other features of the metabolic syndrome, but this association is weak in genotype 3 patients. Genotype 3 is thought to induce steatosis in a direct fashion, as steatosis in these patients correlates with viral load, and reverses with response to treatment ([Negro, 2006](#)). HCV is thought to induce steatosis by impairing secretion and degradation, and increasing the neosynthesis of lipids. The HCV core protein, which localizes to the surface of lipid droplets and mediates the viral assembly in close conjunction with cellular fatty acid metabolism ([Miyanari et al., 2007](#)), and also some HCV non-structural proteins, have all been shown to interfere with vLDL secretion ([Wetterau et al., 1997; Domitrovich et al., 2005](#)). HCV infection has also been associated with an upregulation of the neosynthesis of lipids ([Waris et al., 2007](#)), inhibition of fatty acid oxidation ([Dharancy et al., 2005](#)), and increased release of fatty acids from fat cells ([Negro, 2006](#)). Overall, the effects of HCV proteins on lipid synthesis, secretion and oxidation seem to be most potent in the context of genotype 3, but also occur in the context of other genotypes.

The development of severe steatosis and HCC was shown in PPARalpha-homozygous mice with liver-specific transgenic expression of the core protein gene, while tumours were not observed in the other transgenic mouse genotypes. This result suggested that persistent activation of PPARalpha, a central regulator of triglyceride homeostasis, is essential for the pathogenesis of hepatic steatosis, and HCC induced by HCV infection ([Tanaka et al., 2008](#)).

Besides changes in the lipid metabolism, core and several of the non-structural HCV proteins induce systemic oxidative stress and related signalling by various mechanisms ([Tardif et al., 2005](#)).

With respect to insulin resistance, all HCV genotypes have been shown to interfere with glucose homeostasis, often at early stages of

infection ([Negro, 2006](#)), but the underlying mechanisms and the degree of insulin resistance seem to be again genotype-dependent. HCV has been shown to interfere with insulin signalling by proteasomal degradation of the insulin receptor substrates, (IRS)-1 and -2 ([Aytug et al., 2003](#)).

The feedback circle between steatosis, insulin resistance and oxidative stress is an important denominator for disease progression in NAFLD as well as viral hepatitis, and induces tissue damage and inflammation and consequently, activation of hepatic stellate cells (HSCs). Activated HSCs become responsive to both proliferative and fibrogenic cytokines, and undergo epithelial-to-mesenchymal transdifferentiation (EMT) into contractile myofibroblast-like cells, that synthesize extracellular matrix (ECM) components, which accumulate over time to form fibrous scars, or ‘fibrosis’. Ultimately, nodules of regenerating hepatocytes become enclosed by scar tissue, which defines cirrhosis. Activation of HSCs is regulated by products and effectors of oxidative stress and growth factors, cytokines, adipokines, and chemokines. The cytokine TGF- $\beta$ , a potent inhibitor of epithelial cell growth and tumour suppressor, can also exert pro-oncogenic functions, and is a key regulator of EMT. Importantly, recent findings imply that TGF- $\beta$  induces EMT not only in HSCs but possibly also in hepatocytes ([Matsuzaki et al., 2007](#)). TGF- $\beta$  signalling is upregulated in fibrotic HCV patients, and stimulates ECM deposition and accumulation. Insulin resistance may link fibrosis and steatosis, as it stimulates HSCs to deposit ECM. Several signalling cascades are implicated and modulated during fibrogenesis, including SMADs, PI3K-Akt and various MAPK pathways, such as p38 and JNK. While SMADs are indispensable for the EMT process, TGF- $\beta$  signalling via SMAD synergizes with other signalling pathways to mediate pro-oncogenic EMTs. JNK activation by the pro-inflammatory cytokine interleukin-1 $\beta$  can shift TGF- $\beta$  signalling away from a tumour-suppressive to a pro-oncogenic

profile with augmented fibrogenesis, increased cell motility, and transactivation of cell cycle regulatory genes ([Matsuzaki et al., 2007](#)). Thus, in the context of chronic inflammation, the interplay between endoplasmic reticulum/oxidative stress, steatosis and insulin resistance induces a pro-oncogenic microenvironment that drives fibrogenic processes and genomic instability; and even though HCV has been reported to display direct transforming capacities, the liver microenvironment is thought to determine significantly the transformation process because HCC develops in chronic HCV infection only over long periods of time.

So far, it has not been possible to correlate hepatocarcinogenesis with a consistent pattern of proto-oncogene activation, but several growth factor signalling axes are frequently found to be dysregulated, including insulin-growth factor (IGF), hepatocyte growth factor, Wnt, TGF- $\alpha$ /EGF and TGF- $\beta$  signalling ([Breuhahn et al., 2006](#); [Levrero, 2006](#)). The interplay between these various pathways and their respective roles and contributions to the development of HCC remain to be unravelled.

#### ***4.3.3 Role of HCV in lymphomas and other tumours***

The mechanisms by which lymphoma is induced by HCV remains the subject of debate. Several clinical studies have shown that the HCV genome may be detected in peripheral lymphoid cells as well as dendritic cells ([Bain et al., 2001](#)). However, evidence of true viral genome replication in extrahepatic sites is still lacking.

Some early studies showed that HCV may infect cultured peripheral blood mononuclear cells *in vitro* ([Shimizu et al., 1998](#)), but these observations were not confirmed by other groups.

Few studies showed that the HCV genome sequence from extrahepatic isolates may cluster differently from liver isolates providing

indirect evidence for viral replication in these cells ([Roque-Afonso et al., 2005](#)).

Clinical studies have shown that HCV eradication by pegylated interferon and ribavirin treatment may lead to the cure of cryoglobulinemia, a B-cell proliferation disorder, and to the regression of HCV-associated splenic lymphoma ([Hermine et al., 2002](#)).

Several non-exclusive hypotheses have been discussed regarding the transforming role of HCV in the context of lymphoma: 1) antigen-driven proliferation induced by continuous activation of B cells ([Suarez et al., 2006](#)); 2) a direct role of HCV replication and expression in infected B cells.

Further molecular and cellular biology studies are warranted to decipher the mechanisms of HCV-induced lymphomas.

Regarding the role of HCV in the development of cholangiocarcinoma, both clinical evidence and strong experimental data are lacking.

#### **4.4 Interaction between HCV and environmental agents**

Regarding interactions between HBV and HCV please refer to the *Monograph* on HBV in this volume.

#### **4.5 Animal models for HCV-associated cancers**

Chimpanzees and tree shrews do not, or only partially, develop HCV-associated pathologies upon infection. And given the long delay with which HCC develops in chronic hepatitis, these models are unsuitable to study HCV-induced HCC in the first place. In the absence of animal models that develop HCC in the context of an HCV infection, various groups have described the use of mouse models. Mice expressing HCV replicons, polyproteins or the single HCV proteins alone or in combination, using various

liver specific promoters, have been described by many groups ([Levrero, 2006](#); [McGivern & Lemon, 2009](#)).

To date, studies using transgenic animals expressing HCV cDNA suggest that HCV proteins are not directly cytopathic ([Kawamura et al., 1997](#); [Pasquinelli et al., 1997](#); [Wakita et al., 1998](#)). Only three different HCV core transgenic lines have been shown to develop liver steatosis and HCC ([Moriya et al., 1998, 2001](#)), and one group has been able to demonstrate that upon HCV polyprotein expression, the rate of liver cancer in transgenic mice increases in the absence of intrahepatic inflammation, suggesting a metabolic or genetic host susceptibility for HCV-associated HCC ([Lerat et al., 2002](#)).

NS5A transgenic mice, despite the abundant interactions of NS5A with host-cell factors, do not have any significant phenotype ([Majumder et al., 2002, 2003](#)).

## 4.6 HCV, host immune system, and genetic susceptibility

While many studies have been reported regarding the role of the humoral and cellular responses in the control of HCV infection, as well as micro-array analysis of primary liver tumours showing differential expression of many cellular genes in the tumours, no relevant data are available at the time of writing concerning specific immune or genetic mechanism involved in HCV-induced HCC.

## 4.7 Synthesis

Although there is strong evidence that HCV is one of the leading causes of HCC, there is still much to understand regarding the mechanism of HCV-induced transformation. While liver fibrosis resulting from long-lasting chronic inflammation and liver regeneration resulting from immune-mediated cell death are likely

factors contributing to the development of HCC, the direct role of HCV proteins remains to be determined. Many *in vitro* studies have shown that HCV expression may interfere with cellular functions that are important for cell differentiation and cell growth. However, most studies were performed in artificial study models which can only give clues for potential mechanisms that need to be confirmed in more relevant models. Furthermore, the difficulty to localize HCV proteins as well as infected cells *in vivo* in the liver of infected patients contribute to the complexity of our current understanding.

For all these reasons, at the time of writing, the current view is that there is moderate experimental evidence for a direct oncogenic role of HCV. Further studies are warranted to clarify these issues.

## 5. Evaluation

There is *sufficient evidence* in humans for the carcinogenicity of chronic infection with HCV. Chronic infection with HCV causes hepatocellular carcinoma and non-Hodgkin lymphoma. Also, a positive association has been observed between chronic infection with HCV and cholangiocarcinoma.

Chronic infection with HCV is *carcinogenic to humans (Group 1)*.

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# KAPOSI SARCOMA HERPESVIRUS

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Kaposi sarcoma herpesvirus was considered by a previous IARC Working Group in 1997 ([IARC, 1997](#)). Since that time, new data have become available, these have been incorporated into the *Monograph*, and taken into consideration in the present evaluation.

## 1. Exposure Data

### 1.1 Taxonomy, structure, and biology

#### 1.1.1 Taxonomy

First detected by [Chang et al. \(1994\)](#) in Kaposi sarcomas associated with the acquired immune deficiency syndrome (AIDS) (see [IARC, 1996](#)) by representational difference analysis, this virus was termed Kaposi-sarcoma-associated herpesvirus, KSHV. KSHV is also associated with primary effusion lymphoma and some cases of multicentric Castleman disease (see Section 2). In keeping with the systematic nomenclature adopted for all human herpesviruses, the formal designation human herpesvirus 8 (HHV-8) was proposed by the herpesvirus subcommittee of the International Committee on the Taxonomy of Viruses. In this *Monograph*, the term KSHV is used throughout.

On the basis of phylogenetic analyses ([Moore et al., 1996a; Russo et al., 1996](#)), KSHV is a gamma-2 herpesvirus (rhabdovirus), and represents the first ‘human’ member of this group. There are many more gamma-2 herpesvirus species in old and new world non-human primates.

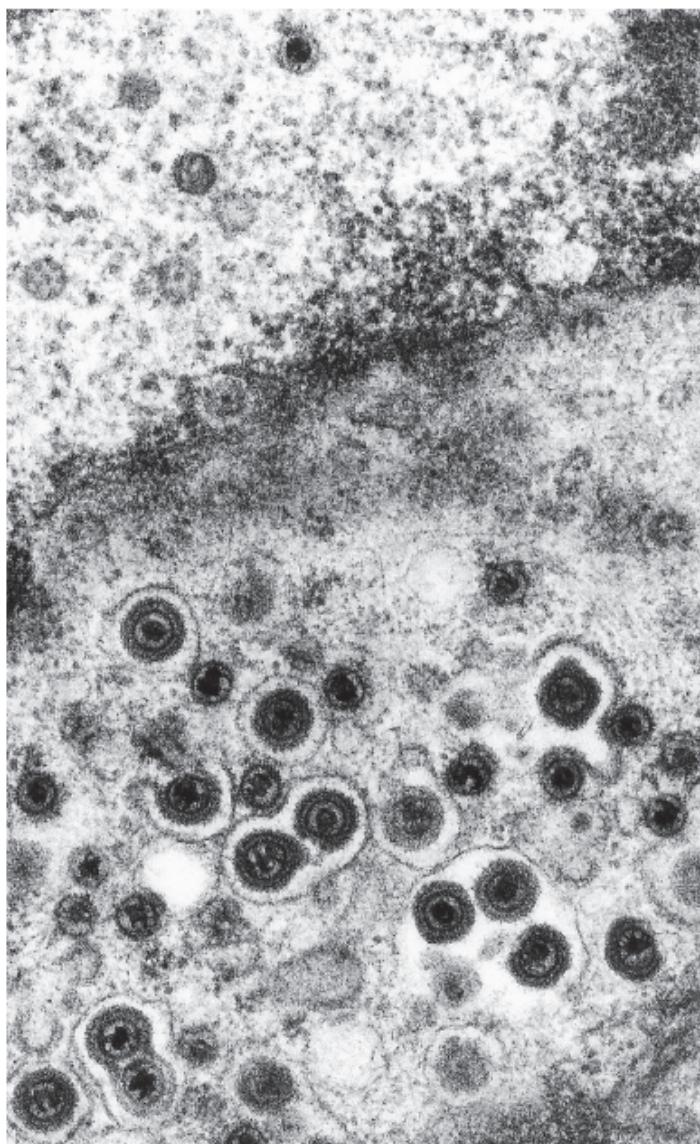
#### 1.1.2 Structure of the virion

KSHV has the typical morphological characteristics of a herpesvirus (Fig. 1.1; [Arvanitakis et al., 1996](#); [Renne et al., 1996a](#); [Orenstein et al., 1997](#)) with 100–150 nm particles surrounded by a lipid envelope, and an electron-dense central core ([Renne et al., 1996b](#)). Cryo-electron microscopy (Cryo-EM) and Cryo-EM tomography studies suggest that KSHV capsomers are hexamers and pentamers of the major capsid protein (encoded by the open reading frame [ORF] 25), with the small capsid protein (encoded by ORF 65), binding around the tips of both hexons and pentons ([Trus et al., 2001](#); [Deng et al., 2008](#)).

#### 1.1.3 Structure of the viral genome

KSHV has a double-stranded DNA genome. The genomic structure of the virus ([Russo et al., 1996](#); [Neipel et al., 1997a](#)) is similar to that of other primate rhabdoviruses, e.g. *Herpesvirus saimiri* ([Albrecht et al., 1992](#)) or Rhesus Rhabdovirus ([Searles et al., 1999](#); [Alexander et al., 2000](#)), with a single, contiguous 140.5-kb-long unique region containing all the identified viral genes ([Russo et al., 1996](#); [Neipel et al., 1997a](#); Fig. 1.2). This region is flanked on either side by a terminal-repeat (TR) region composed of a variable number of repeats of 801-bp length with a high

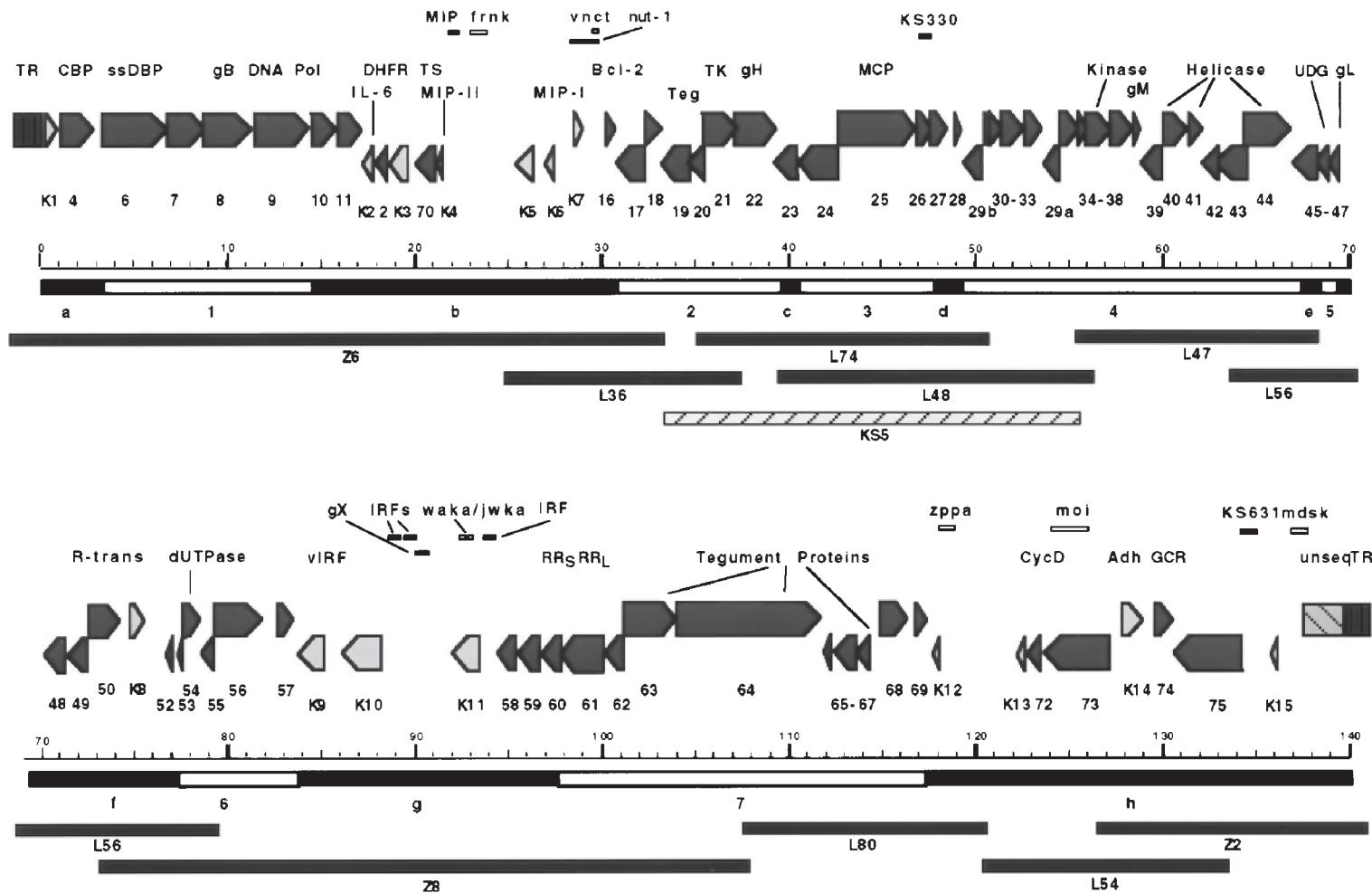
**Fig. 1.1 Electron microscopic view of KSHV capsids in a cross-section of a spleen Kaposi sarcoma**



Cytoplasmic viral capsids obtain their lipid outer membrane by budding into cisternae; the electron dense central core represents viral DNA.  
Original magnification: x53 000.

From [Orenstein et al. \(1997\)](#), AIDS 11: F35–F45, “Copyright © 1997 Lippincott-Raven Publishers.”

**Fig. 1.2 Annotated long unique region and terminal repeats of the KSHV genome**



The orientation of identified open reading frames in the long unique region is denoted by the direction of arrows, with *Herpesvirus saimiri* homologous with open reading frames as shaded areas and those not homologous as lighter areas. Seven blocks (numbered) of conserved herpesviral genes with non-conserved interblock regions (lettered) are shown under the kilobase marker. Features and putative coding regions not specifically designated are shown above the open reading frame map. Repeat regions (frnk, vncnt, waka/jwka, zppa, moi, mdsk) are shown as light lines, and putative coding regions and other features are not designated as open reading frames are shown as solid lines.

From Russo *et al.* (1996), PNAS 93: 1486–1487, “Copyright (2010) National Academy of Sciences, USA.”

G:C (84.5%) content. Due to the variable number of repeat subunits (some of the repeat subunits may be truncated), the overall length of the TR region varies, and with it, the overall size of the KSHV genome. The latter has been calculated to be approximately 165 kb on the basis of studies of the viral genome from productive primary effusion lymphoma cells ([Arvanitakis et al., 1996](#); [Renne et al., 1996b](#)), and mapping of the whole genome ([Russo et al., 1996](#)).

#### (a) Terminal-repeat region

The TR region is a conserved feature of herpesviruses, and is involved in the packaging of the viral DNA into new virions during the lytic cycle of replication. Depending on the viral strain, KSHV has approximately 20–30 TR units. A particular feature of the KSHV TR unit is that it contains two binding sites for the KSHV latent nuclear antigen (LANA) and the latent (episomal) origin of replication ([Garber et al., 2001](#); [Hu et al., 2002](#)). By binding to multiple TR subunits, LANA tethers circular viral episomes to mitotic chromosomes during mitosis ([Ballestas et al., 1999](#); [Barbera et al., 2006](#)). LANA is also required for the replication of viral episomes by recruiting a range of cellular factors involved in DNA replication (see Section 4.1). Currently, the TR region is not known to contain any protein-coding ORFs in contrast to, for example, EBV ([Longnecker & Neipel, 2007](#)).

#### (b) Long unique region

The KSHV 140.5-kb long unique region encodes approximately 90 predicted ORFs ([Russo et al., 1996](#); [Neipel et al., 1997a](#); Fig. 1.2). The ORFs were named according to the corresponding *herpesvirus saimiri* genes with which they share a significant level of sequence similarity. Unique genes that are not homologous with *herpesvirus saimiri* have a K prefix. The long unique region has blocks of genes conserved among all subfamilies of herpesviruses ([Chee et al., 1990](#)), which include genes that encode herpesvirus structural

proteins and replication enzymes. Between the conserved herpesvirus gene blocks lie blocks of genes that are either found in rhabdoviruses or are unique to KSHV ([Russo et al., 1996](#)). Several of these share significant sequence similarity with cellular genes, and were presumably carried away at some point during the evolution of these viruses.

The long unique region also contains genes for untranslated RNAs. Among these is the *PAN/nut-1* transcript, a nuclear untranslated RNA, whose function is not yet clear ([Sun et al., 1996](#); [Zhong & Ganem, 1997](#)). In addition, KSHV encodes at least 12 microRNAs, which are generated from one transcript, and located downstream of the *ORFK13/vFLIP* ([Cai et al., 2005](#); [Samols et al., 2005](#)).

The probable function of these genes in the virus life cycle and tumour formation is discussed in Section 4.1.

#### 1.1.4 Host range and tropism

Humans are the natural hosts for KSHV. Epidemiological studies indicate that KSHV is more prevalent in sub-Saharan Africa, several countries of southern Europe, the North African Mediterranean coast, and several countries of South America compared to northern Europe, North America, and Asia (see Section 1.2).

*In vivo*, KSHV has been detected in endothelial and spindle cells of Kaposi sarcoma lesions, in circulating endothelial cells, primary effusion lymphoma cells, B cells, macrophages, dendritic cells, oropharynx and prostatic glandular epithelium and keratinocytes ([Ambroziak et al., 1995](#); [Boshoff et al., 1995](#); [Cesarman et al., 1995a](#); [Moore & Chang, 1995](#); [Corbellino et al., 1996](#); [Li et al., 1996](#); [Sirianni et al., 1997](#); [Staskus et al., 1997](#); [Stürzl et al., 1997](#); [Reed et al., 1998](#); [Pauk et al., 2000](#)).

Of the cell types targeted by KSHV *in vivo*, primary endothelial cells of different differentiation (vascular, lymphatic, endothelial precursor

cells), monocytes, dendritic cells, fibroblasts, epithelial cells and keratinocytes can be infected *in vitro* ([Renne et al., 1998](#); [Blackbourn et al., 2000](#); [Cerimele et al., 2000](#); [Wang et al., 2004a](#); [Rappoccio et al., 2006](#)). It was shown that B cells can only be infected *in vitro* at a specific differentiation stage ([Rappoccio et al., 2006, 2008](#)).

### 1.1.5 Viral life cycle

KSHV, like all herpesviruses, can establish lifelong latent infections in their human host. Latently infected cells provide a perpetual reservoir from which progeny viruses can be amplified for dissemination within the host and transmission between hosts. The peripheral blood CD19-positive B cells have been identified as a long-term latency reservoir for KSHV; other cells such as endothelial cells may also be a site for KSHV latency, but they do not appear to provide a long-term latent reservoir for the virus. Nonetheless, infected dermal endothelium spindle cells may release progeny virus that can subsequently infect local keratinocytes and the eccrine epithelium in the tumour. Lytic reactivation from latently KSHV-infected cells that results in the release of progeny virions is a critical pathogenic step in multiple human diseases. In immunocompetent KSHV carriers, the immune system plays an essential role in tempering lytic reactivation of the virus (see [Lukac & Yuan, 2007](#) for a detailed review).

## 1.2 Epidemiology of infection

In the previous IARC monograph ([IARC, 1997](#)), preliminary epidemiological data were presented based largely on Polymerase Chain Reaction (PCR) and initial serological studies. Subsequently, substantial additional data have become available with the advent of new serological techniques and large-scale studies.

### 1.2.1 Prevalence, geographic distribution

#### (a) Laboratory methods in epidemiological studies of KSHV

Difficulties have arisen in developing reliable serological tests to assess KSHV infection, and the interpretation of some published KSHV prevalence data is therefore challenging. KSHV encodes multiple antigenic proteins that may be expressed in the latent or lytic phase of the virus life cycle ([Chandran et al., 1998](#)). The major antigenic proteins are the LANAs encoded by ORF 73, and the lytically expressed K8.1 encoded by ORF 65. First-generation serological assays were developed based on the immunofluorescence of latently infected primary effusion lymphoma cells ([Gao et al., 1996](#); [Kedes et al., 1996](#); [Simpson et al., 1996](#)) or primary effusion lymphoma cells induced by treatment with tetradecanoyl phorbol ester acetate (TPA) to produce lytic antigens ([Lennette et al., 1996](#)). More recently, enzyme-linked immunosorbent assays (ELISAs) have been developed using recombinant proteins or peptides.

Concordance between assays detecting antibodies to these antigens has improved but remains moderate. Infected subjects may have antibodies to only lytic or latent antigens, and may develop antibody responses to lytic antigens years before they develop antibodies to LANA, and the reverse can also occur ([Biggar et al., 2003](#), [Minhas et al., 2008](#)). In addition, antibody titres are very high in Kaposi sarcoma patients but very low in asymptomatic subjects ([Biggar et al., 2003](#)). [The Working Group concluded that if the serum samples of Kaposi sarcoma patients are used as “gold standard” positive controls then the sensitivity of an assay is likely to be overestimated. At the other end of the spectrum, antibody levels in asymptomatic subjects are so low that establishing a clear assay cut-off is difficult. For these reasons, current assays for KSHV antibody detection, while suitable for comparisons between populations,

are inadequate for diagnostic purposes in low-risk populations. Comparisons between studies using different assays or even different cut-offs are often problematic.]

(b) *Seroprevalence estimates and geographic distribution*

Despite the difficulties discussed above in estimating the precise prevalence of KSHV, prevalence is in general low (< 10%) in northern Europe, the USA and Asia, elevated in the Mediterranean region (10–30%), and high in sub-Saharan Africa (> 50%) ([Enbom et al., 2002](#); [Dukers & Rezza, 2003](#)). A study of 1000 blood donors in the USA tested by six independent laboratories reported estimates ranging from 0.5–5% ([Pellett et al., 2003](#)). More recently, a study of approximately 14000 adults recruited for the National Health and Nutrition Examination survey (NHANES) III, designed to be representative of the general population, estimated KSHV prevalence to be around 7% ([Engels et al., 2007](#)). In northern Europe, KSHV is reported to be 2–3% in adults ([Simpson et al., 1996](#); [Marcelin et al., 1998](#); [Preiser et al., 2001](#)). Prevalence in Asia is similar to that in the USA and northern Europe. A large international study recently reported a prevalence of 5% for the Republic of Korea, 8–10% for Thailand, and 11–15% for Viet Nam ([de Sanjose et al., 2009](#)).

KSHV prevalence is higher in adults in Mediterranean countries such as Italy than in northern Europe or the USA ([Gao et al., 1996](#); [Whitby et al., 1998](#)), with prevalence higher in Southern Italy and in the Po Valley (13–20%) than in most of Northern Italy ([Calabro et al., 1998](#); [Whitby et al., 1998](#); [Cattani et al., 2003](#); [Serraino et al., 2006](#)). The prevalence in Spain is reported to be 3.65% ([de Sanjose et al., 2009](#)), in Greece 7.6% ([Zavitsanou et al., 2007](#)), and in Israel 10% ([Davidovici et al., 2001](#)). In South America, a high prevalence of KSHV infection is reported in Amerindians but not in the general

population ([Biggar et al., 2000](#); [Whitby et al., 2004](#); [Cunha et al., 2005](#)).

The prevalence of KSHV in Uganda is 40–50% ([Gao et al., 1996](#); [Wawer et al., 2001](#); [Hladik et al., 2003](#); [Newton et al., 2003a](#)). Similar estimates are reported for Kenya (~43%) ([Baeten et al., 2002](#); [Lavrey et al., 2003](#)), Zambia (40%) ([Klaskala et al., 2005](#)), and the United Republic of Tanzania (~ ≥50%) ([Mbulaiteye et al., 2003a](#)). KSHV prevalence appears to be higher in Malawi (54–67%), Botswana (76%), and The Demographic Republic of Congo (82%) ([Engels et al., 2000](#); [De Santis et al., 2002](#); [Whitby et al., 2004](#)).

KSHV prevalence is somewhat lower in South Africa (30%) ([Sitas et al., 1999](#); [Dedicoat et al., 2004](#); [Malope et al., 2007](#)), and in West African countries such as Burkino Faso (~12.5%) and Cameroon (~25%) ([Volpi et al., 2004](#); [Collenberg et al., 2006](#)). The Gambia had a low incidence of Kaposi sarcoma before and during the AIDS epidemic but has a high KSHV prevalence (~75%) ([Ariyoshi et al., 1998](#)). The prevalence of KSHV in Nigeria is intermediate (~45%) ([de Sanjose et al., 2009](#)).

### 1.2.2 Transmission and risk factors for infection

KSHV is primarily transmitted via saliva. In countries where KSHV is highly prevalent, infection occurs during childhood and increases with age ([Whitby et al., 2000](#); [Dedicoat et al., 2004](#); [Malope et al., 2007](#)). The peak age of acquisition is generally between 6–10 years ([Whitby et al., 2000](#); [Mbulaiteye et al., 2004](#)), and the risk of infection is increased if family members, especially mothers, are infected ([Plancoulaine et al., 2000](#); [Dedicoat et al., 2004](#); [Malope et al., 2007](#); [Minhas et al., 2008](#)). Other reported risk factors for infection in childhood include human immunodeficiency virus (HIV) infection ([Malope et al., 2007](#); [Minhas et al., 2008](#)), environmental factors such as source of water ([Mbulaiteye et al., 2005](#)), and insect bites ([Coluzzi et al., 2003](#)). In

the USA, Europe and Australia, KSHV prevalence is elevated in homosexual men, especially those infected by HIV ([Martin et al., 1998](#); [Smith et al., 1999](#); [Grulich et al., 2005](#)). There is considerable evidence that the risk of infection with KSHV is associated with the number of sexual partners of an individual, and other sexual risk factors ([Martin et al., 1998](#); [Smith et al., 1999](#); [Grulich et al., 2005](#)). It is likely that the transmission of KSHV between homosexual men is also via saliva ([Martin, 2003](#); [Martró et al., 2007](#)). KSHV is not generally associated with sexual risk factors in heterosexuals ([Smith et al., 1999](#); [Engels et al., 2007](#); [Malope et al., 2008](#); [de Sanjósé et al., 2009](#)).

KSHV can be detected in peripheral blood suggesting that blood-borne transmission is possible. Some studies have suggested that injecting drug use was not associated with a risk of KSHV infection ([Renwick et al., 2002](#); [Bernstein et al., 2003](#)), but others have shown an increased risk of KSHV infection in injecting drug users, especially with prolonged use ([Cannon et al., 2001](#); [Atkinson et al., 2003](#)). KSHV transmission via blood transfusion is also likely to be rare, but evidence of both risk and actual transmission has been reported ([Mbulaiteye et al., 2003b](#); [Dollard et al., 2005](#); [Hladik et al., 2006](#)). KSHV transmission by organ donation has also been reported ([Parravicini et al., 1997](#), [Concato et al., 2008](#)).

## 2. Cancer in Humans

### 2.1 Kaposi sarcoma

At the time of the previous IARC Monograph ([IARC, 1997](#)), KSHV was classified as ‘probably carcinogenic to humans’ (Group 2A) on the basis ‘that the evidence was compelling but as yet limited’. Since then, a large number of studies have assessed the association between KSHV and Kaposi sarcoma (see Table 2.1 available at <http://monographs.iarc.fr/ENG/Monographs/vol100B/100B-04-Table2.1.pdf>,

[vol100B/100B-04-Table2.2.pdf](http://monographs.iarc.fr/ENG/Monographs/vol100B/100B-04-Table2.2.pdf), and Table 2.2 available at <http://monographs.iarc.fr/ENG/Monographs/vol100B/100B-04-Table2.2.pdf>).

To date, data on the association between KSHV and Kaposi sarcoma come from 22 cohort studies and 80 case-control studies – all show broadly consistent evidence of an association between KSHV infection and Kaposi sarcoma. In most studies, the relative risks for the association between KSHV infection and Kaposi sarcoma are typically greater than 10. Of the 22 cohort studies, conducted in nine countries across three continents, 13 were among cohorts of HIV-infected people and included data on 561 cases. The largest study included 189 cases ([Newton et al., 2006](#)). Nine cohorts of transplant recipients yielded a total of 48 cases ([Parravicini et al., 1997](#); [Regamey et al., 1998](#); [Francès et al., 1999, 2000](#); [Rabkin et al., 1999](#); [Cattani et al., 2001](#); [Emond et al., 2002](#); [Marcelin et al., 2004](#); [García-Astudillo & Leyva-Cobián, 2006](#)). No data were available from cohort studies on “classical” or “endemic” Kaposi sarcoma; to date, all studies have included only immunosuppressed subjects. Two studies used PCR to identify evidence of KSHV in peripheral blood mononuclear cells (PBMC) ([Whitby et al., 1995](#); [Moore et al., 1996b](#)), most studies used serology, and one study used both ([Engels et al., 2003](#)). One study demonstrated a statistically significant increasing risk of Kaposi sarcoma with increasing titre of antibodies against KSHV, before diagnosis, both for a lytic and a latent assay ([Newton et al., 2006](#)).

Of the 80 case-control studies, nearly half included data on cases not infected by HIV – i.e. “classical,” “endemic”, and transplant-associated Kaposi sarcoma. A variety of assays were used to detect evidence of infection, including PCR of tumour lesions, semen, prostate tissue, saliva, and bronchial alveolar lavage. Serological studies used assays against lytic or latent antigens, or both. Two studies ([Sitas et al., 1999](#); [Newton et al., 2003b](#)) had substantial numbers of Kaposi sarcoma cases among HIV-uninfected

individuals and together with one other study ([Albrecht et al., 2004](#)) were able to demonstrate increasing risks of Kaposi sarcoma associated with increasing titres of anti-KSHV antibodies ([Brown et al., 2006a, b](#)). In addition, among KSHV-seropositive people, the presence of KSHV DNA in PBMC was associated both with an increased risk of Kaposi sarcoma, and with an increased risk of disease progression ([Laney et al., 2007](#)). Broadly, results were consistent across all studies, demonstrating a clear association between infection with KSHV and Kaposi sarcoma.

## 2.2 Primary effusion lymphoma

Primary effusion lymphoma is a very rare subgroup of B-cell non-Hodgkin lymphomas presenting as pleural, peritoneal, and pericardial (body cavity) lymphomatous effusions. These comprise less than 2% of HIV-related lymphomas ([Sullivan et al., 2008](#)). There is already strong evidence that KSHV is a causal agent of primary effusion lymphoma ([IARC, 1997](#)). Because of the rarity of primary effusion lymphoma, much of the information comes from case reports, with a few studies that have examined biopsy tissues, aspirates or cell lines. Primary effusion lymphomas were described in HIV-immunosuppressed individuals by [Cesarman et al. \(1995a\)](#) in association with KSHV, and by [Nador et al. \(1996\)](#) as a distinct disease entity, and almost all occurred in association with HIV ([Gaidano et al., 2000](#)). Because of the identification of KSHV in all of the cases presenting as primary lymphomatous effusions in early studies, the presence of this virus has been incorporated as a diagnostic criterion for primary effusion lymphoma. However, it has since been recognized that other lymphoma subtypes such as Burkitt or diffuse large B-cell lymphoma can also present in body cavities, and these lack the presence of KSHV. In addition, some KSHV-positive lymphomas occur as solid tumour masses with or without accompanying effusions.

However, KSHV-associated lymphomas have a multitude of morphological and immunophenotypical features that are characteristic, and so, have been grouped as a single clinicopathological entity. Cases without an effusion are considered to be extracavitary variants of primary effusion lymphoma ([Said & Ceserman, 2008](#)).

See Table 2.3 available at <http://monographs.iarc.fr/ENG/Monographs/vol100B/100B-04-Table2.3.pdf>, and Table 2.4 available at <http://monographs.iarc.fr/ENG/Monographs/vol100B/100B-04-Table2.4.pdf>.

Some primary effusion lymphomas occur in association with post-transplant immunosuppression ([Kapelushnik et al., 2001](#)). Many case reports show primary effusion lymphoma to be in association with Kaposi sarcoma or multicentric Castleman disease, both known to be caused by KSHV. [Ascoli et al. \(2001\)](#), for example, also identified four patients with multicentric Castleman disease and primary effusion lymphoma, all of which had evidence of KSHV DNA in pleural effusions. In a prospective cohort study of 60 HIV-positive patients, three developed primary effusion lymphoma in association with multicentric Castleman disease and/or Kaposi sarcoma, the latter conditions appear to be risk factors for the development of this disease ([Oksenhendler et al., 2002](#)). [Komanduri et al. \(1996\)](#) and numerous case reports (e.g. [Ascoli et al., 1999a, b](#)) described HIV-positive cases of primary effusion lymphoma that had evidence of KSHV infection. Likewise, four patients with primary effusion lymphoma and five cell lines were all reported to be KSHV-positive ([Judde et al., 2000](#)). [Boulanger et al. \(2005\)](#) identified 15 HIV-positive patients with primary effusion lymphoma, all of which were KSHV-positive. Of interest, in this study, six primary effusion lymphoma patients had neither Kaposi sarcoma nor multicentric Castleman disease. [Asou et al. \(2000\)](#) found KSHV to be present in 21/21 patients with primary effusion lymphoma, compared with 0/139 patients with other AIDS- and non-AIDS-related lymphomas,

and mucosa-associated lymphoid tissue (MALT) lymphomas.

During 1996–2008, a total of 95 subjects were identified in the literature as case reports. The majority of these were KSHV-positive and HIV-positive. Nineteen subjects were KSHV-negative and HIV-negative; these were unusual in that all but three were elderly, seven of these were characterized by having had some form of genetic abnormality, three had cirrhosis (two of those were due to infection with the hepatitis C virus), two had some other idiopathic immunodeficiency, and one had a renal transplant. These cases do not fulfill the diagnostic criteria for primary effusion lymphoma ([Said & Ceserman, 2008](#)), and probably represent another form of non-Hodgkin lymphoma involving body cavities. Primary effusion lymphoma has also been reported in people with no obvious immune suppression or HIV infection. See Table 2.5 available at <http://monographs.iarc.fr/ENG/Monographs/vol100B/100B-04-Table2.5.pdf>.

### 2.3 Multicentric Castleman disease

Strictly speaking, multicentric Castleman disease is not a cancer, but a rare polyclonal lymphoproliferative disease that can progress to plasmablastic lymphoma ([Dupin et al., 2000](#)). In addition, [Oksenhendler et al. \(2002\)](#) found that 14/60 HIV-infected patients with multicentric Castleman disease developed non-Hodgkin lymphoma, three of which were primary effusion lymphoma. Several studies have associated multicentric Castleman disease with KSHV. Multicentric Castleman disease was recognized in 1956 ([Castleman et al., 1956](#)), but appears to have increased in incidence as a result of the HIV epidemic, although multicentric Castleman disease in association with KSHV has also been documented in HIV-negative patients ([Hernández et al., 2005](#)).

Table 2.6 (available at <http://monographs.iarc.fr/ENG/Monographs/vol100B/100B-04-Table2.6.pdf>) shows case series that have detected KSHV in patients with multicentric Castleman disease ([Soulier et al., 1995](#); [Barozzi et al., 1996](#); [Corbellino et al., 1996](#); [Gessain et al., 1996](#)). Several hospital-based studies have shown an association between KSHV and multicentric Castleman disease, irrespective of the way in which KSHV is detected and of differences in tissue type (Table 2.7 available at <http://monographs.iarc.fr/ENG/Monographs/vol100B/100B-04-Table2.7.pdf>; [Soulier et al., 1995](#); [Parravicini et al., 1997](#); [Bélec et al., 1999a](#); [Asou et al., 2000](#)). For example, [Soulier et al. \(1995\)](#) found KSHV to be present in all 14/14 HIV-positive cases and 7/17 HIV-negative cases compared with 1/34 controls. Treatment of three multicentric Castleman disease patients with ganciclovir, an inhibitor of KSHV lytic replication, has been reported to ameliorate multicentric Castleman disease ([Casper et al., 2004](#)), while treatment with cidofovir, an inhibitor of KSHV DNA polymerase, was not effective ([Corbellino et al., 2001](#); [Berezne et al., 2004](#)).

### 2.4 Multiple myeloma

A small number of early studies suggested a possible association between KSHV and multiple myeloma ([Rettig et al., 1997](#); [Said et al., 1997](#)), but this was not confirmed by subsequent large well designed serology and PCR-based studies (see Table 2.8 available at <http://monographs.iarc.fr/ENG/Monographs/vol100B/100B-04-Table2.8.pdf>). In addition, the geographic distribution of multiple myeloma is different from that of Kaposi sarcoma and of KSHV, strongly implying different etiologies ([Cottoni & Uccini, 1997](#); [Globocan, 2008](#); [Hjalgrim et al., 1998](#)). [The Working Group noted that a map of the global distribution of Kaposi sarcoma was not available from Globocan 2008 because it is only possible to derive estimates for sub-Saharan African countries.]

Several studies containing a clinical series of patients with multiple myeloma and a comparison group of other hospital patients without *monoclonal gammopathy of undetermined significance* (MGUS, thought to be a precursor of multiple myeloma) or blood donors, have not found an association between markers of KSHV and multiple myeloma ([Mackenzie et al., 1997](#); [Marcelin et al., 1997](#); [Masood et al., 1997](#); [Rettig et al., 1997](#); [Whitby et al., 1997](#); [Agbalika et al., 1998](#); [Santarelli et al., 1998](#); [Bélec et al., 1999b, c](#); [Azzi et al., 2001](#); [Beksac et al., 2001](#); [Patel et al., 2001](#); [Santón Roldán et al., 2002](#); [Zhu et al., 2002](#); [Hermouet et al., 2003](#); [Tsai et al., 2005](#)). [Rettig et al. \(1997\)](#) reported the presence of KSHV sequences using PCR in all 15 of the patients with multiple myeloma, in 2/8 cases with MGUS, and in 0/26 control patients. [The Working Group noted that the majority of studies are based on series of hospital patients which are compared to a series of hospital patients admitted with several unrelated conditions or to blood donors (or both), and therefore the comparison group may not have been adequate. Most studies were typically small in size and did not adjust for age.]

Some of these studies were also reviewed by [Tarte et al. \(1998, 1999\)](#), and many used several serological assays against KSHV LANAs. In two of these studies ([Agbalika et al., 1998](#); [Bélec et al., 1999b](#)), 0/25 cases of multiple myeloma was seropositive versus 0/25 in controls. Neither of these studies attempted to account for age (through matching or adjusting the data).

The biological significance of some of the clinical series, especially those using small PCR fragments has been questioned by [Zong et al. \(2007\)](#). Four studies ([Schönrich et al., 1998](#); [Sitas et al., 1999](#); [Tedeschi et al. 2001, 2005](#)) had age-matched controls or were based on a case-control/comparison design ([Sitas et al., 1999](#)), or had a case-control study design nested within a cohort ([Tedeschi et al., 2001, 2005](#)) (see Table 2.9 available at <http://monographs.iarc.fr/ENG/Monographs/vol100B/100B-04-Table2.9.pdf>).

[Schönrich et al. \(1998\)](#) found KSHV lytic antibodies were present in 4/99 multiple myeloma patients versus 2/67 controls. [Sitas et al. \(1999\)](#) measured the age- and gender-adjusted KSHV immunofluorescence assay seropositivity in 108 black South African incident cancer patients with newly diagnosed multiple myeloma compared with 3185 cancer patients and 85 blood donors. The age- and gender-adjusted KSHV seroprevalence in multiple myeloma patients versus controls was, respectively, 24% versus 32%.

[Tedeschi et al. \(2001\)](#) measured the seroprevalence of KSHV in 47 multiple myeloma cases and 224 age-matched controls from a cohort of 20243 men and 18814 women recruited between 1968–72, and followed until the end of 1991 through the Finnish Cancer Registry. Odds ratios (age-adjusted) for KSHV and multiple myeloma were calculated using IFA lytic antibodies (OR, 2.02; 95%CI: 0.94–4.33; latent OR, 10.0; 95%CI: 0.91–110.3), and Western blot confirmation (any one of ORF 65, 73 and K8.1A; OR, 0.89; 95%CI: 0.25–3.25); none of the associations was found to be statistically significant. In another nested case-control study, [Tedeschi et al. \(2005\)](#) identified 329 cases of multiple myeloma, and matched these to 1631 controls, matched for age and gender. The cases and controls arose from about 1133000 individuals from several Nordic cohorts who had donated blood samples. Seropositivity was 12% in multiple myeloma subjects versus 15% in control subjects. No association was found between multiple myeloma and KSHV lytic (OR, 0.8; 95%CI: 0.5–1.1), or latent antibody levels (OR, 0.6; 95%CI: 0.1–2.7). [The Working Group noted that after stratifying by detection method (PCR, LANA or lytic antibodies), no significant differences in the percent KSHV positivity were observed between multiple myeloma cases and controls when the Working Group used  $\chi^2$  tests for comparison (data not shown).]

## 2.5 Other cancers

[Sitas et al. \(1999\)](#) measured the seroprevalence of KSHV in 16 major cancer types, including Kaposi sarcoma and multiple myeloma. As expected, the seroprevalence of KSHV among people with Kaposi sarcoma was greater than 80%; among people with all other cancers (oral cavity, oesophagus, lung, stomach, liver, colon/rectum, breast, cervix, prostate, non-Hodgkin lymphoma, Hodgkin disease, leukaemia, myeloma, other minor types [where  $n < 50$  in each cancer type]), the seroprevalence was similar to the general population (about 20–30%). Several studies have examined the presence of KSHV sequences in various other cancers but the results are inconclusive. These include mesenchymal tumours (1/76 cases KSHV-positive) ([Kazakov et al., 2002](#)), and other lymphomas (e.g. [Lazzi et al., 1998, 2006](#); [de Sanjose et al., 2004](#)). A case series was reported suggesting an association with large-cell immunoblastic lymphomas ([Hansen et al., 2000](#)), but this has not been confirmed. No associations were found between KSHV and cancer of the prostate in several cohort and case-control studies ([Sitas et al., 1999](#); [Huang et al., 2008](#)), however, one case-control study did present a significant inverse association ([Sutcliffe et al., 2007](#)). KSHV has not been detected in salivary gland tumours ([Atula et al., 1998](#)), despite the fact that KSHV is thought to be transmitted via saliva.

## 2.6 Kaposi sarcoma and cofactors

Infection with KSHV alone is not sufficient to cause Kaposi sarcoma. The most important cofactor predisposing a KSHV-infected person to Kaposi sarcoma is HIV co-infection or, to a lesser extent, other immunodeficient states such as iatrogenic immune suppression in organ transplant recipients ([IARC, 1997](#)). Nonetheless, the incidence of Kaposi sarcoma in specific geographic areas before the HIV epidemic

points to a role of as-yet-unknown cofactors in the etiology of this cancer ([Dedicoat & Newton, 2003](#)).

### 2.6.1 Suspected unknown cofactors

Before the HIV epidemic, Kaposi sarcoma had a much greater geographic variation in incidence than most other malignancies. Classic (sporadic) Kaposi sarcoma occurred but was rare in countries around the Mediterranean, particularly in Italy, Greece, and the Middle East, and was almost non-existent elsewhere in Europe and in the USA, except in immigrants from these countries ([Grulich et al., 1992](#); [IARC, 1997](#)). In contrast, it represented up to 9% of all cancers in parts of sub-Saharan Africa, such as Uganda, in both men and women ([Oettlé, 1962](#); [D'Oliveira & Torres, 1972](#); [Templeton, 1981](#); [Hutt, 1983](#); [Cook-Mozaffari et al., 1998](#); [Dedicoat & Newton, 2003](#)). KSHV infection is prevalent in many African countries, including places where Kaposi sarcoma was almost unknown before HIV, and is as common in women as in men ([Dedicoat & Newton, 2003](#)). However, the incidence of classical Kaposi sarcoma varied markedly across the African continent, primarily affecting men ([Cook-Mozaffari et al., 1998](#)).

Several exposures have been suggested as possible cofactors for diseases that might explain the geographic variation in incidence before the HIV epidemic, both in Africa and elsewhere. These include malaria and other parasitic infections ([Serraino et al., 2003](#); [Lin et al., 2008](#)); fine volcanic soils, which are posited to cause localized immunosuppression in the lower limbs ([Ziegler, 1993](#)); and exposure to specific plants, or “onco-weeds” that might increase viral replication ([Whitby et al., 2007](#)). Although the existence of cofactors is not disputed, for none of these is the evidence sufficiently strong to conclude that there is a definite increase in risk.

### 2.6.2 HIV infection as a strong cofactor

In parts of Africa where Kaposi sarcoma was relatively common even before the era of HIV, the HIV epidemic has led to an explosion in the incidence of the disease ([Curado et al., 2007](#)). In the mid-1990s, the incidence of Kaposi sarcoma increased about 20-fold in Uganda, Zimbabwe, and other sub-Saharan African countries, such that it is now the most common cancer in men, and the second most common in women ([Wabinga et al., 1993, 2000](#); [Bassett et al., 1995](#); [Dedicoat & Newton, 2003](#)). As a result of the HIV epidemic, the incidence of Kaposi sarcoma has also increased in countries where it was previously relatively rare, but where KSHV was prevalent. For example, during 1988–96, the incidence of Kaposi sarcoma increased at least 3-fold in South Africa, and has continued to increase as the HIV epidemic grows. Data from Johannesburg, South Africa, show that incidence rates of Kaposi sarcoma have doubled in men, but have increased 7-fold in women, such that the gender ratio of 7:1 in males versus females in 1988 has now declined to only 2:1 ([Sitas & Newton, 2001](#)). [The Working Group noted that this is an artefact of Kaposi sarcoma incidence being higher in men.] Therefore, in the presence of HIV infection, the role of other etiological cofactors may be less relevant for the development of Kaposi sarcoma than before the spread of HIV ([Dedicoat & Newton, 2003](#)).

### 2.6.3 Host genetic susceptibility

Host genetic variation has been investigated in the etiology of Kaposi sarcoma. In particular, emphasis has been placed on genes that may be relevant to the modulation of host immunity against KSHV ([Brown et al., 2006a, c](#); [Alkharsah et al., 2007](#)), but data in this area remain sparse. More recent evidence suggests that variations in the viral genome itself may also be of relevance, but currently the findings are inconclusive ([Mancuso et al., 2008](#)).

## 3. Cancer in Experimental Animals

In this volume, the Working Group decided not to include a separate section on “Cancer in Experimental Animals” in the *Monographs* on viruses but rather to include description of such studies under Section 4 (below). The reasoning for this decision is explained in the General Remarks.

## 4. Other Relevant Data

### 4.1 Transforming capacity of KSHV

Transformation is a multistep process and KSHV infection has been shown to induce most steps along this progression: a) KSHV infection of primary human endothelial cells leads to morphological alteration and reduced growth-factor dependence ([Ciuffo et al., 2001](#)); b) KSHV infection of immortalized human endothelial cells leads to extended survival, loss of contact inhibition, growth-factor and anchorage independence ([Flore et al., 1998](#); [Moses et al., 1999](#); [Wang & Damania, 2008](#)), and the outgrowth of fully tumorigenic clones ([An et al., 2006](#)); c) KSHV transforms murine endothelial progenitor cells ([Mutlu et al., 2007](#)).

KSHV infection cannot transform mature human B cells in culture ([Kliche et al., 1998](#)). However, this may be due to low infectivity and/or the absence of susceptible cell populations under routine culture conditions, which do not support the growth of haematopoietic cells. Yet, KSHV is clearly required for continued survival of primary effusion lymphoma cells in culture ([Guasparri et al., 2004](#); [Godfrey et al., 2005](#)).

Individual KSHV proteins exhibit transforming capacity in experimental systems (see Sections 4.2 and 4.3), and in transgenic mice (see Section 4.6).

## 4.2 Biochemical and biological properties of KSHV proteins

Several latent or lytic viral proteins are involved in the carcinogenesis process of KSHV (see [Table 4.1](#)).

Five KSHV proteins (K1/VIP, vGPCR, vIRF-1, Kaposin A, LANA) have been reported to have transforming properties in classical transformation assays, others (vCYC, LANA, KbZIP) have been shown to affect cell-cycle regulation or the survival of tumour cells *in vivo* or *in vitro* (vFLIP, vIL6, vIRF-3). Because only some of these proteins are expressed during latency and in the majority of tumour cells, not only 'direct' transformations (as in classical models of virus-mediated cellular transformation) but also indirect (paracrine) effects are thought to play a role in KSHV-mediated oncogenesis ([Ganem, 2007](#)).

### 4.2.1 Latent KSHV proteins

#### (a) LANA/ORF 73

LANA, encoded by ORF 73, is expressed during latency and represents the most consistently detected viral protein in KSHV-associated tumour cells ([Rainbow et al., 1997](#); [Dupin et al., 1999](#); [Katano et al., 2000](#); [Parravicini et al., 2000](#)).

LANA is necessary for replicating the episomal viral DNA; it binds to the latent origin of replication in the TR subunits of the viral genome, and works by recruiting a large variety of cellular interaction partners, among them components of the chromosomal replication machinery such as origin recognition complexes (ORCs), but also cellular proteins linked to transcriptional regulation or proliferation control (see [Table 4.1](#); reviewed in [Verma et al., 2007](#)).

Of relevance to a possible direct role of LANA in oncogenesis are the observations that LANA: (i) inactivates p53-dependent transcriptional activation ([Friborg et al., 1999](#)); (ii) interacts with pRB and enhances oncogenic ras-mediated transformation of rodent fibroblasts ([Radkov](#)

[et al., 2000](#)); (iii) absorbs GSK-3 $\beta$  and thereby reduces the phosphorylation of, thus stabilizing,  $\beta$ -catenin ([Fujimuro et al., 2003](#)); (iv) interacts with Brd2/RING3, a chromatin-binding protein and a lymphomagenic member of the BET protein family ([Platt et al., 1999](#); [Viejo-Borbolla et al., 2005](#)); (v) causes B-cell hyperplasia and B-cell lymphoma when expressed in transgenic mice ([Fakhari et al., 2006](#)).

#### (b) vCYC/ORF 72

In-situ hybridization studies indicate that the KSHV CYC/ORF 72 gene is expressed in the majority of tumour cells *in vivo* ([Davis et al., 1997](#)), in keeping with its classification as a latent gene. v-CYC represents another candidate KSHV oncoprotein because of its homology to the human Cyclin-D/Prad oncoprotein. In general, cyclin-D proteins (D<sub>1</sub>, D<sub>2</sub>, D<sub>3</sub>) associate with specific cyclin-dependent kinases (CDKs), and these complexes phosphorylate pRB family members (reviewed in [Sherr, 1996](#)). An oncogenic cyclin-D homologue is also present in other gammaherpesviruses (reviewed in [Neipel et al., 1997](#)). Ectopic expression of the murine herpesvirus 68 (MHV 68) cyclin in T cells causes T-cell lymphomas in transgenic mice ([van Dyk et al., 1999](#)).

The mechanism of transformation by KSHV vCYC is most likely novel and unique, because it phosphorylates pRB but, unexpectedly, also histone H1, p27<sup>KIP1</sup>, and Bcl-2 ([Chang et al., 1996](#); [Godden-Kent et al., 1997](#); [Li et al., 1997](#); [Ojala et al., 2000](#)). Unlike human cyclin-D, vCYC/CDK6-mediated phosphorylation of pRB is resistant to inhibition by the cyclin-dependent kinase-inhibitors (CDKIs) p16<sup>INK4</sup>, p21<sup>CIP1</sup>, and p27<sup>KIP1</sup> ([Swanton et al., 1997](#)). Moreover, vCYC/CDK6 induces the degradation of p27<sup>KIP1</sup> ([Ellis et al., 1999](#); [Mann et al., 1999](#)).

**Table 4.1 Biochemical and biological properties of KSHV proteins**

KSHV protein	Homology to human protein	Viral Gene	Function in viral life cycle	Biochemical properties	Latent/lytic	Involvement in carcinogenesis	References
K1/VIP <sup>a</sup>		ORF K1	May increase/decrease viral reactivation	Activates several intracellular signalling cascades; Induces angiogenic cytokines; Blocks intracellular transport of BCR-complexes to cell surface	Lytic	Transforming properties Angiogenesis Anti-apoptotic activity	<a href="#">Lee et al. (1998a, b, 2000)</a> , <a href="#">Lagunoff et al. (1999, 2001)</a> , <a href="#">Prakash et al. (2002)</a> , <a href="#">Tomlinson &amp; Damania (2004)</a> , <a href="#">Wang et al. (2004a, 2006)</a>
KCP	Related to a family of mammalian complement regulatory proteins	ORF 4	Inhibits complement activation by virions or virus-infected cells	Cofactor for complement factor I; Accelerates the decay of C3 convertases	Lytic		<a href="#">Mullick et al. (2003)</a> , <a href="#">Spiller et al. (2003)</a> , <a href="#">Mark et al. (2004)</a>
vIL6	Interleukin-6 (IL6) homologue	ORF K2	Viral IL6	Induces proliferation of PEL cell lines Induces VEGF Induces STAT3 phosphorylation	Lytic	Tumour cell survival <i>in vivo</i> and <i>in vitro</i> Angiogenesis Haematopoiesis	<a href="#">Moore et al. (1996a)</a> , <a href="#">Burger et al. (1998)</a> , <a href="#">Aoki et al. (1999)</a> , <a href="#">Aoki &amp; Tosato (1999)</a> , <a href="#">Hoischen et al. (2000)</a> , <a href="#">Kovaleva et al. (2006)</a>
K3/MIR-1/ZMP-B		ORF K3	Downmodulates HLA, ICAM-1, B7.2	E3 ubiquitin ligase	Lytic	Immune evasion	<a href="#">Coscoy &amp; Ganem (2000)</a> , <a href="#">Ishido et al. (2000)</a> , <a href="#">Coscoy et al. (2001)</a> , <a href="#">Cadwell &amp; Coscoy (2005, 2008)</a> , <a href="#">Coscoy (2007) (review)</a>
vCCL-2/vMIP-II	Chemokine homologue related to macrophage inflammatory protein (MIP)-1 $\alpha$	ORF K4	Viral chemokine	Agonist for CCR3, CCR5, CCR8; Induces monocyte chemotaxis	Lytic	Angiogenic properties	<a href="#">Boshoff et al. (1997)</a> , <a href="#">Dairaghi et al. (1999)</a> , <a href="#">Endres et al. (1999)</a> , <a href="#">Nakano et al. (2003)</a>
vCCL-3/vMIP-III	Some homology to chemokines TARC and eotaxin	ORF K4.1	Viral chemokine	CCR4, XCR1 agonist	Lytic	Angiogenic properties	<a href="#">Nicholas et al. (1997)</a> , <a href="#">Stine et al. (2000)</a> , <a href="#">Lüttichau et al. (2007)</a>
K5/MIR-2	Part of a family of membrane-bound-E3-ubiquitin ligases	ORF K5	Downmodulates HLA-A, HLA-B, ICAM-1, CD86, CD1d	E3 ubiquitin ligase	Lytic	Immune evasion	<a href="#">Ishido et al. (2000)</a> , <a href="#">Sanchez et al. (2002)</a> , <a href="#">Coscoy (2007)</a>

**Table 4.1 (continued)**

KSHV protein	Homology to human protein	Viral Gene	Function in viral life cycle	Biochemical properties	Latent/lytic	Involvement in carcinogenesis	References
vCCL-1/ vMIP-I	Chemokine homologue related to macrophage inflammatory protein (MIP)-1 $\alpha$	ORF K6	Viral chemokine	CCR8 agonist; Induces monocyte chemotaxis (VEGF production)	Lytic	Angiogenic properties Anti-apoptotic activity	<a href="#">Nicholas et al. (1997)</a> , <a href="#">Boshoff et al. (1997)</a> , <a href="#">Nakano et al. (2003)</a>
K7/ vIAP	Structurally related to a splice variant of survivin	ORF K7	Apoptosis inhibitor	Binds to and inhibits several proteins involved in apoptosis (see <a href="#">Table 4.2</a> ) Induces degradation of I $\kappa$ B, p53, vGCR	Lytic	Anti-apoptotic activity	<a href="#">Feng et al. (2002, 2004, 2008)</a> , <a href="#">Wang et al. (2002)</a>
vBCL-2	Bcl-2 homologue	ORF 16	Viral Bcl-2	Heterodimerizes with human Bcl-2	Lytic	Anti-apoptotic activity	<a href="#">Sarid et al. (1997)</a>
ORF 36		ORF 36	Viral Cdk2-like kinase	Phosphorylates K-bZIP; Activates JNK pathway	Lytic		<a href="#">Polson et al. (2001)</a> , <a href="#">Hamza et al. (2004)</a> , <a href="#">Izumiya et al. (2007)</a>
ORF 45		ORF 45	Virion protein important for lytic replication Inhibits virus-mediated induction of type 1 interferon	Binds to and inhibits phosphorylation of IRF-7	Lytic	Viron infectivity Immune evasion	<a href="#">Zhu et al. (2002a, 2006)</a> , <a href="#">Zhu &amp; Yuan (2003)</a>
K-RTA		ORF 50	Immediate-early transactivator Ubiquitin E3 ligase	Binds to and activates several lytic viral promoters directly or by interacting with RBPJ $\kappa$ Promotes ubiquitination and degradation of IRF7 Represses p53	Lytic	Reactivation of lytic viral replication from latency Immune evasion Anti-apoptotic activity	<a href="#">Sun et al. (1998)</a> , <a href="#">Gradoville et al. (2000)</a> , <a href="#">Gwack et al. (2001)</a> , <a href="#">Lukac et al. (2001)</a> , <a href="#">Liang &amp; Ganem (2003)</a> , <a href="#">Yu et al. (2005)</a>
K-bZIP		ORF K8	Modulates cell cycle and lytic reactivation	EBV Zta homologue; Binds to lytic replication origin; Binds to, antagonizes, and recruits p53 to ND10/PML bodies; Inhibits G1/S transition; Co-regulator of K-RTA	Lytic	Deregulation of cell cycle	<a href="#">Lin et al. (1999, 2003)</a> , <a href="#">Park et al. (2000)</a> , <a href="#">Katano et al. (2001)</a> , <a href="#">Izumiya et al. (2003a, b)</a>

**Table 4.1 (continued)**

KSHV protein	Homology to human protein	Viral Gene	Function in viral life cycle	Biochemical properties	Latent/lytic	Involvement in carcinogenesis	References
ORF 57/MTA		ORF 57	Exports intronless viral RNAs from nucleus and promotes their translation; Required for the formation of viral progeny	Binds to intronless viral mRNA; Recruits hTREX complex	Lytic		<a href="#">Malik et al. (2004)</a> , <a href="#">Nishimura et al. (2004)</a> , <a href="#">Majerciak et al. (2007)</a> , <a href="#">Boyne et al. (2008)</a>
vIRF-1	Interferon regulatory factor homologue	ORF K9	Modulates viral interferon responses	Prevents IRF-3-mediated transcription Inhibits p53-transcriptional activity and prevents p53-dependent apoptosis	Lytic	Transformation of rodent fibroblasts Immune evasion Anti-apoptotic activity	<a href="#">Gao et al. (1997)</a> , <a href="#">Burýsek et al. (1999)</a> , <a href="#">Lin et al. (2001)</a> , <a href="#">Nakamura et al. (2001)</a> , <a href="#">Seo et al. (2001)</a>
vIRF-4	Interferon regulatory factor homologue	ORF K10		No functional data available	Lytic		<a href="#">Offermann (2007) (review)</a>
vIRF3/LANA-2	Interferon regulatory factor homologue	ORF K10.5	Modulates viral interferon responses	Modulates p53 function; Activates IRF-3, IRF-7, c-myc; Inhibits IRF-5	Lytic in endothelial cells; latent in B-cells	Anti-apoptotic activity Immune evasion Cell survival	<a href="#">Rivas et al. (2001)</a> , <a href="#">Cunningham et al. (2003)</a> , <a href="#">Lubyova et al. (2004, 2007)</a> , <a href="#">Wies et al. (2008, 2009)</a>
vIRF-2	Interferon factor homologue	ORF K11		Inhibits interferon induction; Inhibits induction of CD95L	Lytic	Immune evasion Anti-apoptotic activity	<a href="#">Burýsek &amp; Pitha (2001)</a> , <a href="#">Kirchhoff et al. (2002)</a> , <a href="#">Fuld et al. (2006)</a>
Kaposin A <sup>a</sup>		ORF K12		Interacts with cytohesin-1	Latent/lytic	Transforming properties in cultured cells	<a href="#">Zhong et al. (1996)</a> , <a href="#">Muralidhar et al. (1998)</a> , <a href="#">Sadler et al. (1999)</a> , <a href="#">Kliche et al. (2001)</a> , <a href="#">Tomkowicz et al. (2005)</a>
Kaposin B <sup>a</sup>	Alternative reading frame in ORF K12 mRNA		Modulation of cytokine mRNAs regulated by the p38 pathway	Modulates p38/MK2	Latent/lytic		<a href="#">Sadler et al. (1999)</a> , <a href="#">McCormick &amp; Ganem (2005, 2006)</a>

**Table 4.1 (continued)**

KSHV protein	Homology to human protein	Viral Gene	Function in viral life cycle	Biochemical properties	Latent/lytic	Involvement in carcinogenesis	References
miRs			Viral microRNAs; Regulation of cellular genes (e.g. genes involved in B-cell differentiation)	miRK-11 regulates similar genes as cellular miR-155	Latent	Potential involvement in: Angiogenesis Immune modulation Anti-apoptotic activity	<a href="#">Cai et al. 2005</a> , <a href="#">Pfeffer et al. 2005</a> , <a href="#">Samols et al. 2005</a> , <a href="#">Grundhoff et al. 2006</a> , <a href="#">Skalsky et al. 2007</a> , <a href="#">Gottwein et al. 2007</a>
vFLIP	Homologue of FLICE (caspase-8)-inhibitory proteins	ORF K13/ ORF 71	Viral persistence; Spindle cell formation and lymphomagenesis; Inhibits lytic viral replication	Activates NF-κB; Inhibits CD95/Fas-induced apoptosis, anoikis, superoxide-induced cell death; Modulates MHC-I expression	Latent	Cell survival Anti-apoptotic activity Immune evasion	<a href="#">Keller et al. 2000</a> , <a href="#">Grundhoff &amp; Ganem 2001</a> , <a href="#">An et al. 2003</a> , <a href="#">Field et al. 2003</a> , <a href="#">Sun et al. 2003</a> , <a href="#">Guasparri et al. 2004</a> , <a href="#">2006</a> , <a href="#">Grossmann et al. 2006</a> , <a href="#">Lagos et al. 2007</a> , <a href="#">Matta et al. 2007</a> , <a href="#">Ye et al. 2008</a> , <a href="#">Efklidou et al. 2008</a> , <a href="#">Thurau et al. 2009</a>
vCyclin/ vCYC	D-type cyclin homologue	ORF 72	Viral cyclin; Strongly activates CDK6 protein kinase activity	Phosphorylates H1, pRB, BCL-2, p27 <sup>KIP1</sup> in tandem with CDK6	Latent	Deregulation of cell cycle	<a href="#">Chang et al. 1996</a> , <a href="#">Godden-Kent et al. 1997</a> , <a href="#">Li et al. 1997</a> , <a href="#">Swanton et al. 1997</a> , <a href="#">Ellis et al. 1999</a> , <a href="#">Mann et al. 1999</a> , <a href="#">Ojala et al. 2000</a> , <a href="#">Sarek et al. 2006</a> , <a href="#">Koopal et al. 2007</a>

**Table 4.1 (continued)**

KSHV protein	Homology to human protein	Viral Gene	Function in viral life cycle	Biochemical properties	Latent/lytic	Involvement in carcinogenesis	References
LANA		ORF 73	Replication and maintenance of latent viral episome; Partition of episomes to daughter cells	Interacts with histones, p53, pRB, BET proteins, GSK-3β and others; Induces S-phase entry Activates hTERT transcription; Recruits origin-binding proteins	Latent	Anti-apoptotic activity Cell survival Deregulation of cell cycle Transforming properties in cells Tumorigenicity in mice	<a href="#">Rainbow et al. (1997)</a> , <a href="#">Ballestas et al. (1999)</a> , <a href="#">Friberg et al. (1999)</a> , <a href="#">Platt et al. (1999)</a> , <a href="#">Radkov et al. (2000)</a> , <a href="#">Ballestas &amp; Kaye (2001)</a> , <a href="#">Hu et al. (2002)</a> , <a href="#">Fujimuro et al. (2003)</a> , <a href="#">Watanabe et al. (2003)</a> , <a href="#">Verma et al. (2004)</a> , <a href="#">Hu &amp; Renne (2005)</a> , <a href="#">Viejo-Borbolla et al. (2005)</a> , <a href="#">Fakhari et al. (2006)</a> , <a href="#">Ottinger et al. (2006)</a>
vOX2	OX2 homologue	ORF K14	Modulates inflammatory and T-cell responses	Activates or downregulates myeloid lineage cells in a CD200-like manner	Lytic		<a href="#">Chung et al. (2002)</a> , <a href="#">Foster-Cuevas et al. (2004)</a>
vGCR	Homologue of G-protein-coupled receptor	ORF 74	Stimulates cellular proliferation	Activates Akt, MEK/Erk, JNK, p38; Induces angiogenic cytokines	Lytic	Transforming properties in cells Tumorigenicity in mice Angiogenesis Anti-apoptotic activity	<a href="#">Arvanitakis et al. (1997)</a> , <a href="#">Bais et al. (1998)</a> , <a href="#">Yang et al. (2000)</a> , <a href="#">Holst et al. (2001, 2003)</a> , <a href="#">Montaner et al. (2001, 2003)</a> , <a href="#">Guo et al. (2003)</a> , <a href="#">Muthu et al. (2007)</a> , <a href="#">Nicholas (2007) (review)</a>
K15 protein <sup>a</sup>		ORF K15	Recruits endothelial cells to infected cells	Activates NF-κB, MEK/Erk; Induces inflammatory cytokines; Interacts with proteins involved in signal transduction (e.g. TRAFs 1, 2, 3), with members of src family of PTK, and with an apoptotic regulatory protein HAX-1	Lytic (possibly latent in B-cells)	Possibly anti-apoptotic activity Possibly angiogenesis	<a href="#">Glenn et al. (1999)</a> , <a href="#">Choi et al. (2000)</a> , <a href="#">Sharp et al. (2002)</a> , <a href="#">Brinkmann et al. (2003, 2007)</a> , <a href="#">Wang et al. (2007)</a>

<sup>a</sup> protein unique to KSHV

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(c) *vFLIP/ORF 71*

*vFLIP/ORF 71* is transcribed from the LANA promoter, and translated from an internal ribosome entry site located within the v-cyclin coding region ([Grundhoff & Ganem, 2001](#); [Low et al., 2001](#)). It is therefore thought to be expressed during latency and in all tumour cells. The vFLIP protein is an adhesion molecule, a homologue of cellular FLICE (caspase-8)-inhibitory protein (FLIP) ([Hu et al., 1997](#)). It inhibits CD95/FAS-induced apoptosis *in vitro* by blocking caspase-3, -8 and -9 ([Djerbi et al., 1999](#)).

*vFLIP* directly binds IKK $\gamma$  and TRAF2; this leads to a constitutive activation of NF- $\kappa$ B signalling ([Field et al., 2003](#); [Guasparri et al., 2006](#)). In addition, *vFLIP* induces MHC-I expression through NF- $\kappa$ B in KSHV-infected lymphatic endothelial cells ([Lagos et al., 2007](#)), which underscores the physiological importance of the *vFLIP*-NF- $\kappa$ B interaction. Moreover, *vFLIP* transgenic mice develop lymphoma ([Chugh et al., 2005](#)). Eliminating either *vFLIP* or NF- $\kappa$ B activity from primary effusion lymphoma cells induces apoptosis ([Keller et al., 2000](#); [Guasparri et al., 2004](#)), demonstrating that this pathway is essential for lymphomagenesis.

(d) *Kaposin A/ORF K12*

The many transcripts spanning the predicted K12 ORF (originally called T0.7) are translated in different reading frames, giving rise to the proteins kaposin A, B, C ([Zhong et al., 1996](#); [Sadler et al., 1999](#)). In addition, a long transcript extending through *ORF K12* represents the precursor RNA for the KSHV microRNAs ([Cai et al., 2005](#); [Pfeffer et al., 2005](#); [Samols et al., 2005](#)). One of these microRNAs, miRK-10, is located within the kaposin A sequence. These transcripts are expressed in all Kaposi sarcoma spindle cells ([Staskus et al., 1997](#); [Stürzl et al., 1997](#)), and increase after activation of the lytic replication cycle.

[Muralidhar et al. \(1998\)](#) reported that transfection of the kaposin A reading frame into Rat-3 cells induced focus formation, and that these cell lines were tumorigenic in nude mice. There is some discussion as to whether this is due to the kaposin A protein or the miR-K10 located within its sequence. Kaposin A interacts directly with Cytohesin-1, a guanine nucleotide exchange factor for ARF GTPases, and a regulator of integrin-mediated cell adhesion. It was shown that the transformed cellular phenotype induced by Kaposin A in tissue culture could be reversed by a functionally dead Cytohesin-1 mutant ([Kliche et al., 2001](#)).

(e) *vIRF-3/LANA-2*

*vIRF-3*, one of the viral homologues of interferon regulatory factors, is constitutively expressed in latently KSHV-infected cells, and its expression appears to be tissue specific. *vIRF-3* is indeed detected in nearly all primary effusion lymphoma cells and KSHV-associated Castleman disease cells (both of B-lymphocyte origin), but is not expressed in Kaposi sarcoma tumours (endothelial origin) ([Rivas et al., 2001](#)).

*vIRF-3* binds to and antagonizes p53, and affects the regulation of the interferon response (see [Table 4.1](#)).

Knockdown of *vIRF-3* in primary effusion lymphoma cells has been shown to induce apoptosis, suggesting that *vIRF-3* is required for the survival of KHSV-infected B cells ([Wies et al., 2008](#)).

#### 4.2.2 Lytic KSHV proteins

(a) *K1/VIP (variable, ITAM-containing protein)*

*K1/VIP* is a viral type I transmembrane protein, featuring two hypervariable domains in its extracellular region, and an immunoglobulin transactivation motif (ITAM) in its C-terminal, cytoplasmic region ([Lee et al., 1998b](#)). The K1 protein is expressed during the lytic (productive) replication cycle ([Jenner et al.,](#)

[2001](#); [Paulose-Murphy et al., 2001](#); [Nakamura et al., 2003](#)). [Wang et al. \(2006\)](#) reported that, while expression of K1 is not a consistent feature of Kaposi sarcoma, some Kaposi sarcoma biopsies show a marked K1-expression both at the transcript and protein level. K1-expression was also documented by immunohistochemistry on a small subpopulation of mantle zone lymphocytes of KSHV-positive multicentric Castleman disease, and in primary effusion lymphoma cell lines ([Lee et al., 2003](#)).

[Lee et al. \(1998a\)](#) showed that transfection of a K1-expression vector into rodent fibroblasts induced focus formation, and that K1-transfectants induced lymphoma in the common marmoset. [Prakash et al. \(2002\)](#) reported the emergence of sarcomatoid tumours and plasmablastic lymphoma in transgenic mice expressing the K1 protein under the control of an SV40 promoter. These mice showed an increased expression of bFGF; in transgenic B cells, a constitutive activation of NF- $\kappa$ B and increased c-Lyn activity was noted. [Wang et al. \(2006\)](#) showed that retroviral transduction of primary endothelial cells extended their life span.

K1 activates several intracellular signalling cascades leading to increased Ca-influx, increased phosphorylation of Syk, Vav, Cbl, and the p85 subunit of PI3K, increased NF- $\kappa$ B activity, and activation of NFAT and AP1 ([Lee et al., 1998b](#); [Lagunoff et al., 1999, 2001](#)). The activation of PI3K leads to the activation of AKT by K1 ([Tomlinson & Damania, 2004](#)). K1 induces the expression of angiogenic cytokines, including vascular endothelial growth factor (VEGF), and may therefore play a paracrine role in the pathogenesis of Kaposi sarcoma or primary effusion lymphoma ([Wang et al., 2004a, 2006](#)).

#### (b) vIRF-1/ORF K9

vIRF-1 belongs to a group of four viral homologues of interferon regulatory factors ([Russo et al., 1996](#); [Cunningham et al., 2003](#)). Stable vIRF-1 transfectants in murine NIH

3T3 cells show signs of transformation (loss of contact inhibition, growth in soft agar), and cause tumours in nude mice ([Gao et al., 1997](#)). The main function of vIRF-1 appears to be the inhibition of interferon- $\beta$ -regulated genes such as p21<sup>CIP1</sup> ([Gao et al., 1997](#)); it also inhibits the induction phase of the interferon response by binding to cellular IRFs (IRF-3, IRF-7), and to the transcriptional co-activators p300 and CBP, and inhibits the formation of functional IRF-3/CBP/p300 complexes and the induction of interferon  $\beta$  transcription ([Gao et al., 1997](#); [Burýsek et al., 1999](#); [Seo et al., 2000](#); [Lin et al., 2001](#)). Unlike cellular IRFs, vIRF-1 does not bind directly to cellular DNA.

vIRF-1 is expressed during the lytic (productive) replication in tissue culture and is directly transactivated by K-RTA, the central regulator of the viral lytic programme ([Gao et al., 1997](#); [Chen et al., 2000](#)).

#### (c) vGPCR /ORF 74

ORF 74 encodes a homologue of a G-protein-coupled chemokine receptor, and is constitutively active (reviewed in [Nicholas, 2007](#)). It activates a broad range of signalling pathways, including MEK/Erk, JNK, p38, Akt, NFAT, CREB, NF- $\kappa$ B, AP-1, and HIF-1 $\alpha$ ; these are relevant to the promotion of cell proliferation, cell survival, and angiogenic responses via cytokine gene induction (reviewed in [Nicholas, 2007](#); [Hartmann, 2008](#)). Although constitutive, the activity of vGPCR can be modulated both positively and negatively by several cellular chemokines (Gro $\alpha$ , IL8, IP-10, SDF-1 $\alpha$ ), and one viral (vCCL-2) chemokine (reviewed in [Hartmann, 2008](#)).

Multiple lines of evidence point to a role of vGPCR in KSHV-induced neoplasia, in particular Kaposi sarcoma. Early studies showed the proliferation-enhancing, constitutive signalling, and transforming properties of vGPCR ([Arvanitakis et al., 1997](#); [Bais et al., 1998](#)). Subsequently, vGPCR was shown to cause Kaposi-sarcoma-like tumours in transgenic mice ([Yang et al.,](#)

[2000](#)). In this model, vGPCR was only expressed in a few scattered cells, consistent with a paracrine model involving secretion of angiogenic cytokines ([Holst et al., 2001](#); [Guo et al., 2003](#); [Montaner et al., 2003](#)).

In a xenograft model, vGPCR involving a KSHV-transfected murine endothelial cell line was found to be required for tumorigenicity ([Mutlu et al., 2007](#)). The relevance of these results to KSHV-associated tumours in humans remains to be determined.

#### 4.2.3 Genomic instability

Evidence of genomic instability has been noted in primary effusion lymphoma cells (microsatellite instability, chromosomal imbalances) ([Gaidano et al., 1997](#); [Nair et al., 2006](#)), and late Kaposi sarcoma ([Pyakurel et al., 2006](#)). Experimentally, genomic instability has been noted in KSHV-infected primary endothelial cells ([Pan et al., 2004](#)), as well as in cell lines stably transfected with LANA ([Si & Robertson, 2006](#)), and vCYC-transgenic mice ([Verschuren et al., 2004](#)). Abnormal chromosome segregation in KSHV-infected cells was shown to be the consequence of nucleophosmin (NPM1) phosphorylation by CDK6 in concert with vCYC ([Cuomo et al., 2008](#)).

#### 4.2.4 DNA-damage response

Transduction of vCYC into primary endothelial cells by a retroviral vector induces a DNA damage response, resulting in the increased phosphorylation of  $\gamma$ H2AX (a variant form of histone H2A), which is an early response to DNA double-strand breaks. Increase of  $\gamma$ H2AX phosphorylation was also shown in KSHV-infected primary endothelial cells, albeit only after 2 weeks of culture (in spite of vCYC being expressed early on) ([Koopal et al., 2007](#)). Other KSHV proteins might therefore interfere with the triggering of the DNA-damage response.

[Shin et al. \(2006\)](#) reported that vIRF-1 prevents the DNA-damage response and  $\gamma$ H2AX and p53 phosphorylation by binding to and inhibiting ATM kinase, thereby promoting p53 turnover. As noted above, vIRF-1 and LANA interact with p53 and antagonize the transcription of p53-dependent cellular genes, including p21<sup>CIP1</sup> ([Gao et al., 1997](#); [Friborg et al., 1999](#)). In KSHV-infected primary effusion lymphoma cells, vIRF-3 also binds to p53 and inhibits the activation of the p53 promoter ([Rivas et al., 2001](#)). In primary effusion lymphoma cells, LANA, p53 and Hdm2 form a trimeric complex ([Sarek et al., 2007](#)), and the restoration of the p53 function by treatment with an inhibitor of the p53-Hdm2 interaction – Nutlin-3a – induces apoptosis in primary effusion lymphoma cells ([Petre et al., 2007](#); [Sarek et al., 2007](#)).

Most reports have concluded that the inhibition of p53-activated cellular genes by LANA, vIRF-1, and vIRF-3 involves other mechanisms than the degradation or increased turnover of p53. However, one report showed that the recruitment of the Cul5-Elongin BC E3 ligase complex by LANA resulted in the degradation of p53 ([Cai et al., 2006](#)).

#### 4.2.5 Cell proliferation and differentiation

KSHV-infected primary endothelial cells undergo spindle cell formation, which express markers of the lymphatic endothelium. Gene expression array studies have shown that KSHV can alter the transcriptome profile of vascular endothelial cells towards a profile that is typical for lymphatic endothelial cells ([Carroll et al., 2004](#); [Hong et al., 2004](#); [Wang et al., 2004b](#)). This involves the activation of Prox-1, a transcription factor determining lymphatic endothelial cell differentiation, followed by the increased expression of podoplanin and VEGFR-3 – markers for the lymphatic endothelial cell lineage ([Carroll et al., 2004](#); [Hong et al., 2004](#)). The signalling pathways gp130 (the  $\beta$ -chain of the IL6 receptor

used by vIL6) as well as PI3K/Akt and JAK2/STAT3 have been reported to be involved in the induction of Prox-1 in KSHV-infected endothelial cells ([Morris et al., 2008](#)).

These data raise the possibility that KSHV infects blood or circulating endothelial cells, and drives them to differentiate into the lymphatic endothelium as they become spindle cells. This may be of major importance to Kaposi sarcoma tumour formation ([Morris et al., 2008](#)).

The adoption of a spindle morphology in KSHV-infected cells is thought to be due to vFLIP, a homologue of cellular FLIPs, and potent NF- $\kappa$ B inducer; vFLIP is expressed in latently infected endothelial cells, and NF- $\kappa$ B activation appears to be required for the formation of spindle cells ([Grossmann et al., 2006](#); [Sun et al., 2006](#)). vFLIP is also required essentially for primary effusion lymphoma cell survival (see Section 4.2.1).

The viral IL6 homologue, vIL6, is expressed *in vivo* in a subpopulation of primary effusion lymphoma cells and in many KSHV-infected B cells in multicentric Castleman disease lymphoid follicles ([Moore et al., 1996c](#); [Katano et al., 2000](#); [Parravicini et al., 2000](#)). It induces proliferation, angiogenesis, and haematopoiesis in IL6-dependent cell lineages ([Burger et al., 1998](#); [Aoki et al., 1999](#); [Hoischen et al., 2000](#)), and serves as an essential autocrine factor in primary effusion lymphoma cell lines ([Jones et al., 1999](#)). It also induces VEGF, which has been implicated in the pathogenesis of primary effusion lymphoma and of Kaposi sarcoma ([Aoki & Tosato, 1999](#)). A single-chain antibody to vIL6, blocking its interaction with the IL6 receptor complex, was found to inhibit the proliferation of a primary effusion lymphoma cell line and to inhibit vIL6-induced STAT 3 phosphorylation in vIL6-transfected cells ([Kovaleva et al., 2006](#)). Therefore, vIL6 may contribute to primary effusion lymphoma cell proliferation and to the angiogenesis noted in patients with this lymphoma.

Also, the viral D-type cyclin homologue vCYC and LANA each contribute to cell proliferation (see Section 4.2.1)

One of the viral latent transcripts in primary effusion lymphoma cells, miRNA-K12-11, has been found to target the same cellular microRNAs as miRNA-155, a cellular microRNA regulating the germinal centre reaction during B-cell maturation ([Gottwein et al., 2007](#); [Skalsky et al., 2007](#); [Thai et al., 2007](#)). Both miRNA-K12-11 and miR-155 downregulate several pro-apoptotic cellular genes (see [Table 4.2](#)). miRNA-K12-11 may therefore be involved in blocking terminal B-cell differentiation that contributes to the plasmablastic phenotype of primary effusion lymphoma cells or play a role in the protection of primary effusion lymphoma cells against apoptosis.

These findings highlight how KSHV can affect the differentiation of endothelial cells and of B cells.

vIRF3, an interferon regulation factor homologue, is required for primary effusion lymphoma cell survival ([Wies et al., 2008](#)).

Ablation of the human cytokines IL-6, IL-10, and VEGF or of VEGFR inhibits the growth of primary effusion lymphoma and Kaposi sarcoma ([Masood et al., 1997](#); [Nakamura et al., 1997](#); [Aoki & Tosato, 1999](#); [Arora et al., 1999](#); [Jones et al., 1999](#); [Sin et al., 2007](#)). IFN- $\alpha$  inhibits KSHV reactivation and Kaposi sarcoma tumour growth ([Krown et al., 1986](#); [Chang et al., 2000](#)).

#### 4.2.6 Apoptosis

Several KSHV proteins have been shown to protect against apoptosis when transfected individually. Among them are some of the proteins already discussed above, as well as a viral homologue of cellular Bcl2. [Table 4.2](#) shows a summary of their mode of actions.

**Table 4.2 Mode of action of KSHV proteins involved in the protection against apoptosis**

KSHV protein	Mode of action	References
K1/VIP	Activation of PI3K/Akt; Inhibition of FKHR-mediated apoptosis; Inhibition of Fas-induced apoptosis	<a href="#">Tomlinson &amp; Damania (2004)</a> , <a href="#">Wang et al. (2004a)</a> , <a href="#">Uddin et al. (2005)</a> ,
vBcl-2	Inhibition of Bax-mediated apoptosis; Selective interaction with BH3-only proteins	<a href="#">Cheng et al. (1997)</a> , <a href="#">Sarid et al. (1997)</a> , <a href="#">Flanagan &amp; Letai (2008)</a>
vCCL-1/vMIP-I; vCCL-2/ vMIP-II	VEGF-independent anti-apoptotic effect	<a href="#">Liu et al. (2001)</a>
K7/vIAP	Inhibition of apoptosis by binding to Bcl-2 and caspase-3; Promotes p53 and IκB degradation by interacting with Ubiquilin/PLIC1; Binds to cellular CAML (calcium-modulating cyclophilin ligand); Increases cytosolic Ca <sup>2+</sup> response to an apoptotic stimulus	<a href="#">Feng et al. (2002, 2004)</a> , <a href="#">Wang et al. (2002)</a>
K-RTA	Represses p53-dependent transcription and apoptosis through interaction with CBP	<a href="#">Gwack et al. (2001)</a>
vIRF-1	Degradation of p53 by binding to p53 and ATM; Inhibits ATM-mediated phosphorylation of p53 on serine 15; Interacts with GRIM-19 and inhibits interferon or retinoic-acid-induced apoptosis; Inhibits induction of CD95L	<a href="#">Gao et al. (1997)</a> , <a href="#">Nakamura et al. (2001)</a> , <a href="#">Seo et al. (2001, 2002)</a> , <a href="#">Kirchhoff et al. (2002)</a> , <a href="#">Shin et al. (2006)</a>
vIRF-2	Inhibits induction of CD95L; Binds to and inhibits the activation of the IFN-induced ds-RNA-activated kinase (PKR)	<a href="#">Burýsek &amp; Pitha (2001)</a> , <a href="#">Kirchhoff et al. (2002)</a>
vIRF-3/LANA-2	Inhibits apoptosis in PEL cells; Binds to p53 and inhibits p53-induced transcription and apoptosis; Inhibits apoptosis triggered by PKR	<a href="#">Rivas et al. (2001)</a> , <a href="#">Esteban et al. (2003)</a> , <a href="#">Wies et al. (2008)</a>
miRNA K12-11	Downregulation of proapoptotic cellular genes, e.g. LDOC1, Bim, BCLAF1 (Bcl2-associated transcription factor 1), BAZF (NF-κB regulator)	<a href="#">Gottwein et al. (2007)</a> , <a href="#">Skalsky et al. (2007)</a>
LANA	Inhibits p53-induced apoptosis; Counteracts pro-apoptotic effects of simultaneously expressed vCyc; Stabilizes and activates c-Myc	<a href="#">Friborg et al. (1999)</a> , <a href="#">Curreli et al. (2005)</a> , <a href="#">Bubman et al. (2007)</a> , <a href="#">Liu et al. (2007a)</a> , <a href="#">Petre et al. (2007)</a> , <a href="#">Sarek et al. (2007)</a> , <a href="#">Cuomo et al. (2008)</a>
vFLIP	Binds to FLICE complex; Inhibits CD95/Fas-induced apoptosis; Persistent activation of NF-κB	<a href="#">Thome et al. (1997)</a> , <a href="#">Chaudhary et al. (1999)</a> , <a href="#">Djerbi et al. (1999)</a> , <a href="#">Stürzl et al. (1999)</a> , <a href="#">Liu et al. (2002)</a> , <a href="#">An et al. (2003)</a> , <a href="#">Field et al. (2003)</a> , <a href="#">Sun et al. (2003)</a> , <a href="#">Guasparri et al. (2004)</a> , <a href="#">Godfrey et al. (2005)</a>
vGCR	Promotes endothelial cell survival by activating PI3K/Akt pathway	<a href="#">Montaner et al. (2001)</a>
K15	Interacts with HAX-1 (an anti-apoptotic regulatory protein)	<a href="#">Sharp et al. (2002)</a>

FLICE, (FADD [Fas-associated death domain]-like IL-1 $\beta$ -converting enzyme); FLIPs, FLICE-inhibitory proteins  
Compiled by the Working Group

## 4.3 Evidence for a role of KSHV in malignant conversion

### 4.3.1 Kaposi sarcoma

#### (a) Requirement of KSHV expression for cell growth invasion

*In vitro*, KSHV alters the transcriptional programme in infected primary endothelial cells, leading to a redifferentiation of vascular endothelial cells into lymphatic endothelial cells; this results in the formation of spindle cells that are similar to spindle cells in Kaposi sarcoma (see Section 4.2.4). These infected cells, however, cannot be maintained in long-term culture as they show evidence of spontaneous lytic reactivation ([Ciuffo et al., 2001](#); [Lagunoff et al., 2002](#)).

Owing to a lack of an easily tractable *in vivo* model, not many published studies have addressed the question of whether or not KSHV is required for cell growth and invasion *in vivo*. [Mutlu et al. \(2007\)](#) reported that transfection of a bacterial artificial chromosome vector carrying a KSHV genome into murine endothelial cells derived from bone marrow generated a cell that would induce a Kaposi-sarcoma-like tumour when transplanted into mice. Sublines that had lost the KSHV genome in the absence of drug selection lost their tumour-inducing potential, as did KSHV-genome carrying cells, in which the expression of vGPCR had been silenced by siRNA ([Mutlu et al., 2007](#)).

#### (b) Persistence of the KSHV genome

Similarly to the Epstein-Barr virus (EBV), KSHV is capable of replicating its latent episomal genome synchronously with the host cell cycle. However, latent genomes do not appear to persist efficiently in infected primary endothelial cells, nor in epithelial, endothelial or fibroblast cell lines ([Foreman et al., 1997](#); [Renne et al., 1998](#); [Blackbourn et al., 2000](#); [Grundhoff & Ganem, 2004](#)). In several cell lines, only a small subpopulation of cells are able to retain the virus in a stable

manner following acute infection ([Grundhoff & Ganem, 2004](#); [An et al., 2006](#)).

#### (c) Chromosomal abnormalities, and alterations of specific proto-oncogenes

Most cases of Kaposi sarcoma are cytogenetically normal. However, in some cases, the loss of Y-chromosomal sequences and the gain at 11q13 with an amplification of two oncogenes FGF4 and INT<sub>2</sub>, as detected by comparative genome hybridization, have been noted ([Kiuru-Kuhlefelt et al., 2000](#); [Pyakurel et al., 2006](#)). In short-term cultures of Kaposi sarcoma cells, numerical chromosomal abnormalities have been noted ([Delli Bovi et al., 1986](#); [Scappaticci et al., 1986](#); [Saikevych et al., 1988](#)). Defined chromosomal abnormalities (loss of copies of chromosomes 14 and 21; deletions in the short arm of chromosome 3 at 3p14) were also noted in two permanent cell lines, KS-Y1 and KS-SLK, established from Kaposi sarcoma biopsies ([Popescu et al., 1996](#)). Abnormalities at 3p14 were also noted in another permanent Kaposi sarcoma cell line, KS-IMM, in addition to further chromosomal changes ([Casalone et al., 2001](#)).

No p53 mutations have been reported for the majority of Kaposi sarcoma. Host cell tumour-suppressor mRNAs are dysregulated in KSHV-associated-tumours.

### 4.3.2 Primary effusion lymphoma

#### (a) Requirement of KSHV expression for cell growth and invasion

In primary effusion lymphoma cell lines, siRNA- and shRNA-mediated knockdown of several latent genes, in particular vFLIP and vIRF-3, induces apoptotic death ([Godfrey et al., 2005](#); [Guasparri et al., 2006](#); [Wies et al., 2008](#)). This suggests that these viral genes need to be continuously expressed to ensure the survival of primary effusion lymphoma cells.

### (b) Persistence of the KHSV genome

B-Lymphoma cell lines derived from primary effusion lymphoma, contrary to most KSHV-infected cells, retain a stable latent viral genome in high copy numbers (50–100 copies/cell) ([Cesarman et al., 1995a, b](#); [Boshoff et al., 1998](#); [Katano et al., 1999](#); [Morand et al., 1999](#)).

Knockdown of LANA in primary effusion lymphoma cell lines leads to a reduction in the viral genome copy numbers, this in keeping with the accepted role of LANA in episome replication ([Godfrey et al., 2005](#))

### (c) Chromosomal abnormalities

In primary effusion lymphoma cells, complete or partial trisomy 12, trisomy 7, and abnormalities of bands 1q21–25 were noted frequently in addition to mutations in the 5' untranslated region of the *BCL-6* gene ([Gaidano et al., 1999](#); [Wilson et al., 2002](#)). Additional chromosomal changes were noted in the studies by [Wilson et al. \(2002\)](#) and by [Nair et al. \(2006\)](#).

No p53 mutations have been reported for the majority of primary effusion lymphoma.

#### 4.3.3 Multicentric Castleman disease

Multicentric Castleman disease is a poly-clonal lymphoproliferative disease that can be a precursor to frank lymphoma. In addition to LANA, vIL6 is expressed in multicentric Castleman disease B cells (see Section 4.2.4). Because vIL6 is a potent stimulator of B-cell growth, it is likely that this protein plays an important role in the B-cell proliferation seen in multicentric Castleman disease.

### 4.4 Interaction between KSHV and environmental agents

Many agents have an impact on the biology of KSHV:

1. EBV is present in 70–90% of primary effusion lymphomas. EBV-positive and EBV-negative primary effusion lymphomas can be distinguished from each other on the basis of host gene transcription ([Fan et al., 2005](#)). However, no differences in clinical appearance, tumorigenicity in mice or response to therapy have been observed between EBV-positive and EBV-negative primary effusion lymphomas ([Keller et al., 2000](#); [Petre et al., 2007](#)).
2. Cytomegalovirus (CMV) can reactivate KSHV, and the suppression of CMV has been shown to suppress KSHV viral loads ([Martin et al., 1999](#); [Vieira et al., 2001](#)). However, at the time of writing, no evidence for a direct role for CMV in Kaposi sarcoma or primary effusion lymphoma exists.
3. HIV type 1 can reactivate KSHV and enhance KSHV infectivity ([Mercader et al., 2000](#); [Merat et al., 2002](#); [Aoki & Tosato, 2004](#); [Zeng et al., 2007](#)). These phenotypes are likely to be mediated by cell-derived cytokines. HIV tat protein can cause endothelial cell proliferation in experimental models ([Ensoli et al., 1990, 1994](#)). However, Kaposi sarcoma and primary effusion lymphoma develop in the absence of HIV ([Cesarman et al., 1996](#)). Even in HIV-infected patients, these two viruses have never been found in the same cells ([Delli Bovi et al., 1986](#)).

To explain the well known high incidence of endemic KSHV in certain parts of Africa, it has been postulated that environmental agents might affect KSHV reactivation. In fact, natural, chemical and environmental products can reactivate KSHV from latency, most notably sodium butyrate and phorbol esters or plant extracts ([Renne et al., 1996](#); [Zhong et al., 1996](#); [Miller et al., 1997](#); [Zoetewij et al., 1999](#); [Whitby et al., 2007](#)).

## 4.5 Animal models

Following injection to experimental animals, KSHV can infect non-human primates ([Renne et al., 2004](#)), NOD-SCID mice ([Parsons et al., 2006](#)), and humanized SCID mice ([Dittmer et al., 1999](#); [Foreman et al., 2001](#); [Wu et al., 2006](#)). These infections do not result in the formation of tumours. Nevertheless, they confirm the viral tropism (B cells and endothelial cells), and drug susceptibility (ganciclovir) *in vivo*. KSHV homologous viruses exist in the bank vole-mouse (MHV-68), and virtually in all non-human primates ([Ensser & Fleckenstein, 2007](#)). The infection of macaques with rhesus rhadinovirus in the context of Simian immunodeficiency virus (SIV) induces B-cell lymphoma and endothelial-cell hyperplasia ([Mansfield et al., 1999](#); [Wong et al., 1999](#)).

Multiple tumourgraft models of Kaposi sarcoma and primary effusion lymphoma have been established ([Boshoff et al., 1998](#); [Staudt et al., 2004](#); [Wu et al., 2005](#); [An et al., 2006](#); [Mutlu et al., 2007](#); [Sin et al., 2007](#)).

## 4.6 Transgenic mice models

An alternative approach to infection studies is to use transgenic mice where individual KSHV proteins are expressed in the hope of replicating selected aspects of KSHV pathogenesis. There are some limitations to single transgenic models. Whereas lymphoproliferative lesions and lymphomas in mice are easily classified on the basis of histology and marker-gene expression, this is not the case for endothelial cell tumours. They are referred to as Kaposi-sarcoma-like lesions, but can easily be mistaken for fibrosarcomas ([Table 4.3](#)).

### 4.6.1 Transgenic mice for KSHV latent genes

#### (a) LANA/ORF 73

The KSHV latent promoter (LANAp) showed B-cell lineage specificity in transgenic mice ([Jeong et al., 2002](#)). KSHV LANA expression in transgenic mice resulted in 100% B-cell hyperplasias and lymphomas at about twice the rate of background in the C57/BL6 strain of mice ([Fakhari et al., 2006](#)).

#### (b) vCYC/ORF 72

Whereas vCYC single transgenic mice did not develop tumours, lymphomas developed rapidly in a *p53*-null background ([Verschuren et al., 2002, 2004](#)). [The Working Group noted that, presumably, loss of *p53* counteracted the pro-apoptotic signals that are associated with forced vCYC expression.]

#### (c) vFLIP/ORF 71

The vFLIP transgenic mice exhibited an increased incidence of lymphoma ([Chugh et al., 2005](#)).

### 4.6.2 Transgenic mice for KSHV lytic genes

#### (a) vGPCR/ORF 74.

*vGPCR* transgenic mice activated the same signalling pathways as predicted from human culture studies, and exhibited Kaposi-sarcoma-like lesions ([Yang et al., 2000](#); [Holst et al., 2001](#); [Guo et al., 2003](#); [Montaner et al., 2003](#); [Jensen et al., 2005](#); [Grisotto et al., 2006](#)). Tumour formation required the chemokine binding as well as the constitutive signalling activities of *vGPCR* ([Holst et al., 2001](#)). *vGPCR* was required for lesion initiation, though it was not essential once a fully malignant tumour had formed ([Grisotto et al., 2006](#)).

#### (b) K1/VIP

*K1* transgenic mice also exhibited Kaposi-sarcoma-like lesions and lymphomas ([Prakash et al., 2002, 2005](#)).

**Table 4.3 Transgenics for modelling KSHV-associated cancers**

Viral Gene	Promoter	Incidence	Mean time to onset, days	Reference
<b>Primary effusion lymphoma/multicentric Castleman disease</b>				
<i>MHV68vCYC1</i>	Lck	40%	240	<a href="#">van Dyk et al. (1999)</a>
<i>vCYC</i>	E $\mu$	17%	300	<a href="#">Verschuren et al. (2002)</a>
<i>vCYC/p53<sup>del</sup></i>	E $\mu$	100%	80	<a href="#">Verschuren et al. (2004)</a>
<i>vFLIP</i>	H2Kb	11%	600	<a href="#">Chugh et al. (2005)</a>
<i>LANA</i>	LANAp	10%	300	<a href="#">Fakhari et al. (2006)</a>
<i>K1/VIP</i>	SV40	15%	$\geq 420$	<a href="#">Prakash et al. (2002, 2005)</a>
<b>Kaposi sarcoma</b>				
<i>vCYC</i>	VEGFR-3	80%	$\geq 200$	<a href="#">Sugaya et al. (2005)</a>
K1	SV40	15%	$\geq 420$	<a href="#">Prakash et al. (2002, 2005)</a>
<i>vGPCR</i>	hCD2-rtTA + TRE-vGPCR (Doxycyclin inducible transgenic system)	100%	$\geq 150$	<a href="#">Grisotto et al. (2006), Jensen et al. (2005)</a>
<i>vGPCR</i>	SV40	30%	$\geq 360$	<a href="#">Guo et al. (2003)</a>
<i>vGPCR</i>	hCD2	100%	90	<a href="#">Holst et al. (2001), Yang et al. (2000)</a>

Modified from [Damania & Dittmer \(2008\)](#), and compiled by the Working Group

## 4.7 Synthesis

The available mechanistic data strongly support an oncogenic role of KSHV in human cancer.

KSHV alters the growth properties of endothelial cells in culture, and induces Kaposi-sarcoma-cell-like morphology (spindle cells).

One or several KSHV gene products are expressed in all KSHV-associated cancers in all KSHV-infected tumour cells.

At the molecular level, KSHV-encoded gene products associated with latent viral infection induce cell proliferation, block apoptosis, induce genomic instability or modulate cell migration and tumour progression.

Mechanistic data strongly support an oncogenic role of KSHV in primary effusion lymphoma and in Kaposi sarcoma in immunocompromised

(post-transplant patients, AIDS patients), as well as in immunocompetent individuals.

KSHV proteins like vIL6 induce B-cell proliferation, and are expressed in KSHV-associated multicentric Castleman disease, strongly suggesting that infection with KSHV is causally associated with this lymphoproliferative disease.

## 5. Evaluation

There is *sufficient evidence* in humans for the carcinogenicity of KSHV. KSHV causes Kaposi sarcoma and primary effusion lymphoma. Also, a positive association has been observed between exposure to KSHV and multicentric Castleman disease.

For multiple myeloma, there is *evidence suggesting lack of carcinogenicity*.

KSHV is *carcinogenic to humans (Group 1)*.

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# HUMAN IMMUNODEFICIENCY VIRUS-1

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Human immunodeficiency virus-1 was considered by a previous IARC Working Group in 1996 ([IARC, 1996](#)). Since that time, new data have become available, these have been incorporated in the *Monograph*, and taken into consideration in the present evaluation.

## 1. Exposure Data

### 1.1 Taxonomy, structure, and biology

#### 1.1.1 Taxonomy

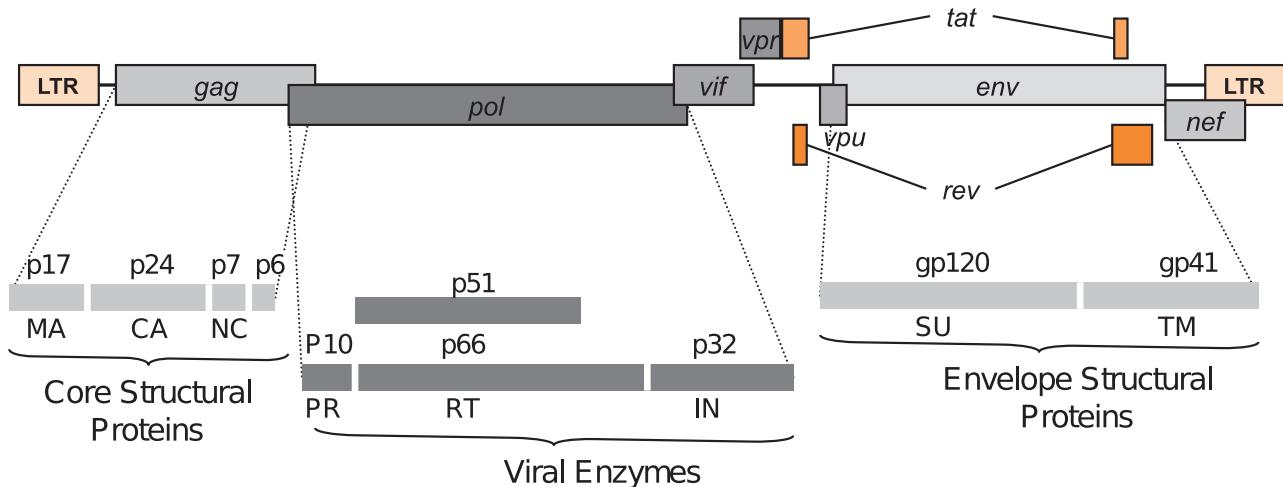
The human immunodeficiency virus type 1 (HIV-1) was first isolated in 1983 ([Barré-Sinoussi et al., 1983](#); [Gallo et al., 1983](#)), and firmly associated with the acquired immunodeficiency syndrome (AIDS) in 1984 ([Gallo et al., 1984](#); [Montagnier et al., 1984](#)). A second related virus, called HIV-2, was subsequently discovered in West Africa ([Clavel et al., 1986](#)). HIV-1 and HIV-2 belong to the family of *Retroviridae* and subfamily *Orthoretrovirinae*. Retroviruses are enveloped RNA viruses that replicate via a DNA intermediate. They rely on the enzyme reverse transcriptase to transcribe their genome from RNA into DNA, which can then be integrated into the host's genome with an integrase enzyme, becoming part of the cellular DNA, and replicating with it. HIV-1 and HIV-2 are the two known human retroviruses that belong to the genus *Lentivirus* (lentus, Latin for “slow”).

#### 1.1.2 Structure of the virion

HIV-1 virions contain two copies of a single-stranded RNA genome within a conical capsid surrounded by a plasma membrane of host-cell

origin containing viral envelope proteins. The RNA genome is 9750 nucleotides long ([Ratner et al., 1985](#); [Wain-Hobson, 1989](#)), and the virions measure approximately 120 nm in diameter. A detailed three-dimensional structure of HIV-1 envelope-glycoprotein spikes, which are required for the infection of host cells, has recently been elucidated by cryoelectron microscopy tomography ([Zhu et al., 2006](#)).

The HIV-1 RNA is tightly bound to the nucleocapsid proteins, p6 and p7, which protect it from digestion by nucleases. This viral core further contains reverse transcriptase, integrase, and protease. The entire complex is surrounded by an icosahedral capsid (p24). A myristoylated matrix protein (p17) surrounds the capsid. Also enclosed within the virion particle are the proteins Vif, Vpr, and Nef (Fig. 1.1). The envelope is formed when the capsid buds from the host cell, taking some of the host-cell membrane with it. Embedded within the lipid bilayer are the viral envelope glycoproteins that form the HIV-1 spikes: the external surface glycoprotein (gp120), and the transmembrane glycoprotein (gp41) ([Turner & Summers, 1999](#); [Bukrinskaya, 2004](#); [Freed & Martin, 2007](#)).

**Fig. 1.1 Genomic organization of HIV-1**

Prepared by the Working Group

### 1.1.3 Structure of the viral genome

The HIV-1 genome, flanked by a long terminal repeat, contains the following genes (Fig. 1.1):

- a) *gag* (group-specific antigen): encodes p24 (viral capsid); p6 and p7 (nucleocapsid proteins); and p17 (matrix protein).
- b) *pol*: encodes the viral enzymes, which are reverse transcriptase (transcribes the viral RNA into double-stranded DNA), integrase (allows integration of the DNA produced by reverse transcriptase into the host genome), and protease (cleaves the proteins derived from *gag* and *pol* into functional proteins).
- c) *env* (envelope): encodes gp160, which is the precursor of the gp120 and gp41 proteins present in the viral envelope of mature virions. This protein forms spikes that allow the virus to attach to and fuse with target cells.
- d) *tat*, *rev*, *nef*, *vif*, *vpr*, *vpu*: each of these genes encodes for a single protein with the same name. Their function is described in Section 4.

The structural biology of HIV-1 has been re-

viewed ([Turner & Summers, 1999](#); [Bukrinskaya, 2004](#); [Freed & Martin, 2007](#)).

### 1.1.4 Host range

Humans are the natural hosts of both HIV-1 and HIV-2. Related viruses, e.g. simian immunodeficiency virus (SIV), naturally occur in African non-human primates. HIV-1 and HIV-2 have been shown to originate in West-Central Africa, and crossed species (zoonosis) from a non-human primate to humans. HIV-1 was found to be closely related to an SIV strain found in chimpanzees (*Pan troglodytes*) (SIVcpz) in Cameroon ([Gao et al., 1999](#)). HIV-2 is more closely related to the SIV of sooty mangabeys (*Cercocebus atys*) (SIVsm) ([Hirsch et al., 1989](#)), a primate species indigenous to West Africa.

### 1.1.5 Target cells

HIV-1 enters cells through interaction with the CD4 receptor and a chemokine co-receptor (CXCR4 or CCR5). The virus infects CD4-positive T cells and macrophages expressing these receptors ([Broder & Collman, 1997](#)). HIV-1 can also

infect dendritic cells ([Knight et al., 1990](#)), which are thought to mediate transmission ([de Witte et al., 2008](#)).

HIV-1 can be assigned to one of three classes based on its ability to use the two co-receptors. Class R5 comprises the viruses that use CCR5 but not CXCR4; they were previously called non-syncytia-inducing (NSI) or M-tropic viruses. The viruses that use CXCR4 are in class X4; they were previously called syncytia-inducing (SI) or T-tropic viruses. Viruses that can use either CCR5 or CXCR4 are referred to as R5X4 or dual viruses ([Coakley et al., 2005](#)). Primary lymphocytes and macrophages express both co-receptors, so co-receptor use does not strictly define cell tropism ([Goodenow & Collman, 2006](#)). Thus, while X4 virus infects T-cell lines, and R5 virus infects macrophage cell lines, in primary cells, these definitions are not as clear. CD4-positive T cells in lymphoid tissues can express both CCR5 and CXCR4, and are the main target for replication *in vivo*. CCR5 is expressed predominantly on the CD45R0<sup>+</sup> memory subset of CD4-positive T lymphocytes, while CXCR4 is expressed on CD4-positive CD45R0<sup>-</sup> and on CD4-positive CD45RA<sup>low</sup> naïve cells ([Bleul et al., 1997](#)).

Within single patients, mixed populations of the virus exist, with any combination of R5, X4 or R5X4. Phenotypic assays and genotyping can be used to determine tropism, as the primary determinants of co-receptor tropism are located in the V3 region of the gp120 envelope protein. Most individuals have the R5 virus at the time of diagnosis, whereas the presence of the X4 and dual virus is associated with progression to AIDS ([Goodenow & Collman, 2006](#)).

HIV-1 can be present in a variety of tissues, which is to be expected given the distribution of T cells, macrophages, and dendritic cells throughout the body. HIV-1 has been detected in tissues from infected patients by means of immunohistochemistry, *in-situ* hybridization, and transmission electron microscopy. HIV-1 has been shown to be associated with germinal

centre follicular dendritic cells in lymph nodes, tonsils and adenoids, and mucosa-associated lymphoid tissue (MALT) as well as in T and B cells ([Teruya-Feldstein et al., 1995](#); [Griffin et al., 1996](#); [Pantaleo et al., 1998](#); [Orenstein et al., 1999](#)). HIV-1 frequently infects the brain, and the microglial cells are the main location for viral replication in the central nervous system ([Shaw et al., 1985](#); [Vazeux et al., 1987](#)). In reproductive organs of infected men, HIV-1 is present in cells of lymphocytic/monocytic morphology in the seminiferous tubules and interstitium of the testis, in the epididymal epithelium, and in connective tissue of the epididymis and prostate ([Pudney & Anderson, 1991](#)). In semen-cell subpopulations isolated by use of an immunoaffinity technique with magnetic beads, T cells were found to be most common cell type infected with HIV-1 (75% of samples), followed by macrophages (38%). Viral DNA was not detected in spermatozoa or in immature germ-cell populations ([Quayle et al., 1997](#)).

### *1.1.6 Life cycle, replication, and regulation of gene expression*

The HIV-1 virus first binds to target cells through semi-specific or nonspecific interactions between the viral envelope and cell-surface glycans or adhesion factors. The gp120 envelope glycoprotein then interacts with the CD4 molecule on the surface of the target cells. This induces a conformational change in gp120, which facilitates its binding to a co-receptor molecule (CCR5 or CXCR4), and the formation of a complex with the transmembrane glycoprotein gp41. Further conformational changes in the gp120-gp41 complex then lead to exposure of the fusion-peptide region of gp41, and its insertion into the host-cell membrane, which results in fusion of the virus with the host cell. Reverse transcription starts immediately after entry. A complex containing protein and viral cDNA (pre-integration complex) is transported

to the host-cell nucleus where the viral integrase enzyme catalyses the integration of viral cDNA into the hosts genomic DNA to form the provirus. The provirus may remain latent for years, producing few or no new copies of HIV-1, which has hampered the treatment of individuals infected with HIV-1, as antiretrovirals can only target replicating virus. When HIV-1 replicates, the provirus uses the cellular RNA polymerase II to create RNA copies of the HIV-1 genome, as well as viral mRNA that encodes the HIV-1 proteins. Transcription of the viral genome is driven by a promoter in the 5' long terminal repeat of the integrated provirus. Tat increases the amount of viral RNA by increasing transcriptional initiation and/or elongation, and Rev regulates the splicing and transport of viral RNA from the nucleus to the cytoplasm. The core and envelope proteins are first synthesized as precursor poly-peptides, which are then cleaved by proteases. Genomic RNA is subsequently packaged into virions. As the virion matures, Gag–Gag and Gag–Pol protein complexes are cleaved by the viral protease into subunit proteins, resulting in the mature virion, which is directed to the cell surface by N-terminal myristylation of Gag. The virion is then released from the plasma membrane where it acquires its envelope. This completes the HIV life cycle (see review by [Freed & Martin, 2007](#)).

## 1.2 Epidemiology of infection

### 1.2.1 Prevalence, geographic distribution

Based on national surveillance systems, the joint United Nations programme on HIV/AIDS (UNAIDS) updates every year the geographic distribution of HIV-1 worldwide. In 2007, the HIV-1 prevalence ranged from less than 0.5% in most developed countries to up to 30% in Central and Southern Africa ([UNAIDS, 2007](#)).

### 1.2.2 Transmission, and risk factors for infection

HIV-1 infection is transmitted through three main routes: sexual intercourse, blood contact, and from mother to infant. HIV-1 infectivity, i.e., the average probability of transmission to another person after that person is exposed to an infected host, is determined by the interplay of three main groups of factors: host-related factors, environmental factors, and agent factors. The probability of HIV-1 transmission is highest for blood transfusion (> 0.95), followed by mother-to-child transmission (about 0.10), intermediate for needle-sharing (about 0.01), and lowest for woman-to-man sexual transmission (about 0.001) ([Royce et al., 1997](#)).

#### (a) Sexual contact

It has been well documented, since the first years of the epidemic, that HIV-1 is transmitted through unprotected (i.e., without condom) vaginal or anal intercourse from man to woman and from woman to man, and through anal intercourse from man to man. Worldwide, the majority of new HIV-1 infections originate from sexual exposures to virus transmitted from semen, with the risk of transmission being related to several virological, biological, and behavioural factors (e.g., HIV-1 subtype, mode of sexual exposure, condom use, penile circumcision, mucosal inflammation, the co-existence of other sexually transmitted diseases, stage of HIV-1 infection, hormonal factors or host genetic background). Before the introduction of Highly Active Anti-Retroviral Therapy (HAART), the quantification of the risk of HIV-1 sexual transmission related to these factors was reviewed by [Royce et al. \(1997\)](#). Most of the accumulated evidence was already described in the previous IARC Monograph ([IARC, 1996](#)).

Higher viral load and genital ulceration are among the main determinants of sexual HIV-1 transmission, and this also applies during the

HAART era. In a Ugandan population, the probabilities of transmission per single coital act increased from 0.0001 at viral loads of less than 1700 copies/mL to 0.0023 at 38500 copies ( $P = 0.002$ ), and were 0.0041 with genital ulceration versus 0.0011 without ( $P = 0.02$ ) ([Gray et al., 2001](#)). The impact of antiretroviral therapy on HIV-1 transmission has been extensively assessed. A 70% reduction in risky sexual behaviour (e.g., not using condoms with HIV-1-negative partners or of unknown HIV-1 status) and a 98% reduction in HIV-1 transmission rate (from 45.7 to 0.9/1000 person-years) was reported from a prospective study conducted among 926 infected adults enrolled in an antiretroviral therapy programme in Uganda ([Bunnell et al., 2006](#)). A study on 393 monogamous heterosexual couples conducted in Spain noted a reduction of approximately 80% in the heterosexual transmission of HIV-1 when HAART became available ([Castilla et al., 2005](#)). HIV-1 prevalence declined from 10.3% during the pre-HAART period (1991–95) to 1.9% in the late HAART period (1999–2003) (odds ratio[OR], 0.14, 95%CI: 0.03–0.66), a decrease that was not influenced by potential confounders like condom use, duration of partnership, CD4-positive lymphocyte count and AIDS-defining diseases ([Castilla et al., 2005](#)).

HIV-1 sexual transmission from men to men occurs through anal intercourse, with seminal plasma viral load and blood plasma viral load associated with an increased risk of transmission ([Butler et al., 2008](#)), though other uncommon routes of transmission have also been suggested – mainly via oral sex ([Richters et al., 2003](#)). A systematic review of the literature, however, concluded that current data are insufficient to precisely estimate the risk of orogenital transmission of HIV-1 due to the small number of studies – the probability estimate was about 0.02–0.45% per single orogenital act ([Baggaley et al., 2008](#)).

### (b) Blood contact

HIV-1 transmission through blood-to-blood contact occurs through the transfusion of HIV-1-infected blood iatrogenically, occupationally, or through needle-sharing by intravenous drug users ([IARC, 1996](#)).

Iatrogenic transmission of HIV-1 is now extremely rare in developed countries due to the recruitment of safe donors, deferral of high-risk donors, and screening. The introduction of nucleic acid testing, and of a new method for computing the residual risk of transfusion-transmitted infections, has allowed precise estimates for the infectious window period ([Soldan et al., 2005](#); [O'Brien et al., 2007](#)). Accordingly, the risk (per million transfusions) of an HIV-1-infected donation entering the blood supply was estimated at 1.91 in Italy ([Gonzalez et al., 2005](#)), at 0.14 in the United Kingdom ([Soldan et al., 2005](#)), and at 0.13 in Canada ([O'Brien et al., 2007](#)). Conversely, in many parts of Africa and in other developing countries, blood screening and banking programmes have been difficult to implement and to sustain. In Kenya, the prevalence of HIV-1 among blood donors ranged from 2–20%, with an estimated 2% of transfusions that transmitted HIV-1 infection to HIV-1-negative blood recipients ([Moore et al., 2001](#)). Reasons for such elevated transfusion-transmitted HIV-1 infections include inconsistent refrigeration, data entry errors, equipment failure, and a lack of quality assurance programmes.

Occupational transmission of HIV-1 in the health care setting has also been documented. The Health Protection Agency has registered, as of March 2005 (worldwide), 106 cases of HIV-1 infections certainly acquired through occupational exposures (of those, 57 occurred in the United States of America and 35 in Europe). Moreover, for another 238 cases, an occupational source of HIV-1 infection was considered highly probable ([Health Protection Agency, 2005](#)). The occupational transmission of HIV-1 occurs

through skin injury with needles or bistouries or from splash exposure to mucosal membranes. Estimates from studies conducted in health settings of the USA and Italy have indicated that HIV-1 transmission occurs in 0.3% of percutaneous exposures, and in 0.1% of muco-membranous exposures ([Jagger et al., 2003](#)).

The sharing of injection equipment by intravenous drug users represents a major mode of HIV-1 transmission worldwide. Since the early 1980s, a high prevalence of HIV-1 has been reported among this group from many parts of the world; and in many areas, HIV-1 prevalence among intravenous drug users was raised to 50% or more within the first years of the epidemic. Such outbreaks continue to occur, and rapid spread has been documented in the newly independent states of the former Soviet Union ([Rhodes et al., 2002](#)). In England and Wales, HIV-1 prevalence declined from 5.9% in 1990 to 0.6% until 1999, and thereafter it increased to 1.4% in 2003 ([Hope et al., 2005](#)). The timely introduction of comprehensive harm reduction measures, particularly needle exchange programmes, has prevented the rapid spread of HIV-1 in several northern European countries and Australia. Furthermore, trends in the prevalence of HIV-1 have reversed in several areas, like Northern Italy and New York City, and have been partially attributed to behavioural change, improved access to treatment, and needle exchange programmes ([Hurley et al., 1997](#); [Des Jarlais et al., 2000](#); [Sabbatini et al., 2001](#)). In Asia, 30% of intravenous drug users were reported to be infected with HIV-1 in India and Thailand (Razak *et al.*, 2003; [Panda et al., 2005](#)). In Yunnan province, China, HIV-1 infection was documented in 59.9% of 314 intravenous drug users, and it was positively associated with frequency of injection ([Yao et al., 2009](#)).

#### (c) Mother-to-child transmission

Despite substantial reductions in mother-to-child transmission of HIV-1 infection achieved in North America and Europe ([Fiscus et al.,](#)

[1999](#); [Townsend et al., 2008](#)), paediatric HIV-1 infection remains a major worldwide pandemic. It is estimated that about 1800 new HIV-1 infections are transmitted daily from mother to infants (UNICEF, <http://www.unicef.org/media/files/RegionalSummary.doc>) during pregnancy, labour, delivery, and postpartum through breastfeeding. Several randomized clinical trials were conducted in developing countries (where the majority of pregnant women have no access to antiretroviral therapies to treat their own HIV-1 infection), to assess mother-to-child transmission rates through the use of antiretroviral regimens (reviewed by [Kourtis et al., 2006](#)). Although not completely defined yet, the timing and mechanisms of mother-to-child transmission are important to quantify transmission rates, and to implement prevention strategies. In the absence of any intervention, it is estimated that in-utero and intra-partum transmission of HIV-1 occurs in approximately 25% of infants born to HIV-1-positive women ([Connor et al., 1994](#)). The administration of zidovudine from 14 weeks of gestation through to delivery, and to the newborn for 6 weeks, decreased intra-partum and delivery HIV-1 transmission by 67%, from 25.5% to 8.3% ( $P < 0.01$ ) in a randomized, double-blind, placebo-controlled efficacy trial ([Connor et al., 1994](#)). Similarly, a study conducted in Thailand that used a shortened zidovudine regimen starting at 36 weeks of gestation prevented 50% of HIV-1 transmission ([Shaffer et al., 1999](#)). Adding a single maternal/infant nevirapine dose to zidovudine further reduced the in-utero and intra-partum transmission risk ([Lallemand et al., 2004](#)). Overall, the findings from in-utero and intra-partum transmission studies indicate that the risk of mother-to-child transmission increases steadily towards the late stages of pregnancy, with nearly 80% of new HIV-1 infection occurring from 36 weeks to delivery ([Kourtis et al., 2006](#)). Thus, the HIV-1 transmission risk is reduced by up to 70% by elective Caesarean delivery, as compared to vaginal delivery

([European Mode of Delivery Collaboration, 1999](#)). In developed countries, where the majority of pregnant HIV-1-infected women are treated with HAART, mother-to-child transmission rates are less than 10% ([Cooper et al., 2002](#)). In resource-limited settings, postnatal transmission via breastfeeding may be as high as 18% ([Miotti et al., 1999; Fawzi et al., 2002](#)). Several intervention studies have demonstrated, in this context, the efficacy to extend antiretroviral prophylaxis to reduce HIV-1 transmission. Studies have indicated that 8.9 transmissions per 100 child-years of breastfeeding occur, with cumulative probabilities of transmission at 6 months being about 5% lower than that at 18 months (the standard duration of breastfeeding in African countries) ([Coutsoudis et al., 2004](#)). The extension of antiretroviral prophylaxis of breastfed infants born to HIV-1-positive mothers with nevirapine or with nevirapine plus zidovudine for the first 14 weeks of life significantly reduces the rate of postnatal HIV-1 infection in 9-month-old infants from 10.6% in controls to 6.4% (nevirapine) or to 5.2% (nevirapine plus zidovudine) ([Kumwenda et al., 2008](#)).

## 2. Cancer in Humans

The studies included in this section were published since the previous *IARC Monograph* ([IARC, 1996](#)), a calendar period that coincided with the introduction of HAART in developed countries, and later in developing countries. The introduction of HAART has dramatically improved the survival of HIV-1-infected patients and has reduced the incidence of several diseases associated with HIV-1 infection, including some types of cancer. In the years following the introduction of HAART (the post-HAART era), the spectrum of cancers associated with HIV-1 infection has substantially changed. This section focuses on those cancers for which the data

are most strongly suggestive of a true increase in risk. It should be noted that HIV-1 infection causes cancer indirectly through immune deficiency, and the increased expression of the effects of oncogenic infections. In this way, the patterns of cancer are generally similar to those in other immunodeficient populations, such as solid organ transplant recipients. In addition, it is possible that immune deficiency results in impaired immune surveillance and the emergence of cancers that are usually controlled by the immune system, as originally hypothesized by Thomas and Macfarlane Burnet in the early 1950s ([Beral & Newton, 1998; Kinlen, 2004](#)).

### 2.1 Kaposi sarcoma

Kaposi sarcoma herpesvirus (KSHV) is now recognized as a necessary condition for the development of Kaposi sarcoma, with HIV-1-related immunosuppression increasing the risk of developing Kaposi sarcoma by several orders of magnitude (in the thousands). In addition to the studies referenced in the previous *IARC Monograph* ([IARC, 1996](#)), there have been a large number of cohort studies (in developed countries) and a few case-control studies (all from Africa) that confirmed the strong association between HIV-1 infection and Kaposi sarcoma. Kaposi sarcoma has become the most frequently reported cancer in many sub-Saharan African countries. However, the relative risks (RRs) reported from Africa, though still elevated, are substantially lower than those reported in developed countries. The reasons for this are unclear but may reflect differences in background risk and competing mortality.

Effective antiretroviral therapy in individuals with HIV-1-related immunosuppression usually results in a substantial and rapid reduction in the risk of Kaposi sarcoma. Nevertheless, Kaposi sarcoma incidence rates remain very substantially raised above population rates (and are no longer declining), and Kaposi sarcoma remains

a considerable cause of morbidity and mortality in people infected with HIV-1. According to a calendar-period meta-analysis on 47936 HIV-1-positive people in North America, Europe and Australia, the relative risk for Kaposi sarcoma in the early post-HAART era (1997–99) was 0.32 (95%CI: 0.26–0.40) when compared to the pre-HAART era (International Collaboration on HIV-1 and Cancer, 2000). Similar declines by calendar period were reported in Italy from 1986 through to 1998 ([Franceschi et al., 2003](#)), and in the USA (RR, 0.19; 95%CI: 0.12–0.30, 1997–2002 versus 1989–96) ([Bedimo et al., 2004](#)). When the follow-up of HIV-1-positive patients was continued through to the late post-HAART period (2002), time trends incidence rates for Kaposi sarcoma were observed to level off in the last study years ([Patel et al., 2008](#)). In the USA, the risk for Kaposi sarcoma declined 83.5% during 1990–95 and 1996–2002 (standardized incidence ratio [SIR], 22100 and 3640, respectively;  $P < 0.0001$ ). The pattern presented a steady decline that began in the 1980s and continued through 1990–95, with a further fall in risk offsetting the 1996–2002 period from the 1990–95 period (RR, 0.41; 95%CI: 0.28–0.60). Subsequently, during the HAART era itself, the risk for Kaposi sarcoma remained constant ([Engels et al., 2006](#)).

A similar temporal pattern was observed for Kaposi sarcoma in Australia ([Grulich et al., 2001](#)), and in Europe ([Franceschi et al., 2003](#); [Clifford et al., 2005](#)). In Switzerland, the incidence of Kaposi sarcoma in the Swiss HIV-1 cohort study following the advent of HAART fell abruptly in 1996–98 to reach a plateau. Individual data on HAART use showed that the risk for Kaposi sarcoma declined steeply in the first months after HAART initiation, and continued to be low for another 7–10 years (hazard ratio [HR], 0.06; 95%CI: 0.02–0.17) ([Franceschi et al., 2008](#)).

In addition to making Kaposi sarcoma a relatively rare event, HAART use has also diminished the variation in Kaposi sarcoma risk by

host characteristics, including gender, age group, HIV-1-transmission category, and CD4-positive cell count. Increases in SIR with declining number of CD4-positive count were seen in most of the studies with relative risks estimates according to strata of CD4-positive cell counts. In the Swiss HIV-1 cohort study, the SIR for Kaposi sarcoma was 571 (95%CI: 449–716) among persons with CD4-positive counts of less than 100 cells/mm<sup>3</sup>, but 76.5 (95%CI: 52.3–108) among persons with CD4-positive counts of  $\geq 500$  cells/mm<sup>3</sup> ([Clifford et al., 2005](#)). In the USA, the risk for Kaposi sarcoma increased by 36% for each fall of 100 CD4-positive cells/mm<sup>3</sup> (95%CI: 29–43%) ([Mbulaiteye et al., 2003](#)). A longitudinal study of 2002 HIV-1-infected persons in Italy with known date of seroconversion followed up to 2004 found a relative risk of 0.11 (95%CI: 0.06–0.19) for Kaposi sarcoma associated with a CD4-positive count  $\geq 350$  cells/mm<sup>3</sup> at enrolment (vs  $< 200$ ) ([Serraino et al., 2005](#)). Homosexual men were at higher risk than injecting drug users (RR, 6.67; 95%CI: 3.58–12.42), and women were at lower risk of Kaposi sarcoma than men (RR, 0.23; 95%CI: 0.06–0.83) ([Serraino et al., 2005](#)).

See Table 2.1 available at <http://monographs.iarc.fr/ENG/Monographs/vol100B/100B-05-Table2.1.pdf>, Table 2.2 available at <http://monographs.iarc.fr/ENG/Monographs/vol100B/100B-05-Table2.2.pdf>, and Table 2.3 available at <http://monographs.iarc.fr/ENG/Monographs/vol100B/100B-05-Table2.3.pdf>.

## 2.2 Non-Hodgkin lymphoma

Non-Hodgkin lymphoma has been part of the AIDS case definition since 1985 ([CDC, 1985](#)). A recently published meta-analysis that included population-based prospective studies comparing rates of non-Hodgkin lymphoma in people with HIV-1 or AIDS to the general population reported an SIR of 77 in HIV-1 patients, and of 8 in solid organ transplant recipients ([Grulich et al., 2007a](#)). In Africa, the relative increase in people

with HIV-1 appears much less, and was about 6-fold in case-control studies in Uganda, and in a South African case-control study ([Newton et al., 2001](#); [Stein et al., 2008](#)). Non-Hodgkin lymphoma also occurs at increased rates in people with primary immune deficiency ([Beral & Newton, 1998](#); [Mellekjær et al., 2002](#)).

There is no evidence that HIV-1 causes non-Hodgkin lymphoma through a direct effect. Rather, the profound depletion of CD4-positive T lymphocytes that is caused by HIV-1 allows the dysregulation of control of B cells, and the expression of the effects of lymphotrophic viruses ([Engels, 2007](#)). As in the general population, more than 90% of cases of non-Hodgkin lymphoma due to AIDS are of the B-cell phenotype. There are three B-cell lymphoma subtypes that occur most commonly. First, primary brain lymphoma occurs at profound levels of immune deficiency, and occurs several thousand times more commonly in people with AIDS than in the general population ([Coté et al., 1996](#)). Second, large-cell immunoblastic lymphoma occurs in the severely immunodeficient, and occurs several hundred times more commonly than in the general population ([Engels & Goedert, 2005](#)). Third, Burkitt lymphoma can occur at any stage of immune deficiency and, again, occurs nearly a hundred times more frequently than in the general population ([Engels et al., 2008](#); [Stein et al., 2008a](#)). A rare lymphoma subtype that occurs in people with HIV-1 is primary effusion lymphoma, which presents as either a pleural or peritoneal effusion. It is associated with infection with KSHV, is more likely to present in a person with Kaposi sarcoma ([Mbulaiteye et al., 2002](#)), and has also been described in association with the KSHV-related disease, multicentric Castleman disease ([Ascoli et al., 2001](#)). In addition to these B-cell subtypes, a record linkage study in the USA demonstrated a 15-fold increase in the incidence of T-cell non-Hodgkin lymphoma ([Biggar et al., 2001](#)). The pathological spectrum of T-cell non-Hodgkin lymphoma in

HIV-1 is diverse, and tends to occur at very low CD4-positive counts ([Arzoo et al., 2004](#)).

Of the non-Hodgkin lymphoma subtypes that are associated with HIV-1, the incidence of two primary brain lymphoma and diffuse large B-cell lymphoma is correlated closely with the severity of immune deficiency. These two types of lymphoma are uncommon when CD4-positive counts are maintained at relatively normal levels. Similarly, in organ transplant recipients, the risk of non-Hodgkin lymphoma is proportional to the intensity of immune suppression ([Grulich et al., 2007b](#)). On the other hand, the third non-Hodgkin lymphoma subtype associated with HIV-1, Burkitt lymphoma, can occur at any level of immune deficiency. All three B-cell lymphoma subtypes are associated with markers of immune activation, such as serum immunoglobulin ([Martínez-Maza & Breen, 2002](#)), soluble CD44 ([Breen et al., 2005](#)), and IL10 ([Breen et al., 2003](#)).

There was initially considerable debate about whether or not non-Hodgkin lymphoma rates decreased after the introduction of HAART ([Grulich, 1999, 2000](#); [Matthews et al., 2000](#); [Powles et al., 2000](#); [Tirelli et al., 2000](#); [Ives et al., 2001](#); [Vilchez et al., 2002](#)), but by around the turn of the millennium, it became clear that rates of non-Hodgkin lymphoma due to AIDS were declining ([International Collaboration on HIV and Cancer, 2000](#); [Besson et al., 2001](#); [Grulich et al., 2001](#); [Franceschi et al., 2003](#); [Clayton & Mughal, 2004](#); [Kinlen, 2004](#); [Stebbing et al., 2004](#); [Engels et al., 2006](#); [Gingues & Gill, 2006](#); [Chiappini et al., 2007](#); [Long et al., 2008](#); [Polesel et al., 2008](#)). Further case-control ([Bonnet et al., 2006a](#)) and cohort studies ([Carrieri et al., 2003](#); [Stebbing et al., 2004](#); [Clifford et al., 2005](#); [Kirk et al., 2007](#); [Serraino et al., 2007](#); [Polesel et al., 2008](#)) have shown that individual receipt of HAART is associated with a reduced risk of non-Hodgkin lymphoma. It is remarkable that the risk of non-Hodgkin lymphoma appears to decrease markedly within months of starting HAART ([Kirk et al., 2007](#); [Polesel et al., 2008](#)).

Of the subtypes of AIDS-related non-Hodgkin lymphoma, rates have declined most dramatically for primary brain non-Hodgkin lymphoma ([International Collaboration on HIV and Cancer, 2000](#); [Besson et al., 2001](#); [Kirk et al., 2001](#); [Inungu et al., 2002](#); [Bower et al., 2006](#); [Diamond et al., 2006](#); [Haldorsen et al., 2008](#)). Rates of large-cell immunoblastic lymphoma have also declined substantially, though not as markedly. Rates of Burkitt lymphoma appear to have changed little in most studies ([International Collaboration on HIV and Cancer, 2000](#); [Engels et al., 2006](#); [Babel et al., 2007](#); [Barclay et al., 2007](#)), although a decline in risk was reported in a large European cohort ([Kirk et al., 2001](#)). Despite these recent very large declines in non-Hodgkin lymphoma risk, in the most recent studies, rates of non-Hodgkin lymphoma remain 10-fold or more greater than population rates ([Engels et al., 2008](#); [Patel et al., 2008](#)). Before 1996, AIDS-related non-Hodgkin lymphoma was almost universally fatal ([Tirelli et al., 2000](#)). Since the advent of HAART, the mean CD4-positive count at non-Hodgkin lymphoma presentation has increased substantially, and the prognosis has improved remarkably ([Evison et al., 1999](#); [Besson et al., 2001](#); [Little et al., 2001](#); [Baiocchi et al., 2002](#); [Gérard et al., 2002](#); [Vaccher et al., 2003](#); [Robotin et al., 2004](#); [Bower et al., 2005](#); [Lascaux et al., 2005](#); [Lim et al., 2005](#); [Mounier et al., 2006](#); [Miralles et al., 2007](#)). Treatment schedules and responsiveness are now similar to that in the HIV-1-negative population ([Clayton & Mughal, 2004](#); [Lim et al., 2005](#)).

## 2.2.1 The Epstein-Barr virus in AIDS-related non-Hodgkin lymphoma

The Epstein-Barr virus (EBV) can be detected in AIDS-related non-Hodgkin lymphoma in virtually all cases of primary brain lymphoma ([Hansen et al., 2000](#); [Tirelli et al., 2000](#)), around 40% of the cases are large cell lymphoma, and around 30% of the cases are Burkitt lymphoma ([Tirelli et al., 2000](#)). In solid organ transplant

recipients, the risk of non-Hodgkin lymphoma is much higher in EBV-seronegative organ transplant recipients who contract primary EBV infection from the donated organ ([Shapiro et al., 1999](#); [Swinnen, 2000](#)). In this population, high EBV plasma viral load predicts the development of non-Hodgkin lymphoma, and regression of lymphoma is associated with declining EBV in plasma. In addition, cytokine polymorphisms favouring a pro-inflammatory state increase the risk of lymphoma after transplantation ([Babel et al., 2007](#)). In people with HIV-1, EBV viral loads are increased from the early stages of HIV-1 infection ([Pirou et al., 2004](#)). In one study, high plasma EBV viral load was found in people with EBV-positive but not EBV-negative AIDS lymphoma, and viral loads fell with successful therapy ([Fan et al., 2005](#)). In a French study, plasma EBV declined with chemotherapy for non-Hodgkin lymphoma ([Bonnet et al., 2006b](#)). EBV DNA is found in the cerebral spinal fluid in 80–100% of cases of primary brain lymphoma in people with AIDS ([DeLuca et al., 1995](#); [Brink et al., 1998](#); [Antinori et al., 1999](#); [Bossolasco et al., 2002](#); [Fan et al., 2005](#)). There is interest in using anti-EBV therapies in the treatment of primary brain lymphoma in HIV-1 ([Aboulafia et al., 2006](#)). In one small study, latent antigen EBNA-1-specific CD4 T cells were lost before the diagnosis of AIDS lymphoma, but not in those who progressed to other AIDS illnesses, suggesting an important role of immunity to EBNA-1 ([Pirou et al., 2005](#)). A Japanese study documented a decline in the proportion of non-Hodgkin lymphoma positive for EBV, from 88% in the pre-HAART era down to 58% in the HAART era ([Hishima et al., 2006](#)). This is consistent with the declining occurrence of opportunistic EBV-related non-Hodgkin lymphoma. Studies which have examined anti-herpesvirus agents as potential preventive agents for non-Hodgkin lymphoma have produced conflicting results ([Fong et al., 2000](#); [Grulich et al., 2000, 2001](#)), and high-dose aciclovir was not protective against death from non-Hodgkin

lymphoma in a meta-analysis of trials of high-dose aciclovir in people with HIV-1 ([Ioannidis et al., 1998](#)). [The Working Group noted that the power was limited to show this effect, and it was difficult to disentangle the subtypes of lymphoma from the individual trials.]

Despite an association of hepatitis C virus (HCV) infection with non-Hodgkin lymphoma in the general population, a cohort study did not find an association of non-Hodgkin lymphoma with HCV infection in those with HIV-1 ([Waters et al., 2005](#)).

See Table 2.4 available at <http://monographs.iarc.fr/ENG/Monographs/vol100B/100B-05-Table2.4.pdf>, Table 2.5 available at <http://monographs.iarc.fr/ENG/Monographs/vol100B/100B-05-Table2.5.pdf>, and Table 2.6 available at <http://monographs.iarc.fr/ENG/Monographs/vol100B/100B-05-Table2.6.pdf>.

## 2.3 Hodgkin lymphoma

In the general population of developed countries, Hodgkin lymphoma is one of the most common malignancies diagnosed in people under the age of 45 years with upward trends recorded since the late 1990s ([Hjalgrim et al., 2001](#)). Four subtypes have been distinguished within classical Hodgkin lymphoma: nodular sclerosis, mixed cellularity, lymphocyte-rich, and lymphocyte-depleted. These subtypes cannot be distinguished by the immunophenotype of the tumour cells, but are different in terms of characteristics such as the sites of involvement, clinical features, growth pattern, and frequency of EBV infections ([Stein et al., 2008b](#)).

The epidemiology of Hodgkin lymphoma is characterized by a bimodal incidence curve – with a first peak around the age of 30 years and the second peak around the age of 50 – that has been taken as suggestive of an infectious etiology ([World Cancer Report, 2008](#)). A particularly important etiological role has been attributed to EBV, whose genome is found in

higher percentages among Hodgkin lymphoma cases that are HIV-1-positive compared to those that are HIV-1-negative ([Carbone et al., 1999](#); [Frisch et al., 2001](#); [Rezk & Weiss, 2007](#)). Epidemiological studies conducted during the first years of the HIV-1 epidemic lacked statistical power to assess a significantly increased risk of Hodgkin lymphoma among the HIV-1-infected population ([Biggar et al., 1987](#)). However, with the spread of the epidemic and longer survival of HIV-1-infected persons, the scientific evidence has accumulated showing that HIV-1-positive persons have, overall, a 10-fold higher risk of developing Hodgkin lymphoma than HIV-1-negative persons of the same sex and age. Such evidence comes from the different types of epidemiological studies conducted worldwide.

In the pre-HAART period, a cohort study of 6704 homosexual men conducted in the USA was the first to demonstrate a statistically significant excess risk for Hodgkin lymphoma in HIV-1-positive persons (RR, 5.0; 95%CI: 2.0–10.3) ([Hessol et al., 1992](#)). A nearly 10-fold higher risk (95%CI: 8–11) was documented thereafter, in Italy, in a cohort study on 1255 HIV-1-positive persons with a known date of seroconversion ([Serraino et al., 1997](#)), by a record linkage of national AIDS registry and population-based cancer registries (SIR, 8.9; 95%CI: 4.4–16.0) ([Franceschi et al., 1998](#)), and, in Australia, through a similar record linkage study (RR, 7.8; 95%CI: 4.4–13.0) ([Grulich et al., 2002](#)). A meta-analysis of seven reports of HIV-1-associated cancer risk, involving 444172 people with HIV-1/AIDS in the USA, Australia, Scotland, Italy, Switzerland, and England ([Grulich et al., 2007a](#)) reported an overall relative risk of 11.0 (95%CI: 8.4–14.4). Although to a lesser extent than in developed countries, significant excess risk for Hodgkin lymphoma in people with HIV-1 infection or AIDS were also noted in Uganda (RR, 5.7; 95%CI: 1.2–17) ([Mbulaiteye et al., 2006](#)), and in South Africa (RR, 1.6; 95%CI: 1.0–2.7) ([Stein et al., 2008a](#)).

The excess risk for Hodgkin lymphoma in HIV-1-infected persons was not consistently observed across Hodgkin lymphoma histological types. A comparative study based on a clinical series of 92 cases of Hodgkin lymphoma in HIV-1-positive persons, showed a 4-fold increased frequency of the mixed cellularity type, and a 12-fold increased frequency of the lymphocyte depletion type in HIV-1-positive cases compared with the general population ([Serraino et al., 1993](#)). Similarly, a meta-analysis of 17 studies on Hodgkin lymphoma in HIV-1-positive individuals showed statistically significant differences in the proportion of distribution of all types, with odds ratios of 0.4 (95%CI: 0.3–0.6) for lymphocyte predominance, 0.3 (95%CI: 0.2–0.4) for nodular sclerosis, 3.2 (95%CI: 2.6–3.8) for mixed cellularity, and 6.3 (95%CI: 4.5–8.8) for the lymphocyte depletion type ([Rapezzi et al., 2001](#)). In the USA, very elevated SIRs according to histological type were reported by a large record linkage study of AIDS and cancer registries investigating the association between cancer and immunosuppression. With regard to Hodgkin lymphoma, only the mixed cellularity type (RR, 18.3; 95%CI: 15.9–20.9) and the lymphocytic depletion type (RR, 35.3; 95%CI: 24.7–48.8) were associated with a significantly increased risk ([Frisch et al., 2001](#)).

In contrast with non-Hodgkin lymphoma, whose incidence has declined with immune restoration due to the use of HAART ([International Collaboration on HIV and Cancer, 2000](#)), time trends in relative risks for Hodgkin lymphoma have generally shown upward trends in recent years. [Although the Working Group notesd that SIRs have methodological limitations when used to compare changes among HIV-1-positive persons.] Whereas a cohort study of 8074 HIV-1-positive persons in Italy and France showed no significant variations in the risk of Hodgkin lymphoma between those treated (RR, 9.4; 95%CI: 2.0–27.6) or not treated (RR, 11.1; 95%CI: 6.2–18.3) with HAART ([Serraino et al.](#),

[2007](#)), the elevation in risk following HAART were noted in most investigations. Hodgkin lymphoma risk was higher in the post-HAART period (RR, 31.7) than in the pre-HAART period (RR, 22.8) according to a cohort study of 77025 HIV-1-positive persons in France ([Herida et al., 2003](#)). As seen in France, the findings of a record linkage between the Swiss HIV-1 cohort and cancer registries pointed to a higher risk for Hodgkin lymphoma in HIV-1-positive persons treated with HAART (RR, 36.2), when compared to those who were never treated (RR, 11.4) ([Clifford et al., 2005](#)). Another record linkage study of 57350 HIV-1-infected persons recruited from 1991–2002 with cancer registries in the USA indicated that the incidence of Hodgkin lymphoma increased 3-fold in the study period (RR, 2.7; 95%CI: 1.0–7.1; 1996–2002 versus 1991–95) ([Engels et al., 2008](#)). These results, and those from another investigation from the USA showing increasing incidence rates of Hodgkin lymphoma with a higher count of CD4-positive cells in HIV-1-positive persons treated with HAART ([Biggar et al., 2006](#)), might suggest that the excess risk for Hodgkin lymphoma is more pronounced in HIV-1-infected individuals with moderate immunosuppression, where the mixed cellularity type is more frequent. It has been hypothesized that this finding could be the result of the strong association of Hodgkin lymphoma with EBV infection ([Frisch et al., 2001](#)).

See Table 2.7 available at <http://monographs.iarc.fr/ENG/Monographs/vol100B/100B-05-Table2.7.pdf>, Table 2.8 available at <http://monographs.iarc.fr/ENG/Monographs/vol100B/100B-05-Table2.8.pdf>, and Table 2.9 available at <http://monographs.iarc.fr/ENG/Monographs/vol100B/100B-05-Table2.9.pdf>.

## 2.4 Cervical and anogenital cancers

### 2.4.1 Cancer of the cervix

HIV-1-positive women constitute a growing part of the population groups affected by the epidemic in developed countries, but they are particularly numerous in low- and middle-resource countries, where the infection with HIV-1 and the human papillomavirus (HPV) is common ([Franceschi et al., 2006](#)). Because both HIV-1 infection and HPV infection are sexually transmitted, the two infections often co-exist ([Strickler et al., 2005](#); [Clifford et al., 2006](#)). HPV is a necessary condition for cervical cancer to occur, and as a result of HIV-1-induced immune impairment in HIV-1-infected women, there is an increased probability that HPV infection will become persistent ([Strickler et al., 2005](#)), and evolve into cancerous lesions of the cervix uteri ([Frisch et al., 2000](#); [Dal Maso et al., 2003a](#)). Invasive cervical cancer was the last type of cancer included among the AIDS-defining diseases in 1993, but an association with HIV-1 infection started to emerge in Europe and in the USA several years later ([Franceschi et al., 1998](#); [Serraino, 1999](#); [Ahdieh et al., 2000](#); [Frisch et al., 2000](#)). The magnitude of the excess was weaker than that found for the other AIDS-defining cancers (i.e., Kaposi sarcoma and non-Hodgkin lymphoma), with increases 5–10-fold in developed countries, depending on the study site and characteristics of the populations under study. The relative risk for invasive cervical cancer among women living with HIV-1 infection varies from country to country, depending on factors like premature death due to other causes or early detection of cancer that prevents the progression of pre-invasive lesions to the invasive stage ([Franceschi & Jaffe, 2007](#)).

A meta-analysis of seven reports of HIV-1-associated cancer risk, involving 444172 people with HIV-1/AIDS in the USA, Australia, Scotland, Italy, Switzerland, and England ([Grulich et al.,](#)

[2007a](#)) reported an overall relative risk of 5.8 (95%CI: 3.0–11.3), with risk estimates in individual studies ranging from 1.0 (95%CI: 0.2–2.9) in the United Kingdom ([Newnham et al., 2005](#)) to 21.9 (95%CI: 13.0–34.7) in Italy ([Dal Maso et al., 2003a](#)). An even higher risk for cervical cancer was reported among HIV-1-infected women in Spain (SIR, 41.8; 95%CI: 19.9–77.1) ([Galceran et al., 2007](#)). In Italy and Spain, the particularly elevated cervical cancer risk could be attributed to the concomitant high prevalence of intravenous drug users among HIV-1 women (who are at a particularly elevated risk of cervical cancer), and to poorly organized screening strategies in this population. Conversely, elevated rates of cervical cancer in the general population and competing risks of deaths are likely to explain the observation that risks from studies conducted in Africa tended to be of lower magnitude than those from developed countries ([Serraino, 1999](#)). For instance, a comparison of cancer rates between 1988–2002 in Uganda showed that the relative risk of invasive cervical cancer among women with HIV-1, when compared to women in the general population, was 2.4 (95%CI: 1.1–4.4) ([Mbulaiteye et al., 2006](#)).

Trends in SIRs of cervical cancer indicate that the introduction of HAART has not influenced the occurrence of this cancer among HIV-1-infected women. This observation was documented in the first years after HAART became available by a pooled analysis of cancer incidence data from 23 prospective studies that included 47936 HIV-1-seropositive individuals from North America, Europe, and Australia ([International Collaboration on HIV and Cancer, 2000](#)), as well as by several other single investigations. Among 2331 HIV-1-infected women in Italy and France, the risk of cervical cancer was 15.7 (95%CI: 9.1–25) in those treated with HAART, and 11.8 (95%CI: 3.8–27.5) in those who were not ([Serraino et al., 2007](#)). Also, in Italy, no change was noted by a record linkage study of AIDS and cancer registries with risks varying from 51.0 (95%CI:

23.1–97.3) in the pre-HAART period from 1985–96 to 41.5 (95%CI: 28.0–59.3) in the post-HAART era from 1997–2002 ([Dal Maso et al., 2009](#)). Similar findings were documented in the USA ([Bedimo et al., 2004](#); [Biggar et al., 2007](#); [Patel et al., 2008](#)). Cervical cancer incidence rates were higher in 1996–2002 (86.5/100000 person–years) than in 1990–95 (64.2/100000 person–years) (RR, 1.41; 95%CI: 0.81–2.46), but this was not statistically significant ([Biggar et al., 2007](#)). Data from two large prospective cohort studies in the USA, the Adult and Adolescent Spectrum of HIV Disease (ASD) Project and the HIV Outpatient Study (HOPS) had statistically significant SIRs of 11.8 in 1992–95, 13.3 in 1996–99, and 10.1 in 2000–03) ([Patel et al., 2008](#)).

(a) *Cancer of the cervix in immunosuppressed individuals*

The incidence of cervical cancer also increased in transplant recipients [SIR, 2.50; 95%CI: 1.33–4.27] ([Vajdic et al., 2006](#)). Pre-invasive cervical cancer lesions altered in HIV-positive women led to the decision that the cohort study estimates were not just due to confounding.

See Table 2.10 available at <http://monographs.iarc.fr/ENG/Monographs/vol100B/100B-05-Table2.10.pdf>, Table 2.11 available at <http://monographs.iarc.fr/ENG/Monographs/vol100B/100B-05-Table2.11.pdf>, and Table 2.12 available at <http://monographs.iarc.fr/ENG/Monographs/vol100B/100B-05-Table2.12.pdf>.

#### 2.4.2 *Cancer of the anus*

A recent meta-analysis of population-based cohort studies estimated that rates of anal cancer are raised almost 30 times in people with HIV-1/AIDS, and are raised about 5 times in transplant recipients ([Grulich et al., 2007a](#)). Anal cancer is closely related to anal infection with high-risk subtypes of HPV ([IARC, 2007](#)). Determining whether anal cancer is associated with HIV-1 infection is complicated by the fact

that HIV-1-negative homosexual men are much more likely than others to develop anal HPV infection and anal cancer ([Daling et al., 1987](#)). Nevertheless, cohort data do suggest that the rate of anal cancer is higher in HIV-1-positive than in HIV-1-negative homosexual men ([D'Souza et al., 2008](#)). Anal cancer occurs at younger ages in HIV-1-positive cases than in the HIV-1-negative cases ([Chiao et al., 2008](#); [D'Souza et al., 2008](#)). Among HIV-1-positive persons, lower CD4-positive counts are associated with a higher probability of infection with multiple HPV types ([Orlando et al., 2008](#)). HPV infection of the anal canal is extraordinarily prevalent in both people with HIV-1, and in transplant recipients ([Drobacheff et al., 2003](#); [Patel et al., 2007](#); [Orlando et al., 2008](#)). Rates of HPV infection of over 50% have been described even in HIV-1-positive women ([Palefsky et al., 2001](#)), in whom anal cancer is relatively uncommon.

Many studies have noted increases in the incidence of anal cancer during the HAART era ([Bower et al., 2004](#); [Diamond et al., 2005](#); [Hessol et al., 2007](#); [D'Souza et al., 2008](#); [Patel et al., 2008](#); [Piketty et al., 2008](#)); this may be related to increased screening for anal cancer and its precursors, to an increase in risky behaviour of patients feeling safer by taking HAART, and/or to increased longevity allowing enough time for the cancer to develop ([Hessol et al., 2007](#); [Piketty et al., 2008](#)). [The Working Group noted that although initially the introduction of a screening programme (as is beginning to occur in some specialized centres) may lead to an increase in the reported prevalence of a cancer, in the longer term, the detection and treatment of pre-invasive disease should lead to a decline in the incidence of invasive malignancy in the screened population.] In the USA, record linkage of HIV/AIDS and cancer registries showed that the AIDS- and HIV-1-cancer matched rates of anal cancer were approximately the same in the pre- and post-HAART eras ([Engels et al., 2006](#); [Engels et al., 2008](#)). Several studies concluded that the use of

HAART does not appear to reduce anal cancer risk ([Clifford et al., 2005](#); [Hessol et al., 2007](#); [D'Souza et al., 2008](#)).

See Table 2.13 available at <http://monographs.iarc.fr/ENG/Monographs/vol100B/100B-05-Table2.13.pdf>, Table 2.14 available at <http://monographs.iarc.fr/ENG/Monographs/vol100B/100B-05-Table2.14.pdf>, and Table 2.15 available at <http://monographs.iarc.fr/ENG/Monographs/vol100B/100B-05-Table2.15.pdf>.

#### *2.4.3 Cancers of the vulva, vagina, and penis*

A recent meta-analysis reported that rates of cancers of the vulva and vagina (SIR, 6.45; 95%CI: 4.07–10.2) and penis (SIR, 4.42; 95%CI: 2.77–7.07) are increased in persons with HIV-1 ([Grulich et al., 2007a](#)). [The Working Group noted that the increase in transplant recipients is of a similar magnitude, suggesting that confounding by sexual behaviour does not completely explain the increased risk in people with HIV-1.] However, fewer than 50 cases of these cancers have been described in cohort studies of people with HIV-1 (See Table 2.16 available at <http://monographs.iarc.fr/ENG/Monographs/vol100B/100B-05-Table2.16.pdf>).

### **2.5 Cancer of the skin**

There has been limited research on non-melanoma skin cancer in people with HIV-1, and a recent meta-analysis estimated that the rate was increased 4-fold ([Grulich et al., 2007a](#)). In contrast, in solid organ transplant recipients, rates of non-melanoma skin cancer are raised around 30-fold ([Grulich et al., 2007a](#)). While the majority of cases of non-melanoma skin cancer in transplant recipients is squamous cell carcinoma, this does not appear to be the case in people with HIV-1, in whom basal cell carcinoma predominates ([Bedimo et al., 2004](#)). However, it is not clear whether data on basal cell carcinoma and squamous cell carcinoma are comparable in

terms of completeness and allowance for differences in age distribution. In the US AIDS cancer match (record linked AIDS and cancer registries), increased rates of the rare Merkel cell carcinoma have been described (based on six cases), and this is also a cancer which has been described as occurring at increased rates in transplant recipients ([Engels et al., 2002](#)).

See Table 2.17 available at <http://monographs.iarc.fr/ENG/Monographs/vol100B/100B-05-Table2.17.pdf>, and Table 2.18 available at <http://monographs.iarc.fr/ENG/Monographs/vol100B/100B-05-Table2.18.pdf>.

### **2.6 Cancer of the conjunctiva**

An association of HIV-1 with conjunctival cancer was first suggested by clinical observations of an increased number of diagnoses of this cancer in Africa at the time of the onset of the HIV-1 epidemic ([Kestelyn et al., 1990](#); [Waddell et al., 1996](#)). Moreover, data from the Ugandan cancer registry demonstrated a 6-fold increase in incidence in the late 1980s compared to the incidence rates in 1970–88 ([Ateenyi-Agaba, 1995](#)). Data in Table 2.19 (available at <http://monographs.iarc.fr/ENG/Monographs/vol100B/100B-05-Table2.19.pdf>) and Table 2.20 (available at <http://monographs.iarc.fr/ENG/Monographs/vol100B/100B-05-Table2.20.pdf>) demonstrate a consistent marked increase in risk of conjunctival cancer among HIV-1-infected people, with a relative risk of around 10. One study described a case of conjunctival cancer that completely regressed after HAART was instituted with the occurrence of immune reconstitution ([Holkar et al., 2005](#)). Several studies suggest that mucosal HPV types are not involved in the etiology of this tumour, but a role for cutaneous types remains uncertain ([de Koning et al., 2008](#)).

Previous observations that conjunctival carcinoma was more common in tropical regions led to a hypothesis that exposure to solar ultraviolet (UV) radiation would be important

([Guech-Ongey et al., 2008](#)). The risk is increased in those with a past history of skin cancer, and in an ecological analysis, rates were strongly correlated with ambient UV exposure ([Newton, 1996](#)). In the USA, rates of AIDS-associated conjunctival cancer are higher in regions with higher ambient UV radiation ([Guech-Ongey et al., 2008](#)). Squamous cell carcinoma lesions contain classic UV-radiation-induced p53 mutations ([Waddell & Newton, 2007](#)). A direct role of HIV-1-associated immune deficiency is supported by the fact that the incidence is also increased in kidney transplant recipients (based on five cases) ([Vajdic et al., 2007](#)). In addition, a case report of spontaneous regression of biopsy-proven cancer in an African woman commencing HAART, co-incident with an improvement in CD4 count, has been described ([Holkar et al., 2005](#)). In the largest African series of 476 cases diagnosed in Uganda in 1995–2001, 64% of cases were HIV-1-positive, and the median CD4 count in these was 111 cells/mL ([Waddell et al., 2006](#)). [The Working Group noted that the median CD4 count was based on a subset of 159 cases.]

## 2.7 Cancer of the lung

Cancer of the lung is one of the most common cancers that occurs in men and women of the general population in developed countries ([World Cancer Report, 2008](#)). Most (> 90%) of the lung cancer cases are registered in cigarette smokers, and the risk of developing the disease is strongly associated with duration and intensity of the habit. Higher prevalence of smokers among HIV-1-infected people, as compared to their referent general population, has been hypothesized, but not well documented ([Parker et al., 1998](#); [Bower et al., 2003](#)). In the USA, among a national representative sample of persons receiving care for HIV-1 infection in the late 1990s, 73% had ever smoked and 51% were current smokers, which is a much higher proportion than the 20–30% of the general US

population who currently smoke ([Giordano & Kramer, 2005](#)). However, there was no evidence of an excess risk for lung cancer in HIV-1-infected people until prolonged survival due to the use of HAART had enhanced the likelihood of these persons developing cancer types (including lung cancer) rarely noted in the pre-HAART era. The epidemiological evidence of a statistically significant excess risk for lung cancer was consolidated in the post-HAART era, with relative risks in the range of 2–4 ([Bower et al., 2003](#); [Dal Maso et al., 2003a](#); [Chaturvedi et al., 2007](#); [Patel et al., 2008](#)). The magnitude of the excess risk was, in Italy, 2.4 (95%CI: 1.5–3.7) during 1985–98 ([Dal Maso et al., 2003a](#)); in the USA, 4.5 (95%CI: 4.2–4.8) during 1992–95, and 2.8 (95%CI: 2.4–3.1) during 2002–05 ([Frisch et al., 2001](#)); and in Switzerland, 3.2 (95%CI: 1.7–5.4) during 1985–2003 ([Clifford et al., 2005](#)). A meta-analysis of seven reports of HIV-1-associated cancer risk, involving 444172 people with HIV-1/AIDS in the USA, Australia, Scotland, Italy, Switzerland, and England reported an overall relative risk of 2.7 (95%CI: 1.9–3.9) ([Grulich et al., 2007a](#)).

Studies that assessed the risk of lung cancer in HIV-1-infected people according to individual antiretroviral treatment failed to demonstrate an effect of HAART on lung cancer risk, thus indicating that lung cancer is not strongly associated with severe immunosuppression ([Clifford et al., 2005](#); [Serraino et al., 2007](#)). In the United Kingdom, incidence rates of lung cancer increased from 0.8 (95%CI: 0.2–3.2)/10<sup>5</sup> patient-years follow-up in the pre-HAART era to 6.7 (95%CI: 3.1–13.9)/10<sup>5</sup> patient-years follow-up in the post-HAART era ([Bower et al., 2003](#)), while in Italy, incidence rates were 10.7 (95%CI: 6.2–17.2)/10<sup>4</sup> person-years and 14.1 (95%CI: 3.7–36.4)/10<sup>4</sup> person-years, respectively ([Dal Maso et al., 2003b](#)). An update of these data indicated that the SIR for lung cancer among Italian people with AIDS has nearly doubled from the pre- to the post-HAART period, from 2.1 (95%CI: 1.2–3.3) during 1985–96 to 4.1 (95%CI: 2.9–5.5) during

1997–2004 ([Dal Maso et al., 2009](#)). [The Working Group noted that several aspects regarding the role of HIV-1-induced immunosuppression in the etiology of lung cancer still need to be clarified, because factors like duration and intensity of smoking have not been controlled for, and could deeply confound the association between HIV-1 infection and lung cancer.]

Several studies have attempted to control for confounding by smoking but residual confounding is possible in these studies ([Engels et al., 2006](#); [Kirk et al., 2007](#)). In a population where smoking rates did not differ by HIV-1 status, lung cancer was not related to HIV-1 status ([Stein et al., 2008a](#)). In the USA, it has been estimated that nearly twice as many cases of lung cancer would be observed in HIV-1-infected persons than the general population, simply because of the higher prevalence of smoking in that population ([Giordano & Kramer, 2005](#)). Only one study regarding the risk of lung cancer among HIV-1-infected persons ([Phelps et al., 2001](#)) has included a control group with a history of smoking similar to that of the study group. Nearly 90% of the persons included in the study had ever smoked, and no statistically significant increase in the risk of lung cancer was reported. Interestingly, one study found a higher SIR for lung cancer among injecting drug users not infected with HIV-1 than among HIV-1-infected persons, which is further evidence that the relationship of HIV-1 infection to lung cancer is confounded by an exposure, most likely smoking ([Serraino et al., 2000](#)). [The Working Group noted that, clearly, persons with HIV-1 infection are at increased risk for lung cancer, compared with persons without HIV-1 infection. Much of that risk is due to the high prevalence of smoking in the HIV-1-infected population. Studies of large cohorts that can adequately adjust for smoking are needed to determine if HIV-1 infection itself increases the risk of lung cancer; in addition, Kaposi sarcoma can appear in the lungs and be potentially misdiagnosed as lung cancer.]

See Table 2.21 available at <http://monographs.iarc.fr/ENG/Monographs/vol100B/100B-05-Table2.21.pdf>, and Table 2.22 available at <http://monographs.iarc.fr/ENG/Monographs/vol100B/100B-05-Table2.22.pdf>.

## 2.8 Cancer of the liver

HIV-1-positive persons, in particular injecting drug users, have a greatly increased prevalence of HBV and HCV infections compared to the general population, and hence are at a higher risk for liver cancer (i.e., hepatocellular carcinoma [HCC]) ([Thio et al., 2002](#); [Hisada et al., 2005](#); [Kramer et al., 2005](#); [McGinnis et al., 2006](#)). Studies on HBV/HCV natural history have shown that HIV-1-related immune suppression worsens the risk of cirrhosis and of liver-related death ([Di Martino et al., 2001](#); [Graham et al., 2001](#); [Thio et al., 2002](#); [Kramer et al., 2005](#)), but a direct effect of HIV-1-related immunodeficiency on HCC risk has not yet been demonstrated ([Frisch et al., 2001](#); [Kramer et al., 2005](#); [McGinnis et al., 2006](#)). Following the widespread use of HAART, liver disease has become a progressively more important cause of morbidity and mortality among HIV-1-infected persons ([Louie et al., 2002](#); [Weber et al., 2006](#)). Findings from cohort investigations, record linkage of HIV-1/AIDS registries with population-based cancer registries and case-control studies conducted across Europe, the USA and Australia have documented 2–20-fold excess risks for HCC. A 5.2-fold elevated risk (95%CI: 3.3–8.2) was reported in a meta-analysis of seven population-based studies of people with HIV-1 ([Grulich et al., 2007a](#)). Excess risks were more pronounced among HIV-1-infected injecting drug users (SIR, 50.5; 95%CI: 15.9–11; [Clifford et al., 2005](#)) (SIR, 24.3; 95%CI: 2.3–89.3; [Serraino et al., 2000](#)); than among other HIV-1-transmission categories, and among HIV-1-infected persons with low CD4-positive cell counts ([Clifford et al., 2008](#)). There is evidence indicating that the advanced

immunosuppression associated with HIV-1 disease progression does not influence the occurrence of HCC ([Frisch et al., 2001](#)), but the role of HAART still needs to be better defined. Relative risks for HCC were higher in the HAART era than in preceding years ([Hessol et al., 2007](#)), though studies based on individual data on HAART use either reported lack of association ([Serraino et al., 2007](#)) or a significantly reduced risk of HCC in people treated with HAART (RR, 0.3; 95%CI: 0.1–0.9) ([Hessol et al., 2007](#)). Data from African studies do not report an increase in risk in association with HIV-1 for that cancer ([Newton et al., 2001](#); [Mbulaiteye et al., 2006](#); [Stein et al., 2008a](#)).

See Table 2.23 available at <http://monographs.iarc.fr/ENG/Monographs/vol100B/100B-05-Table2.23.pdf>, and Table 2.24 available at <http://monographs.iarc.fr/ENG/Monographs/vol100B/100B-05-Table2.24.pdf>.

## 2.9 Other cancers

### 2.9.1 Cancer of the lip

A meta-analysis that included only studies that linked HIV-1/AIDS and cancer registries found an SIR for cancer of the lip of 2.80 (95%CI: 1.91–4.11), and in solid organ transplant recipients, the SIR was 30.0 (95%CI: 16.3–55.3) ([Grulich et al., 2007a](#)). In the US AIDS cancer match, there was an increased risk of lip cancer, and a trend towards increasing risk across the pre- and post-AIDS periods, suggesting a link with advancing immune deficiency ([Frisch et al., 2001](#)).

### 2.9.2 Cancer of the head and neck

A meta-analysis that included only studies that linked HIV-1/AIDS and cancer registries found an SIR for oral cavity and pharyngeal cancer of 2.32 (95%CI: 1.65–3.25), and in solid organ transplant recipients, the SIR was 3.23 (95%CI: 2.40–4.35) ([Grulich et al., 2007a](#)). In

people with HIV-1, HPV is particularly frequent, and it is also possible that the increase in rates is due to confounding factors, in particular, increased rates of smoking ([Frisch et al., 2001](#); [Haigentz, 2005](#); [Silverberg & Abrams, 2007](#)).

### 2.9.3 Cancer in transplant patients

Data on cohort studies of transplant recipients are presented in Table 2.25 (available at <http://monographs.iarc.fr/ENG/Monographs/vol100B/100B-05-Table2.25.pdf>). Transplant recipients share immune suppression with people with HIV-1, but are not at an increased risk of oncogenic sexually transmitted viruses. [The Working Group concluded that if cancer patterns are similar between these two populations, it is likely that immune deficiency is the main factor involved.]

## 3. Cancer in Experimental Animals

In this volume, the Working Group decided not to include a separate section on “Cancer in Experimental Animals” in the *Monographs* on viruses but rather to include description of such studies under Section 4 (below). The reasoning for this decision is explained in the General Remarks.

## 4. Other Relevant Data

The first indication of the AIDS pandemic came in October of 1981 from the observation that a specific type of cancer, namely Kaposi sarcoma, was occurring with increased frequency in young homosexual men ([Friedman-Kien, 1981](#)). This report was quickly followed by the recognition that the same risk group was affected by increased opportunistic infections and immunodeficiency ([Gottlieb et al., 1981](#); [Siegal et al., 1981](#)), and the discovery of an AIDS-associated

virus, HIV (see Section 1). While HIV-1-infection has never been shown to transform any cell type, it is now clear that infected individuals are at a greatly increased risk for cancer development. The prevailing opinion is that HIV-1 acts indirectly, mainly through immunosuppression.

## 4.1 Biochemical and biological properties of relevant HIV-1 proteins

The proteins encoded by HIV-1 are illustrated in Fig. 1.1 (see Section 1.1). Several HIV-1 proteins are conserved as is the case for all other retroviruses. These include the structural proteins and viral enzymes described in Section 1.1. Although none of the proteins encoded by HIV-1 has been unequivocally shown to be directly oncogenic, some are unique to lentiviruses, and are associated with immunodeficiency, thereby indirectly promoting cancer development. In addition, there is evidence that some of the HIV-1-encoded proteins may promote cancer by other indirect mechanisms that are not dependent on immunodeficiency. Some of the proteins that are unique to HIV-1 and related immunodeficiency lentiviruses are discussed below.

### 4.1.1 Tat

This multifunctional protein is an important regulator of viral transcription. Tat recruits cellular transcription factors to the HIV-1 promoter. Tat interacts with the protein-kinase complexes Cdk9/cyclin T1 and Cdk2/cyclin E, with p300/CBP, p300/CBP-associated factor and hGCN5, and with protein phosphatases, and multiple other cellular proteins. Tat vastly increases the level of transcription of the HIV-1 DNA by inducing a positive feedback loop ([Gatignol, 2007](#)). It can also affect the course of HIV-1-associated disease indirectly, as it is secreted by infected cells, and can enter uninfected

cells ([Gupta & Mitra, 2007](#)). Extracellular Tat has many functions, which are thought to play a major role in enabling HIV-1 to escape immune surveillance, and to act as a viral toxin contributing to the pathology of AIDS. Extracellular Tat is able to regulate cytokine-gene expression ([Marone et al., 2000](#)), and immune cell hyperactivation ([Kwon et al., 2008](#)). Arguments in favour of Tat being involved in oncogenesis include:

- Tat can induce apoptosis in neighbouring uninfected cells when secreted from infected cells ([Li et al., 1995](#); [Westendorp et al., 1995](#); [Alimonti et al., 2003](#)). This is thought to be due to the ability of Tat to upregulate the expression of Fas ligand mRNA in macrophages and increase the susceptibility of bystander CD4-positive T cells to crosslinking-induced death. This may contribute to the massive depletion of CD4-positive T cells by apoptosis, leading to the severe immunodeficiency seen in AIDS.
- Tat has been shown to stimulate the growth of Kaposi sarcoma cells ([Vogel et al., 1988](#); [Aoki & Tosato, 2007](#); see Section 4.3).

Nevertheless, it appears that although Tat may contribute to carcinogenesis, it is probably involved only as a cofactor. While early studies found an increased incidence of lymphomas in *Tat*-transgenic mice ([Corallini et al., 1993](#)), these do not faithfully replicate the pathology of AIDS-related malignancies ([Altavilla et al., 2004](#)).

Other effects of extracellular Tat include repression of major histocompatibility complex class I transcription ([Weissman et al., 1998](#)), and upregulation of the expression of CXC-chemokine receptor 4 (CXCR4) on resting CD4-positive T cells ([Secchiero et al., 1999](#)).

#### 4.1.2 Rev

The HIV-1 Rev protein regulates post-transcriptional processing of viral mRNAs. Rev primarily functions to export unspliced and partially spliced viral RNAs from the nucleus into the cytoplasm ([Suhasini & Reddy, 2009](#)). Currently there is no evidence to support a direct role of this protein in the development of cancer.

A specific feature of primate immunodeficiency viruses is the presence of accessory proteins (e.g., Nef, Vif, Vpr, Vpu), which play a role in helping the virus to evade the various forms of cell-mediated antiviral resistance, thereby ensuring viral persistence and transmission ([Malim & Emerman, 2008](#)).

#### 4.1.3 Nef

Nef is a multifunctional regulatory protein that affects HIV-1 virulence ([Kestler et al., 1991](#); [Deacon et al., 1995](#)). Carriers of HIV-1 strains with Nef deletions have been identified, and they are characterized as slow progressors or long-term non-progressors. The mechanisms whereby Nef favours the development of AIDS remain unclear, but there are some interesting insights ([Foster & Garcia, 2008](#)). Nef downregulates CD4 ([Garcia & Miller, 1991](#)), thus preventing the interaction of budding virions on infected cells with CD4, which would interfere with the production of fully infectious virions. Nef also downregulates the expression of surface major histocompatibility complex class I by altering the endocytotic machinery ([Blagoveshchenskaya et al., 2002](#)), thereby protecting infected cells from destruction by cytotoxic T lymphocytes ([Collins et al., 1998](#)). Nef protects infected cells from apoptosis ([Baur et al., 1994](#)). In addition, Nef manipulates signalling via multiple intracellular kinases, thereby enabling infected dendritic cells and macrophages to attract, and subsequently infect, permissive CD4-positive T cells ([Pope et al., 1994](#); [Swingler et al., 1999, 2003](#)).

Similarly to the HIV-1 Tat protein, infected cells release the Nef protein into the extracellular environment and affect neighbouring cells ([Fujii et al., 1996](#)). Through this mechanism, Nef has been shown to be responsible for some B-cell defects seen in HIV-1-infected individuals by suppressing CD40-dependent immunoglobulin class-switching in bystander B cells ([Qiao et al., 2006](#)). Therefore, Nef affects B-cell differentiation and antigen selection, and contributes to immune dysregulation. These findings suggest that Nef may play a role in lymphomagenesis, but this has not been demonstrated experimentally.

#### 4.1.4 Vif

The accessory protein Vif is involved in the inhibition of cytoplasmic defenses. It is critical for *in vivo* replication of HIV-1, and for the production of infectious virions in a cell-type specific manner ([Malim & Emerman, 2008](#)). There is no evidence that Vif plays a role in AIDS-related malignancies.

#### 4.1.5 Vpr

Truncation of the open reading frame that encodes the Vpr protein results in a slower-replicating virus ([Wong-Staal et al., 1987](#)). *In vitro* analysis has demonstrated various Vpr functions that may contribute to HIV-1 pathogenesis. Vpr has been found to induce cell-cycle arrest in G2, followed by apoptosis, thereby leading to viral cytopathic effects in T cells ([Andersen et al., 2008](#)). There is no evidence that Vpr plays a role in AIDS-related malignancies.

#### 4.1.6 Vpu

The Vpu protein reduces the surface expression of CD4 of the host cell, and modulates the subcellular compartmentalization of the host membrane protein tetherin to help promote viral dissemination and replication ([Malim &](#)

[Emerman, 2008](#)). There is no evidence that Vpu plays a role in AIDS-related malignancies.

## 4.2 HIV-1, host immune system, and carcinogenesis

### 4.2.1 Comparison of AIDS-related and transplantation-associated tumours

#### (a) Immunosuppression

Although individuals with AIDS and those with iatrogenic immunosuppression following organ transplantation have immunodeficiency in common, the immunological abnormalities appear to differ significantly between these two conditions. A direct comparison is difficult because the immune dysregulation in both groups can be qualitatively and quantitatively heterogeneous. Among transplant recipients, immunodeficiency depends on multiple factors. The immunosuppressive regimen can be a major determinant of the immunological dysfunction, and these regimens vary according to the organ transplanted (for example, there is more tolerance for rejection in kidney than in heart/lung transplants), age, and time (as therapeutic approaches have evolved). Cytotoxic CD8-positive T-cell responses, in particular to EBV, have been recognized as a common immunological defect in organ transplant recipients with lymphoproliferative diseases ([Kyaw-Tanner et al., 1994](#)). One study that focused on immune function in renal transplant patients found that the most common abnormality was B-cell lymphopenia (seen in 85% of the patients), followed by reduced production of reactive oxygen species in neutrophils (in 63%), NK-cell lymphopenia (in 50%), and abnormal lymphocyte mitogen response (in 49%). A low CD4 count was only found in about a quarter of the patients ([Hutchinson et al., 2003](#)). A characteristic feature of AIDS is CD4 deficiency, which is well documented to be central to disease progression, but in addition, there

is B-lymphocyte hyperactivation with hypergammaglobulinaemia, and increased release of soluble markers from activated cells ([De Milito, 2004](#)). However, AIDS patients, in particular those with advanced disease, suffer from a severe loss of memory B cells, and an impaired long-term serological memory ([Titaji et al., 2006](#)). In HIV-1-infected individuals, EBV-specific cytotoxic T cells have been found in normal numbers, but their functional capacity decreases as AIDS progresses until, finally, immunological collapse occurs ([Kersten et al., 1997; van Baarle et al., 2001](#)). Thus, the immune dysfunction in AIDS patients and transplant recipients is heterogeneous, and although it is different between these two categories, there is also overlap in immunological defects in selected groups of individuals.

#### (b) Cancer types

An obvious similarity between post-transplant and AIDS patients is the increased incidence in B-cell lymphomas, which often have plasmacytoid differentiation, and are associated with EBV. However, there are significant differences in lymphoma subtypes ([Raphael & Knowles, 1990; Swerdlow et al., 2008](#)). Specific differences include more frequent high-grade lymphomas in the setting of HIV-1 infection, and a more frequent EBV association and polymorphic lesions in transplant recipients (see [Table 4.1](#)).

The second important malignancy that is greatly increased in incidence in HIV-1-infected individuals and transplant recipients is Kaposi sarcoma. This is the most common cancer in patients with HIV-1, and an AIDS-defining condition. A recent study of renal transplant recipients reported a more than 20-fold increased incidence of Kaposi sarcoma compared with the general population ([Kasiske et al., 2004](#)). While Kaposi sarcoma tends to be more aggressive in patients with AIDS than in those with other immunodeficiencies, there are no differences in histological, immunophenotypic, virological or

**Table 4.1 Major categories of lymphoproliferations associated with acquired immunodeficiency**

	Lymphoma subtype and frequency	Approximate relative frequency of lymphoma subtype	Approximate EBV frequency (LMP-1 positivity)	Other common oncogenic alterations
<b>AIDS-related lymphomas</b>	Burkitt and Burkitt-like lymphoma	30%	30–50% (Rare)	c-Myc, p53, BCL-6
	DLBCL-Centroblastic	25%	30% (Rare)	BCL-6
	DLBCL-Immunoblastic <sup>a</sup>	22%	> 80% (Common)	
	Primary effusion lymphoma <sup>a</sup>	2–4%	70–90% (None)	KSHV infection
	KSHV-positive extracavitary lymphoma <sup>a</sup>	2–4%	> 80% (Unknown)	KSHV infection
	Polymorphic B-cell lymphoma (PTLD-like) <sup>a</sup>	Rare	40% (Unknown)	Unknown
	Plasmablastic lymphoma of oral cavity <sup>a</sup>	Rare	80% (Rare)	Unknown
	Primary CNS lymphomas <sup>a</sup>	10–15%	> 95% (Common)	BCL-6
<b>Post-transplant lymphoproliferative disorders</b>	Plasmacytic hyperplasia	30%	> 90% (Common)	None
	Polymorphic PTLD	50%	> 90% (Common)	BCL-6
	Monomorphic PTLD (non-Hodgkin lymphoma/multiple myeloma)	20%	60% (Variable)	BCL-6, c-Myc, N-Ras, p53

<sup>a</sup> These lymphoma subtypes occur much more frequently or almost exclusively in HIV-1-infected individuals, and share frequent EBV-positivity and plasmacytoid differentiation.

DLBCL, diffuse large B-cell lymphoma; PTLD, post-transplant lymphoproliferative disorder; CNS, central nervous system

Adapted from [Ceserman & Chadburn \(2007\)](#)

molecular features of AIDS-associated and post-transplant-related Kaposi sarcoma. Both types may regress (but they not always do) after reconstitution of the immune response.

#### 4.2.2 Immune dysfunction and carcinogenesis

The importance of immunodeficiency is easy to understand in the context of virus-associated malignancies. The three most common cancers in HIV-1-infected individuals are Kaposi sarcoma (caused by KSHV), lymphomas (many EBV-positive), and cervical and anogenital carcinomas associated with HPV infection. These infectious etiologies explain the reasons as to why the associated cancers are greatly increased in individuals with immunodeficiencies. Infection by “oncogenic” viruses is much more common than the diseases caused by those viruses, and the incidence and severity of these cancers is

greatly increased by immunodeficiency. Viruses have evolved to survive through three essential properties: they can go from host to host (transmission), get in and out of the host cells (lytic replication), and remain in the host in live cells without being recognized by its immune system (latency).

Immune dysregulation *per se* may facilitate cancer development. A clear example of this phenomenon is the B-cell hyperactivation manifested by lymphadenopathies seen in HIV-1-infected patients. Lymph nodes from these patients are characterized by a florid follicular hyperplasia, where the germinal centres are greatly enlarged. The germinal centre reaction is where B cells undergo somatic hypermutation of immunoglobulin genes and class-switch recombination. These are dangerous processes in that if uncorrected mistakes are made, oncogenic genetic alterations can occur. These mistakes can lead to translocations involving the *cMyc* oncogene and

mutations in the p53 tumour-suppressor gene, giving rise to Burkitt lymphomas.

### 4.3 HIV-1 and other infectious agents associated with human cancers

The HIV-1 genome is not present in cancer cells, which is in contrast to what is observed with infectious agents that are directly oncogenic. Therefore, any interaction between virus and host is indirect. The interaction between HIV-1 and some of the other infectious agents reviewed in this volume is discussed below.

#### 4.3.1 KSHV

##### (a) *In vivo*

KSHV and HIV-1 are not present in the same cells. In germinal centres of patients with HIV-1 infection, HIV-1 is frequently in dendritic cells and T cells ([Burton et al., 2002](#)), whereas KSHV is present in mantle zone B cells ([Dupin et al., 1999](#); [Amin et al., 2003](#)). In Kaposi sarcoma lesions, HIV-1 is localized to tumour-associated macrophages, whereas KSHV is in the Kaposi sarcoma spindle cells ([Gessain & Duprez, 2005](#)).

##### (b) *At the molecular level*

The only HIV-1 protein for which there is experimental evidence for a potential role in Kaposi sarcoma is Tat. Biologically active Tat is released by HIV-1-infected cells ([Chang et al., 1997](#)), and it can enter readily into neighbouring infected and uninfected cells ([Gupta & Mitra, 2007](#)), a property that has also been found for other cationic peptides. Three endocytic pathways appear to account for this process: macro-pinocytosis, clathrin-mediated endocytosis, and caveolae/lipid-raft-mediated endocytosis ([Duchardt et al., 2007](#)). This indicates that although HIV-1 does not directly infect tumour cells, it has the potential to produce Tat, which may then enter the tumour cells, although this

phenomenon has not been demonstrated to occur *in vivo*. Nevertheless, the potential effects of Tat have prompted investigators to test a role for HIV-1, and specifically the Tat protein, in Kaposi sarcoma development ([Aoki & Tosato, 2007](#)). Tat binds and activates the Flk-1/kinase insert domain receptor (Flk-1/KDR) ([Albini et al., 1996](#); [Morini et al., 2000](#)). Transgenic mice expressing HIV-1 Tat were found to develop vascular lesions that resembled Kaposi sarcoma ([Vogel et al., 1988](#)). Several studies found that Tat has an effect on Kaposi sarcoma cell lines, increasing proliferation *in vitro*, and growth in mice ([Ensoli et al., 1990](#); [Barillari et al., 1999a, b](#); [Morini et al., 2000](#)). However, when cells are removed from Kaposi sarcoma lesions and expanded *in vitro*, they lose the KSHV genome, therefore the question remains whether the “Kaposi sarcoma” cells lacking KSHV used in most of these studies represent a valid model for Kaposi sarcoma.

More recently, investigators have addressed the role of HIV-1 directly on KSHV infection. Individuals with Kaposi sarcoma and HIV-1 infection had a 4-fold higher KSHV viral load in serum than those with Kaposi sarcoma without HIV-1 ([Chandra et al., 2003](#)). HIV-1 can induce lytic replication of KSHV by upregulating the expression of the replication and transcription activator (Rta) protein, which is the main regulator of the lytic switch in this virus ([Varthakavi et al., 2002](#)). The HIV-1 Tat protein was shown to enhance the entry of KSHV into endothelial cells, thereby promoting KSHV cellular transmission ([Aoki & Tosato, 2004](#)). Conversely, KSHV can increase HIV-1 replication in T cells, monocytic cells, and endothelial cells ([Mercader et al., 2001](#); [Caselli et al., 2005](#)). Thus, there is evidence that HIV-1 and KSHV may enhance each other's replication, which could be a mechanism whereby HIV-1 can act as a cofactor in the complex process of Kaposi sarcomagenesis.

### 4.3.2 EBV

There is some experimental evidence for a direct role for HIV-1 in EBV-related lymphomagenesis apart from the epidemiological associations, but most of the increase in incidence is probably due to immune dysfunction. As mentioned above, HIV-1 is not found to be present in lymphoma cells, but the virus can infect primary human B cells, and activate the expression of endogenous EBV ([Astrin & Laurence, 1992](#)). It is also possible that HIV-1-encoded proteins like Tat affect the growth of EBV-immortalized B cells ([Colombrino et al., 2004](#)). Several studies where interactions between HIV-1 and EBV have been tested involve the forced expression of viral proteins or infection with both viruses of cells that are not naturally co-infected ([Kenney et al., 1988](#); [Scala et al., 1993](#); [Zhang et al., 1997](#)).

### 4.3.3 HPV

The third most common malignancy in HIV-1-positive individuals, and an AIDS-defining condition, is cervical carcinoma associated with HPV infection. Anogenital intraepithelial neoplasms and carcinomas are also increased in frequency, and so are skin cancers associated with HPV infection. Infection of HPV-infected cells with HIV-1, or addition of Tat, induces transcriptional activation of the integrated HPV ([Vernon et al., 1993](#); [Dolei et al., 1999](#)), and proliferation of infected cells ([Kim et al., 2008](#)). However, a direct interaction between HIV-1 and HPV has not been demonstrated to occur *in vivo*.

### 4.3.4 HBV and HCV

HIV-1-infected individuals have a greatly increased incidence of infection with HBV and HCV, and are therefore at risk for HCC ([Grulich et al., 2007a](#)). Although interactions between HBV and HIV-1 in co-infection experiments *in vitro* have been demonstrated ([Barak et al., 2001](#);

[Gómez-Gonzalo et al., 2001](#)), these have not been shown to occur *in vivo*.

## 4.4 Animal models for HIV-1-associated cancers

Because HIV-1, like the oncogenic herpesviruses EBV and KSHV, is species-specific, there are no ideal animal models for HIV-1-associated cancers. Four main approaches have been explored: a) the analysis of malignancies occurring in animals infected with the corresponding animal virus (i.e., SIV, simian EBV, etc); b) xenograft studies; c) the use of transgenic mice expressing selected viral oncogenes; and d) the use of chimeric mice infected with human viruses.

### 4.4.1 Lymphomas in animals infected with species-specific viruses

Lymphomas occurring in HIV-1-infected individuals have been compared with those in SIV-infected macaques ([Baskin et al., 2001](#)). Like in humans, most simian AIDS-associated lymphomas are of the B-cell type, and may be classified as either immunoblastic/large-cell lymphoma or Burkitt-like lymphoma. Furthermore, these simian lymphomas tend to be morphologically indistinguishable from the same lymphomas occurring in humans. The systemic simian AIDS-associated lymphomas in rhesus monkeys frequently contain rhesus lymphocryptovirus (LCV), while simian AIDS-associated lymphomas in cynomolgus monkeys contain herpesvirus Macaca fascicularis 1 (HVMF-1), both of which are homologues of EBV ([Li et al., 1994](#); [Habis et al., 1999](#)), thereby replicating virological AIDS-related lymphomas in humans. A rhesus monkey rhadinovirus that is closely related to KSHV has also been described ([Desrosiers et al., 1997](#)). Although lymphoid hyperplasias and sometimes extranodal lymphomas are seen in some of these

monkeys ([Wong et al., 1999](#); [Orzechowska et al., 2008](#)), neither primary effusion lymphoma or Kaposi sarcoma have been observed.

The similarities between simian and human AIDS-related lymphomas make these animal models very attractive for mechanistic studies, but they are limited by the fact that the incidence of lymphoma is relatively low. While this low incidence is not dissimilar to that seen in humans, it makes experiments with the monkeys challenging and expensive. For this reason, mouse models have been developed. The murine gamma-herpesvirus 68 (gamma-HV68) is a member of the gammaherpesvirus family related to EBV and KSHV, and has been very useful in studies aimed at understanding the mechanisms of viral persistence and replication *in vivo*, and the role of several viral proteins. Lymphoproliferative disease, including lymphoma, has been reported in association with gamma-HV68 infection in wild-type mice, but at a low incidence (9%), and after a prolonged incubation period (up to 3 years) ([Sunil-Chandra et al., 1994](#)). More recently, infection with gamma-HV68 of beta2-microglobulin-deficient mice was found to induce an atypical lymphoid hyperplasia that is similar to post-transplantation lymphoproliferative disease, and this model has been used to evaluate the function of some viral genes in pathogenesis ([Tarakanova et al., 2005, 2008](#)).

#### **4.4.2 Xenograft studies**

Transplanting human cancers into immunodeficient mice is a common approach to evaluate the biology and possible treatment strategies for malignancies. Such models of HIV-1-related cancers include those for EBV-associated lymphomas ([Nilsson et al., 1977](#); [Mosier et al., 1990](#); [Mosier, 1991](#)), primary effusion lymphoma ([Picchio et al., 1997](#); [Staudt et al., 2004](#); [Keller et al., 2006](#)), and Kaposi sarcoma ([Masood et al., 1997](#); [Barillari et al., 1999b](#); [Mutlu et al., 2007](#)). Most Kaposi sarcoma models are imperfect, however,

because the cells from Kaposi sarcoma lesions lose the KSHV genome. In an attempt to resolve this, murine and human haematopoietic precursors were infected with a bacterial artificial chromosome containing the entire KSHV genome (KSHV Bac36), and spindle cell sarcomas were obtained, which had some interesting features in common with Kaposi sarcoma ([An et al., 2006](#); [Mutlu et al., 2007](#)).

#### **4.4.3 Viral malignancies in chimeric mice**

Several attempts have been made to develop a mouse model of herpesviral malignancy by infecting chimeric mice. One of the first such studies used BNX immunodeficient mice to graft human B cells, followed by injection of EBV particles, which led to the development of rapidly fatal, polyclonal lymphomas ([Dosch et al., 1991](#)). More recently, immunodeficient mice reconstituted with human haematopoietic stem cells followed by EBV infection have been used to evaluate immune responses to EBV ([Melkus et al., 2006](#)); some of these mice also developed B-cell lymphoproliferative disorders ([Yajima et al., 2008](#)). Immunodeficient mice transplanted with human fetal thymus and liver grafts have been shown to be susceptible to KSHV infection. There was no effect of HIV-1 on KSHV viral replication, or vice versa ([Dittmer et al., 1999](#)). In addition, human haematopoietic stem cells have been infected with KSHV before transfer into immunodeficient mice ([Wu et al., 2006](#)), but these KSHV-infected mice did not develop angioproliferative or lymphoproliferative diseases. This may be due to the fact that these systems do not fully replicate the complexity of Kaposi sarcoma and lymphoma development in humans, or that these diseases would develop in only very rare occasions in mice, as is the case with KSHV-infected people. In contrast, injection of KSHV in human skin engrafted on SCID mice was found to induce Kaposi-sarcoma-like lesions ([Foreman et al., 2001](#)).

## 4.5 Synthesis

HIV-1 increases the cancer risk in humans indirectly, primarily by immunosuppression.

Many of the AIDS-defining malignancies have a different primary cause, e.g. EBV, HPV, and KSHV.

In addition to HIV-1-mediated immunosuppression, other aspects of the HIV-1 biology contribute to the increased cancer incidence in AIDS patients. Suggested mechanisms include HIV-1-mediated immune dysregulation, in particular B-cell hyperactivation, and perhaps effects of the secreted HIV-1 Tat protein. However, unlike what is known about other cancer-associated viruses, there is no evidence that HIV-1-infection by itself leads to cell transformation or immortalization.

## 5. Evaluation

There is *sufficient evidence* in humans for the carcinogenicity of infection with HIV-1. Infection with HIV-1 causes cancer of the cervix, anus, and conjunctiva, and Kaposi sarcoma, non-Hodgkin lymphoma, and Hodgkin lymphoma. Also, a positive association has been observed between infection with HIV-1 and cancer of the vulva and vagina, penis, and hepatocellular carcinoma, and non-melanoma skin cancer.

Infection with HIV-1 is *carcinogenic to humans (Group 1)*.

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# HUMAN PAPILLOMAVIRUSES

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Human papillomaviruses were considered by a previous IARC Working Group in 2005 ([IARC, 2007](#)). Since that time, new data have become available, these have been incorporated in the *Monograph*, and taken into consideration in the present evaluation.

## 1. Exposure Data

### 1.1 Taxonomy, structure, and biology

A concise overview of the taxonomy, structure, and biology of the human papillomavirus (HPV) is given below. For a more comprehensive description, the reader is referred to Volume 90 of the *IARC Monographs* ([IARC, 2007](#)).

#### 1.1.1 Taxonomy

All papillomaviruses belong to the *Papillomaviridae* family, which includes 16 different genera. Of these, the alpha genus contains the viruses associated with the development of mucosal tumours in humans, and the beta genus contains those that are associated with the development of cutaneous tumours (Fig. 1.1).

#### 1.1.2 Structure of the virion

Papillomaviruses are small non-enveloped icosahedral viruses of approximately 50–60 nm in diameter, containing a circular, double-stranded DNA genome (~7000–8000 bp) that exists in a chromatinized state.

#### 1.1.3 Structure of the viral genome

The HPV genome is divided into three regions: the long control region (LCR), which regulates viral gene expression and replication; the early (E) region, which encodes proteins required for viral gene expression, replication and survival; and the late (L) region, which encodes the viral structural proteins. The designations E and L refer to the phase in the viral life cycle when these proteins are first expressed.

#### 1.1.4 Host range and target cells

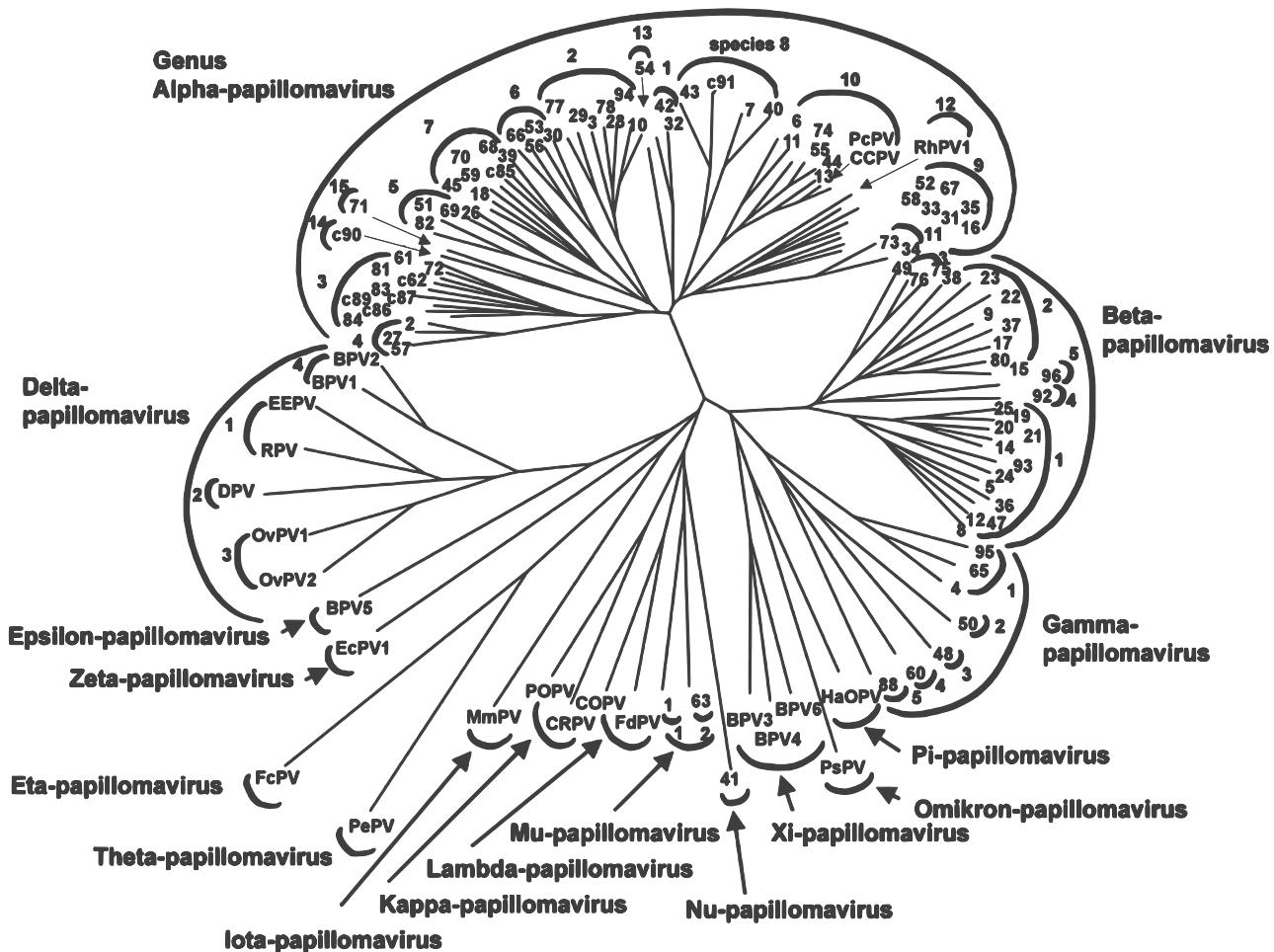
HPVs are restricted in their host range to humans, and primarily infect stratified epithelia at either cutaneous or mucosal sites. Mucosotropic HPVs can be further subdivided into high- and low-risk types depending upon their degree of association with human malignancy.

#### 1.1.5 Function of the gene products

##### (a) E1

E1 is the only enzyme encoded by the virus possessing DNA helicase activity. Once bound to the viral origin of replication, this enzyme recruits the cellular DNA-replication machinery to drive viral DNA replication.

Figure 1.1 Phylogenetic tree containing the sequences of 118 papillomavirus types



Reprinted from Virology 324(1), de Villiers EM, Fauquet C, Broker TR, Bernard HU, zur Hausen H, Classification of papillomaviruses, pp 17–27, 2004, with permission from Elsevier.

## (b) E2

This protein serves three major functions in the viral life cycle. The first is to regulate the expression levels of the other viral gene products, and – depending upon the binding sites occupied in the LCR – to act as a transcriptional repressor or activator. Second, it recruits E1 to the viral origin, thereby enhancing viral DNA replication. Third, it has a critical role in the transfer of the viral genome to daughter cells during division of the host cell.

## (c) E4

E4 is the most abundantly expressed viral protein, the function of which is still obscure. It has been linked to processes aiding viral DNA amplification and viral release.

## (d) E5

E5 is one of three oncoproteins encoded by the virus (see Section 4.2). Its mode of action is still unclear, although it contributes quantitatively to the productive stage of the viral life cycle, and has been closely linked with the regulation of

growth-factor signalling pathways and immune avoidance.

(e) *E6*

E6 is the second HPV-encoded oncoprotein (see section 4.2). It cooperates with E7 to provide an environment suitable for viral DNA replication, principally by overcoming cellular apoptotic processes. The most well characterized target of E6 from high-risk mucosotropic HPV types is the tumour-suppressor protein p53, which is directed by E6 towards degradation.

(f) *E7*

E7 is the third HPV-encoded oncoprotein (see section 4.2). By targeting cell-cycle regulatory pathways controlled by the tumour-suppressor protein pRb and the related proteins p107 and p130, it provides an environment favourable to viral DNA replication by maintaining an S-phase-like state in the differentiating keratinocytes.

(g) *L1 and L2*

L1 and L2 are the major and minor constituents, respectively, of the viral capsid. When overexpressed in various eukaryotic cells, L1 can self-assemble to form virus-like particles (VLPs). These VLPs are the basis for prophylactic vaccines against HPV, through induction of neutralizing antibodies.

### 1.1.6 Life cycle

HPVs are specifically epitheliotropic and their life cycle takes place within stratified squamous epithelia.

(a) *Entry*

It is assumed that HPVs initiate infection by penetrating through microtraumas in the epithelia to reach the basal cells, which are believed to be the target cells for HPV infection. The mechanism for virus entry into the basal cells is not entirely understood. Subsequent steps

in the life cycle of the virus can be divided into three stages: establishment, maintenance, and production.

(b) *Establishment of the non-productive infectious state*

Once an HPV particle enters the host cell, it must rely primarily on the cellular machinery to replicate its DNA. In infected basal cells, the HPV genome becomes established as a low copy-number nuclear plasmid. Within these cells, only early viral gene products are expressed, and this is consequently referred to as the ‘non-productive’ stage of infection.

(c) *Maintenance of the non-productive infectious state*

A hallmark of HPV infection is its long-term persistence over many years, which, in the case of high-risk types, is a prerequisite for the development of cancer. This requires that the viral genome be maintained over multiple cell divisions; how this is achieved is still unclear.

(d) *Productive stage*

This begins when the daughter cells derived from the infected basal cells start to differentiate. The virus delays the terminal differentiation programme of the cell, and redirects the cell’s DNA replicative capacity. This then allows amplification of the viral genome and expression of the late viral genes necessary for the production of progeny virus, and subsequent viral release.

## 1.2 Epidemiology of infection

The epidemiology and natural history of HPV infection were extensively reviewed in the previous *IARC Monograph* ([IARC, 2007](#)).

### 1.2.1 Prevalence, geographic distribution

Most sexually active individuals will acquire at least one genotype of anogenital HPV infection at some time during their lifetime. The most comprehensive data on cervical HPV prevalence in women with normal cytology (the great majority of infections do not produce concurrently diagnosed cytological abnormalities) is provided by a meta-analysis including over 150000 women ([Castellsagué et al., 2007](#); [de Sanjose et al., 2007](#)). After adjusting to the extent possible for study design, age, and HPV DNA detection assays, the estimated worldwide HPV DNA point prevalence was approximately 10%. The highest estimates were found in Africa and Latin America (20–30%), and the lowest in southern Europe and South East Asia (6–7%). Point prevalence estimates are highly dynamic because incidence and clearance rates are high; averaging across age groups can be particularly misleading.

Fig. 1.2 shows the eight most common HPV types (HPV 16, 18, 31, 33, 35, 45, 52, and 58) by geographic region. HPV 16 is the most common type in all regions with levels of prevalence ranging from ~3–4% in North America to 2% in Europe. HPV 18 is the second most common type worldwide.

Generally, similar results for the regional estimates of point prevalence of HPV DNA were observed in an IARC population-based prevalence survey conducted in 15613 women aged 15–74 years from 11 countries around the world ([Clifford et al., 2005a](#)).

The age-specific prevalence curve showed a clear peak in women up to 25 years of age with subsequent decline until an age range of 35–44 years, and an increase again in all regions included in the meta-analysis except Asia ([de Sanjose et al., 2007](#)). In the IARC population-based survey, a first peak was observed in women under 25 years of age, and a second peak after 45 years of age in most Latin American populations, but the HPV prevalence was high across all age groups in a few

places in Asia and in Nigeria ([Franceschi et al., 2006](#)). In this survey, the prevalence of high-risk HPV correlates well with cervical cancer incidence, and the strength of the correlation steadily increases with age ([Maucort-Boulch et al., 2008](#)).

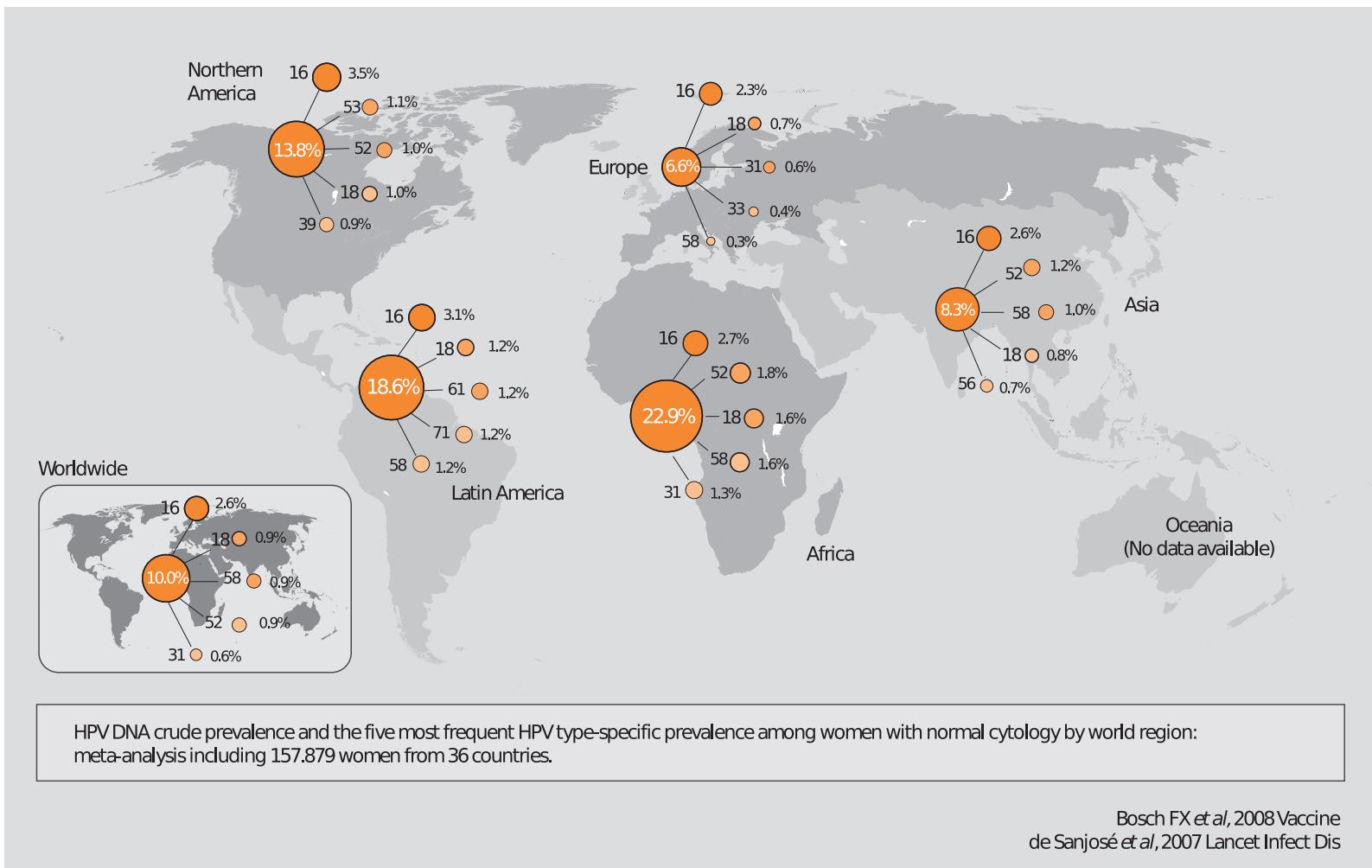
Data on HPV DNA prevalence and natural history of genital HPV infection in men is scant, and difficult to evaluate. There is great variation in the prevalence depending on anatomical sites sampled, sampling methods, and HPV DNA detection assays. In general, the overall HPV prevalence is over 50%, and the proportion of low-risk types is higher in men than in women ([Giuliano et al., 2008](#)). However, the biological or clinical meaning of the HPV DNA detected in the superficial layers of genital skin is not yet clear. Unlike what has been observed in women, no clear age pattern is detected in HPV prevalence rates in men ([Giuliano et al. 2008](#)).

HPV prevalence is lower in the oral cavity than in other anogenital sites. Among women who practiced prostitution, HPV DNA prevalences for specimens from the cervix, vagina, and oral cavity have been observed to be 27.8%, 26.1%, and 15%, respectively ([Cañadas et al., 2004](#)). HPV infections of the skin are extremely common, but the type distribution is different (beta and gamma genera predominating) than the mucosal types in the alpha genus that commonly infect the anogenital tract and the oral cavity.

### 1.2.2 Transmission and risk factors for infection

HPV infections are transmitted mainly through direct skin-to-skin or skin-to-mucosa contact. The viruses are easily transmitted and each genotype has its characteristic tissue tropism and characteristic age-specific peak transmission curve. In line with the unequivocal demonstration of sexual transmission of anogenital HPV, the number of sexual partners has been shown to be the main determinant of anogenital HPV infection both in women and men. The highest

**Figure 1.2 HPV DNA crude prevalence and HR-HPV type-specific prevalence among women with normal cytology by world region: meta-analysis including 157.879 women from 36 countries**



'Other HR' includes the 6 most common HPV types in cervical cancer other than 16 and 18: HPV-31, 33, 35, 45, 52, 58  
Art work: Laia Bruni adapted from [Bosch et al. \(2008\)](#) and [de Sanjosé et al. \(2007\)](#)

incidence of anogenital infection occurs in teens and young adults. Increasing age is linked to decreasing acquisition of anogenital HPV infection as a corollary of fewer new partners and, possibly, immunity to previously cleared infections ([Burchell et al., 2006](#); [Dunne et al., 2006](#)).

HPV infection probably requires access to basal cells through micro-abrasions in the epithelium ([Burchell et al., 2006](#)). Circumcision and condom use have also been associated with a reduced risk of infection in men and their partners ([Burchell et al., 2006](#); [Dunne et al., 2006](#)). Although it has been reported that smoking, use of oral contraceptives, parity, other sexually transmitted agents, age at first sexual intercourse, and host susceptibility may influence the risk of acquisition of HPV infection ([Burchell et al., 2006](#); [Moscicki et al., 2006](#)), the epidemiological evidence is inconsistent.

Non-sexual routes account for a tiny minority of HPV infections, and include perinatal transmission and, possibly, transmission by medical procedures and fomites.

### *1.2.3 Persistence, latency, and natural history of infection*

Most HPV infections clear within 1–2 years. However, estimates of duration of infection for individual types vary from study to study, and depend not only on the statistical methods used (definition of clearance, use of mean or median), but also on the accuracy of the HPV DNA detection methods. Although it has been reported that infections in older women last longer, suggesting greater risk of cancer ([Castle et al., 2005a](#)), this only pertains to detected infections found at the baseline of cross-sectional screening. There is no association between HPV incident infection duration and age, when infections detected during follow-up are followed in cohort studies ([Trottier et al., 2008](#); [Muñoz et al., 2009](#)). Persistent HPV infection is a prerequisite for the development of high-grade precancerous lesions

(cervical intraepithelial neoplasia [CIN]3) and cervical cancer, but for epidemiological purposes there is no consensus on the definition of persistent infection. Most investigators call persistent infections those in which the same HPV type or group of HPV types is detected during two consecutive visits, but these two visits could be 4 months up to 5–7 years apart, leading to serious conceptual problems ([Woodman et al., 2007](#)). A new definition of persistent HPV infection based on the duration of incident infection has been proposed ([Muñoz et al., 2009](#)). Moreover, there are many parameters of the natural history that are unknown (e.g., the precise time of HPV acquisition, and the probable existence of latent infections with possible reactivation as suggested by new detection among sexually inactive older women); overall, the distinction between transient and persistent infection is impossible to establish accurately.

Despite these limitations, persistence defined as HPV positivity at two or more visits has been associated with an increased risk of CIN2/3 lesions in most studies included in a meta-analysis ([Koshiol et al., 2008](#)). In particular, repeat detection of HPV 16 is associated with an extremely high cumulative risk of subsequent CIN3+ diagnosis, exceeding 30% in some cohorts ([Wheeler et al., 2006](#); [Rodríguez et al., 2008](#)). Persistence is not sufficient for carcinogenicity because there are non-carcinogenic types, like HPV 61, that persist without carcinogenic risk ([Schiffman et al., 2007](#)).

Host susceptibility factors and immune responses are obviously important but poorly understood determinants of persistence and progression. Other cofactors are discussed under Section 2.6.

CIN3 can develop very quickly (within 2–3 years) following HPV exposure, especially in young women ([Winer et al., 2005](#); [Ault, 2007](#)). Initially, CIN3 lesions are very small, and it takes a few years for them to grow and to be detectable by cytology and then colposcopy. In young,

intensively screened women, the median age of CIN3 diagnosis was around 23 years, while it was 38 years in a cohort of women from New Zealand where screening and treatment were inadequate ([McCredie et al., 2008](#); [Schiffman & Rodríguez, 2008](#)).

A direct estimate of the rate of progression from CIN3 to invasive cervical cancer has been reported in the cohort of 1063 women from New Zealand for whom treatment for CIN3 was withheld or delayed in an unethical clinical study starting in the 1960s. Cumulative incidence of invasive cervical cancer was 31.3% at 30 years of follow-up among 143 women who had had only diagnostic biopsies, and it was 50.3% in the subset of 92 women who had persistent CIN3 during 24 months. Cancer risk at 30 years was 0.7% for women whose initial treatment for CIN3 was considered adequate ([McCredie et al., 2008](#)). [The Working Group noted that McCredie *et al.* were not responsible for the unethical study but gathered data from that study, and did the final follow-up.] It is unknown which proportion of small early CIN3 lesions will eventually progress to invasive cancer.

#### *1.2.4 Evaluation of HPV vaccination on precancerous lesions occurrence or decrease*

Two prophylactic HPV vaccines are currently marketed. One is bivalent and contains VLP antigens for HPV 16 and 18, and the other is quadrivalent and contains VLP antigens for HPV 16, 18, 6, and 11. Both vaccines are designed to prevent HPV infection and HPV-related disease, and not to treat women with past or current HPV infection or disease. End-points of CIN2/3 or adenocarcinoma *in situ* (AIS) have been widely accepted as a proxy for cervical cancer that can be studied ethically in efficacy trials.

Both vaccines have efficacies of > 90% against CIN2 or higher grade among women aged 15–26 years who had no evidence of past or current

infection with HPV types related to type-specific VLP antigens. Efficacy estimates vary by vaccine, type of study, the population analysed, and duration of follow-up ([Ault et al., 2007](#); [WHO, 2009](#)). In addition, the HPV vaccine trials with the quadrivalent vaccine have shown an efficacy close to 100% against high-grade vulvar (VIN2/3) or vaginal intraepithelial lesions (VaIN2/3) related to HPV 16 or 18, and against genital warts related to HPV 6 or 11 among the per-protocol susceptible population ([Garland et al., 2007](#); [Joura et al., 2007](#)). Although protection with both vaccines has been shown to last 5–6 years, their long-term protection and their impact on the prevention of cervical cancer and of other genital and non-genital HPV-associated tumours remains to be determined.

## 2. Cancer in Humans

### 2.1 Cancer of the cervix

Epidemiological evidence for the carcinogenicity of HPV was originally presented in Volume 64 of the *IARC Monographs* ([IARC, 1995](#)), and was extensively updated in Volume 90 of the *IARC Monographs* ([IARC, 2007](#)), based on data available as of February 2005.

HPV carcinogenicity has been established most convincingly for cancer of the cervix. HPV behaviour is strongly correlated with phylogenetic (i.e. evolutionary or taxonomic) categories ([Schiffman et al., 2005](#)). All HPV genotypes that are known to be cervical carcinogens belong to the alpha genus, in an evolutionary branching or high-risk clade containing a few genetically related species ([Table 2.1](#) and Fig. 2.1). HPV 16 (alpha-9) and HPV 18 (alpha-7) have been classified as cervical carcinogens since 1995. HPV 31 and HPV 33, in alpha-9, were categorized as probably carcinogenic. In 2005, the group of cervical carcinogens was expanded to include the following 13 types: alpha-5 genotype HPV 51,

**Table 2.1 HPV types in the high-risk clade**

Alpha HPV species	Types classified as Group 1 carcinogens in Volume 90	Other Types in Species
5	51	26, 69, 82
6	56, 66	30, 53
7	18, 45, 39, 59	68, 70, 85, 97
9	16, 31, 33, 35, 52, 58	67
11		34, 73

alpha-6 genotypes HPV 56 and HPV 66, alpha-7 genotypes HPV 18, HPV 39, HPV 45, and HPV 59, and alpha-9 genotypes HPV 16, HPV 31, HPV 33, HPV 35, HPV 52, and HPV 58.

There is virtually no epidemiological evidence of cervical carcinogenicity for other species in the alpha genus or for other genera. To save considerable space presenting null evidence, this section will not include data related to HPV species alpha-1, -2, -3, -4, -8, -10 (other than HPV 6 or 11), -13, or -14/15. These species contain HPV types that cause skin or genital warts, minor cytological atypia, and often no apparent disease.

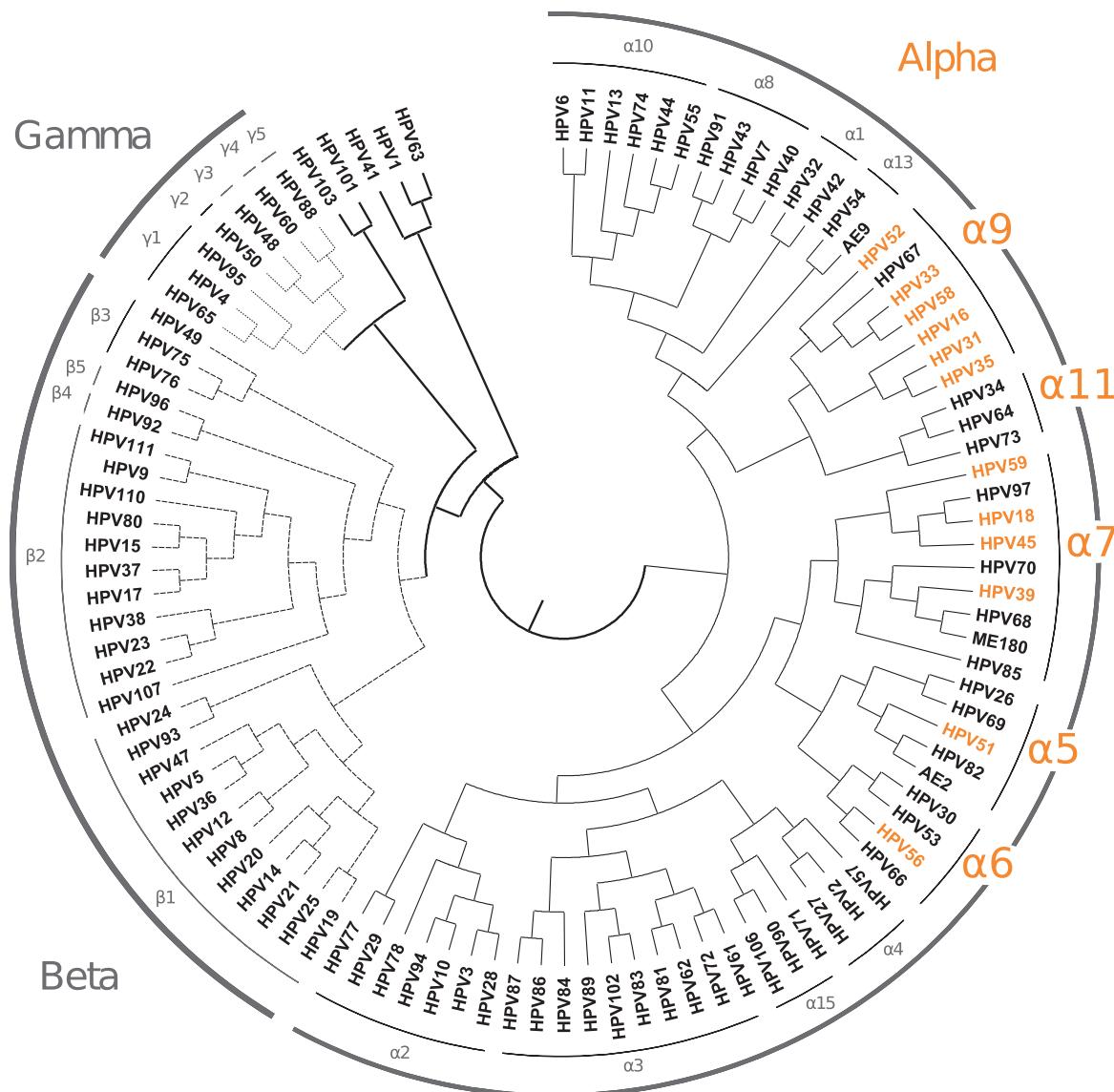
Since the previous *IARC Monograph*, new evidence has further supported that HPV types in the high-risk clade of the alpha genus cause virtually all cases of cervical cancer worldwide ([Smith et al., 2007](#); [Bosch et al., 2008](#)). In case-control studies, the odds ratios (ORs) associating cervical cancer and its immediate precursor, CIN3, with HPV DNA positivity for these high-risk types in pooled probe tests consistently exceed 50. It is persistent infections that are associated with an extremely high absolute risk of CIN3 and cancer. In cohort studies, women who test negative for this group of HPV types as assayed by hybrid capture 2 (HC2, including a mix of the HPV types 16, 18, 31, 33, 35, 39, 45, 51, 52, 56, 58, 59, 68, and several cross-reacting genotypes in the high-risk clade) are at extremely low subsequent risk of cancer for at least 10 years ([Khan et al., 2005](#)).

Because persistent infection with HPV is a nearly necessary cause of cervical cancer, a

reconsideration of HPV and cervical carcinogenicity based on the new (non-cohort) data must be made to decide whether any additional types within the high-risk clade are also carcinogenic, and whether any types that were previously categorized as carcinogenic should be downgraded. The types in the high-risk clade are listed below.

Given the existence of some HPV types that are very carcinogenic, notably HPV 16 and HPV 18, determining which less common and weaker types are also carcinogenic becomes, in epidemiology, an issue of confounding. The alpha genus types share a common route of transmission, and multiple infections are present in a large minority of women, both concurrently and sequentially. None of the traditional approaches to control confounding is entirely successful. Because HPV 16 causes over 50% of cases of cervical cancer ([Clifford et al., 2003](#); [Smith et al., 2007](#)), logistic regression and similar approaches will parsimoniously attribute to HPV 16, cases associated with both HPV 16, and a less important type. HPV 18 is the second most important cervical carcinogen, responsible for approximately 15% of cervical cancer of all histological types combined (and a higher fraction of adenocarcinomas) ([Clifford et al., 2003](#); [Smith et al., 2007](#)). If a type co-occurs with either HPV 16 or HPV 18, its association with cervical cancer might be confounded by either of these powerful carcinogens. For types causing only a very small fraction of cervical cancer, confounding by any of the more important types is possible.

**Figure 2.1 Phylogenetic tree of 100 human papillomavirus types with an highlight of the high-risk alpha species**



Phylogenetic tree of 100 human papillomavirus types inferred from the nucleotide sequences of 5 ORFs (E7, E1, E2, L2 and L1). The tree was constructed using the Markov chain Monte Carlo (MCMC) algorithm in BEAST v1.4.8 ([Drummond & Rambaut, 2007](#)). HPV species were generally classified according to the new classification system for PVs by [de Villiers et al. \(2004\)](#). All subtypes of the alpha PVs were included in the tree, followed as HPV 44 is a subtype of HPV 55, AE9 is a subtype of HPV 54, HPV 64 is a subtype of HPV 34, ME180 is a subtype of HPV 68, and AE2 is a subtype of HPV 82.

In red are highlighted the alpha HPV types previously classified as *carcinogenic to humans (Group 1)* in Volume 90, and the alpha species (high-risk species) to which they belong.

Adapted from an unpublished figure (courtesy of Robert D. Burk and Zigui Chen)

Dealing with confounding by exclusion, i.e. examining the possibility of carcinogenicity of a more minor type among cancer specimens that do not contain a more important type, becomes a problem of misclassification. The main epidemiological criterion used for the classification of an HPV type as a carcinogen, i.e. finding the HPV genotype as a single infection in a cervical scrape or biopsy specimen in a woman with cancer, might sometimes be too lax, and prone to error. Colposcopic biopsies and cytology specimens can be misdirected and fail to obtain the critical cells, whereas the contamination of scrapes and biopsies from lower-grade lesions that often surround cancers can lead to the detection of types other than the causal one. Studies relying on the testing of microdissected cervical malignancies will address these issues, but large-scale highly accurate data are not yet available.

Difficulty with control selection adds another level of complexity in assessing carcinogenicity. As discussed in the section on the HPV natural history of infection (Section 1.2), cervical cancer typically follows age of infection by decades. HPV DNA and RNA transmitted at young ages usually become undetectable and no sensitive serological assay exists to measure HPV exposure. Consequently, odds ratios based on a comparison of HPV DNA prevalence at the time of case diagnosis to age-matched HPV point prevalence in controls do not estimate true relative risks.

See Table 2.2 available at <http://monographs.iarc.fr/ENG/Monographs/vol100B/100B-06-Table2.2.pdf>, Table 2.3 available at <http://monographs.iarc.fr/ENG/Monographs/vol100B/100B-06-Table2.3.pdf>, and Table 2.4 available at <http://monographs.iarc.fr/ENG/Monographs/vol100B/100B-06-Table2.4.pdf>.

Only sparse type-specific prospective data is available on the carcinogenicity of the full range of individual HPV genotypes ([Khan et al., 2005](#); [Schiffman et al., 2005](#); [Wheeler et al., 2006](#)). Studies have categorically shown the unique

carcinogenicity of HPV 16 ([Khan et al., 2005](#); [Bulkmans et al., 2007](#); [Kjaer et al., 2009](#); [Muñoz et al., 2009](#)). HPV 18 causes a lower and more delayed absolute risk of CIN3+ diagnosis. [Khan et al. \(2005\)](#) observed that a 10-year cumulative risk of  $\geq$  CIN3 for the women who were positive by the pooled probe HC2 assay, but negative for HPV 16 or HPV 18, was 3.0% (95%CI: 1.9–4.2) compared with the risk of 0.8% (95%CI: 0.6–1.1) among women who were HC2-negative at baseline. However, there is not convincing long-term prospective evidence for individual HPV types other than HPV 16 and HPV 18.

Finally, the accuracy of detection of HPV genotypes differs between the major polymerase-chain-reaction(PCR)-based systems used to generate most of the data. The epidemiological study of individual HPV genotypes is made more difficult with the variety of methods available for testing. In the past few years, the major HPV genotyping methods have converged towards a common, improved standard of analytical sensitivity and specificity, but none of the main methods is the reference standard ([Gravitt et al., 2008](#)). Sequencing of PCR products is also imperfect because multiple HPV types can infect tissues concurrently, and sequencing distinguishes multiple infections sub-optimally. The methods have evolved over time, producing additional testing variability that is difficult to appreciate as a reader. The residual error in HPV genotyping occurs mainly when multiple infections are present, and for the less important carcinogenic types. As a result, determining the major carcinogenic types can be done rather easily, but ruling out confounding in the context of multiple infections can be quite difficult.

With these caveats, the cervical carcinogenicity of the HPV types listed above varies in strength in a continuum without clear breakpoint, from extremely strong (i.e. HPV 16 and, to a lesser degree, HPV 18) to weak, but still may cause cervical cancer in rare instances (e.g. HPV 68, see below). Evaluators taking one extreme

position could rightfully claim that there is reasonable evidence for the carcinogenicity of virtually all the types in the species listed above, extending further the list established in the previous *IARC Monograph*. Strict interpreters of causal criteria could argue for a return to a more limited list. But based on current evidence, no clear cut-off between *sufficient*, *limited*, and *inadequate* evidence is entirely defensible.

The Working Group chose the following pragmatic approach to creating an imperfect cut-off between *sufficient*, *limited*, and *inadequate* epidemiological evidence for cervical carcinogenicity:

The Working Group considered only types in the high-risk clade because data are *inadequate* for all others. The Working Group evaluated the most recent and accumulated data on cervical cancers from very large single projects (e.g. [Bosch et al., 2008](#)), and especially as summarized in meta-analyses from IARC ([Smith et al., 2007](#), updated as needed by the Working Group). The Working Group excluded from consideration high-grade precancerous lesions (CIN3 and the more equivocal CIN2 which occur in approximately 1% of screened women) often used as ethical surrogate end-points in prospective studies and clinical trials, because there are now enough data for invasive cancers, and because it appears that HPV types have different potential to progress from CIN2/3 to invasive cervical cancer ([Clifford et al., 2003](#)). For comparisons with the background frequency of cervical HPV infection in the general female population, the Working Group noted the prevalence from a large meta-analysis of HPV genotypes found in women with normal cytology ([de Sanjose et al., 2007](#)).

About 10–30% of women with detectable HPV DNA exhibit definite cytological abnormalities, depending on the HPV type, cytological cut-off point and DNA test ([Kovacic et al., 2006](#)). But low-grade or even equivocal lesions represent only a few percent of screening cytological tests; therefore, the population prevalence of an HPV

genotype (in controls) can be approximated by its prevalence in cytologically normal women.

Comparing the prevalence in women with normal cytology ([de Sanjose et al., 2007](#)) to the prevalence in women with invasive cervical cancers compiled by [Smith et al. \(2007\)](#), one can see obvious “case–control” differences. The most clearly carcinogenic genotypes, HPV 16 and HPV 18 in particular, are more common among cervical squamous carcinomas than cytologically normal women or even in low-grade squamous intraepithelial lesions ([Clifford et al., 2005b](#)). HPV 18 is especially common in adenocarcinomas ([Bosch et al., 2008](#)), as are other members of the alpha-7 clade of which HPV 18 is a member ([Clifford & Franceschi, 2008](#)), lending additional support to the importance of genetic similarity in terms of the carcinogenicity of different HPV types. Almost all types of HPV in the high-risk clade – except for HPV 16 and HPV 18 – are (relatively) more common in low-grade lesions.

Including HPV 16 and HPV 18, eight HPV types (alpha-7, HPV types 18 and 45; alpha-9, HPV types 16, 31, 33, 35, 52, and 58) are the most common types found in cancers in both the Catalan Institute of Oncology (ICO) study and the IARC meta-analysis ([Bosch et al., 2008](#); [Clifford & Franceschi, 2008](#)) in all regions of the world providing data. These types are all much more common in cancer case specimens than in controls, providing sufficient epidemiological evidence of carcinogenicity.

To move beyond the most clearly carcinogenic eight HPV genotypes, the Working Group chose the presence of HPV 6 as a surrogate for estimating the percentage of cancers that might contain HPV DNA by accumulated and unknown measurement errors alone. The reasons being that HPV 6, the common cause of benign condyloma acuminata (external genital warts), is an archetype of a low-risk type, is not classified as a cervical carcinogen, and is very uncommonly detected in cervical cancer specimens. [When detected, even without detection

**Table 2.5 Meta-analysis of type-specific HPV DNA prevalence in invasive cervical cancer**

	<u>Invasive cervical cancer</u>			<u>Normal</u>		
	N tested	% pos	95%CI	N tested	% pos	95%CI
<b>HPV 16</b>	14595	54.4	53.6–55.2	76385	2.6	2.5–2.8
<b>HPV 18</b>	14387	15.9	15.3–16.5	76385	0.9	0.8–1.0
<b>HPV 33</b>	13827	4.3	4.0–4.6	74141	0.5	0.4–0.5
<b>HPV 45</b>	9843	3.7	3.3–4.1	65806	0.4	0.4–0.4
<b>HPV 31</b>	11960	3.5	3.2–3.9	74076	0.6	0.6–0.7
<b>HPV 58</b>	10157	3.3	2.9–3.6	72877	0.9	0.8–1.0
<b>HPV 52</b>	9509	2.5	2.2–2.8	69030	0.9	0.8–1.0
<b>HPV 35</b>	9507	1.7	1.5–2.0	74084	0.4	0.3–0.4
<b>HPV 59</b>	13471	1.28	1.09–1.47	64901	0.3	0.2–0.3
<b>HPV 51</b>	13057	1.16	0.97–1.34	67139	0.6	0.6–0.7
<b>HPV 56</b>	13247	0.78	0.63–0.93	68121	0.5	0.5–0.6
<b>HPV 39</b>	13370	1.29	1.10–1.48	64521	0.4	0.3–0.4
<b>HPV 68</b>	11982	0.61	0.47–0.75	63210	0.3	0.2–0.3
<b>HPV 73</b>	9939	0.48	0.35–0.62	44063	0.1	0.1–0.1
<b>HPV 66</b>	12118	0.39	0.28–0.50	59774	0.4	0.3–0.4
<b>HPV 70</b>	10503	0.33	0.22–0.44	35014	0.3	0.3–0.3
<b>HPV 82</b>	9265	0.27	0.16–0.38	42536	0.1	0.0–0.1
<b>HPV 26</b>	6111	0.13	0.04–0.22	44098	0.0	0.0–0.1
<b>HPV 53</b>	8140	0.42	0.28–0.56	44058	0.4	0.4–0.4
<b>HPV 6</b>	14912	0.45	0.35–0.56	58370	0.3	0.2–0.3
<b>HPV 11</b>	8761	0.2	0.1–0.4	58370	0.2	0.2–0.2

Compiled by the Working Group during the meeting

Data for women with normal cytology is from [de Sanjosé et al. \(2007\)](#)

Data for HPV types 16, 18, 31, 33, 35, 45, 52, and 58 is from [Smith et al. \(2007\)](#), but for other HPV types, the data from [Smith et al. \(2007\)](#) was updated by the Working Group using the following 61 published studies: [Andersson et al. \(2005\)](#), [Bardin et al. \(2008\)](#), [Beeren et al. \(2005\)](#), [Bertelsen et al. \(2006\)](#), [Bhatla et al. \(2006\)](#), [Bryan et al. \(2006\)](#), [Bulk et al. \(2006\)](#), [Balkmans et al. \(2005\)](#), [Cambruzzi et al. \(2005\)](#), [Castellsagué et al. \(2008\)](#), [Chan et al. \(2006\)](#), [Chen et al. \(2006\)](#), [Ciotti et al. \(2006\)](#), [Dabić et al. \(2008\)](#), [Daponte et al. \(2006\)](#), [De Boer et al. \(2005\)](#), [de Cremoux et al. \(2009\)](#), [De Vuyst et al. \(2008\)](#), [Del Mistro et al. \(2006\)](#), [Esmaeili et al. \(2008\)](#), [Fanta \(2005\)](#), [Gargiulo et al. \(2007\)](#), [Ghaffari et al. \(2006\)](#), [Gheit et al. \(2009\)](#), [Guo et al. \(2007\)](#), [Hadzisejdć et al. \(2007\)](#), [Hindryckx et al. \(2006\)](#), [Hong et al. \(2008\)](#), [Inoue et al. \(2006\)](#), [Khan et al. \(2007\)](#), [Kjaer et al. \(2008\)](#), [Klug et al. \(2007\)](#), [Kulmala et al. \(2007\)](#), [Lai et al. \(2007b\)](#), [Lee et al. \(2007\)](#), [Liu et al. \(2005\)](#), [Maehama \(2005\)](#), [Odida et al. \(2008\)](#), [Panotopoulou et al. \(2007\)](#), [Peedicayil et al. \(2006\)](#), [Piña-Sánchez et al. \(2006\)](#), [Prétet et al. \(2008\)](#), [Qiu et al. \(2007\)](#), [Ressler et al. \(2007\)](#), [Sigurdsson et al. \(2007\)](#), [Siriakungul et al. \(2008\)](#), [Song et al. \(2007\)](#), [Sowjanya et al. \(2005\)](#), [Sriamporn et al. \(2006\)](#), [Stevens et al. \(2006\)](#), [Su et al. \(2007\)](#), [Tao et al. \(2006\)](#), [Tawfik El-Mansi et al. \(2006\)](#), [Tong et al. \(2007\)](#), [Tornesello et al. \(2006\)](#), [Wentzensen et al. \(2009\)](#), [Wu et al. \(2008\)](#), [Wu et al. \(2006\)](#), [Zhao et al. \(2008\)](#), [Zuna et al. \(2007\)](#)

of a more likely causal type, the Working Group judged that misclassification of some kind was a more likely explanation than causality.] The best published estimate of percentage of detection of HPV 6 in cervical cancers (not necessarily as a single infection) was judged to be 0.5% (95%CI: 0.4–0.6), based on 15000 cases of cancer ([Smith et al., 2007](#); estimated and confirmed by the Working Group update, see [Table 2.5](#)). The Working Group took the pragmatic approach once more and made the following rule—an individual HPV type in the high-risk alpha clade (i.e. with an elevated prior probability of being carcinogenic due to analogy to closely related viral types in the same or closely-related species) was considered to have sufficient epidemiological evidence of carcinogenicity if:

- its prevalence in cancers was significantly greater than that of HPV 6.
- its prevalence in cancer was significantly enriched in comparison to the background estimate for the general population, i.e. women with normal cytology.

By this logic, four more types were judged, as in the previous *IARC Monograph*, to have sufficient epidemiological evidence of cervical carcinogenicity: alpha-5 HPV 51, alpha-6 HPV 56, and alpha-7 HPV types 39 and 59.

The remaining types in the high-risk alpha clade (see [Table 2.1](#)) were considered, as a group, to have limited evidence to support carcinogenicity. If phylogeny can be taken to predict behaviour, it is possible that most of these types can very rarely cause cancer. Indeed, many of the types have been detected, albeit uncommonly (no greater than HPV 6), in cancers. There are not enough data, even after testing of many thousands of specimens, to be sure which types are definitely carcinogenic or not. But, within this group, there are two types, alpha-7 HPV 68 and alpha-11 HPV 73, for which the data are slightly stronger than for the others despite methodological challenges. One of the major PCR-based testing methods (SPF10) cannot distinguish

these two types because their amplicons using those primers are identical. Neither of these two types is optimally detected by MY09-MY11 dot blot ([Gravitt et al., 2008](#)). Nonetheless, the data supporting the carcinogenicity of HPV 68 and HPV 73 are suggestive.

This categorization scheme leads to the re-classification of HPV 66, for which the evidence of carcinogenicity was previously judged sufficient. In the assembly of much more testing data from cancer cases, HPV 66 has been found so rarely that its percentage of detection is less than the relative percentage of detection among the general population. In the Working Group review of each individual article, HPV 66 was found alone in cancers with extreme rarity, well below the possible threshold of confounding and misclassification.

### 2.1.1 Summary

The data accumulated supports:

- The unique carcinogenic strength of HPV 16.
- The importance of HPV 18 and genetically related types ([Clifford & Franceschi, 2008](#)) in causing adenocarcinoma compared with squamous cell carcinoma.
- The weaker but still clear carcinogenic potential of six additional types in alpha-7 (HPV 45) and alpha-9 (HPV 31, 33, 35, 52, and 58), with some regional variation in the etiological fractions of cancers due to each type. For example, HPV 52 and 58 are relatively more prevalent in Asia than in other regions, HPV 33 is most clearly prevalent in Europe, and HPV 45 has particular regions where it is prominent.
- The small, and less certain, incremental etiological contributions of another group of carcinogenic types from alpha-5 (HPV 51), alpha-6 (HPV 56), and alpha-7 (HPV 39 and HPV 59). Each causes a few percent at most of cervical cancer cases

worldwide, although regional variability has been observed.

- Acknowledgement of an unresolved dividing line between the HPV types with the weakest evidence judged to be sufficient, and those with evidence judged highly suggestive yet limited (alpha-7 HPV 68 and alpha-11 HPV 73).
- A re-evaluation of the evidence for HPV 66. The data were re-evaluated and the evidence was judged to be very limited now that more cases have been studied showing that it is very rarely found in cancers despite being relatively common. HPV 53, also in alpha-6, shows the same pattern of relative common population prevalence with extremely rare cases of occurrence alone in cancer. The Working Group noted that for these types in particular, there could be harm to public health if the types are included as carcinogenic in screening assays, which would decrease the specificity and positive predictive value of the assays with virtually no gain in sensitivity and negative predictive value.
- The existence of a few types within the high-risk clade that have extremely sparse or no evidence of carcinogenicity. For some types there are anecdotal but very interesting cases that merit further study of additional carcinogenic types. For example, the carcinogenicity of alpha-5 HPV 26 has been supported by a recent report of multiple peri-ungual cancers in an immunosuppressed individual, containing high viral loads, and active transcription of HPV 26 alone ([Handisurya et al., 2007](#)). There have been reports of alpha-9 HPV 70 found as single infections in cervical cancer ([Lai et al., 2007a](#)), but the supportive data are sparse. There are only a few reports of HPV 67 in cancer ([Gudleviciene et al., 2006](#); [Wentzensen](#)

[et al., 2009](#)), which is intriguing because this is the only known type in the alpha-9 species that is not categorized as carcinogenic. For a few types in the high-risk clade, no reports of invasive cancers with single-type infections were found, but isolated reports might exist.

## 2.2 Cancer at other anogenital sites

### 2.2.1 Cancer of the vulva

Cancer of the vulva is rare. The tumours are generally of epithelial origin and squamous cell carcinoma is the most common histological type. Tumours can be mainly categorized as keratinizing, non-keratinizing, basaloid, warty and verrucous vulvar tumours. Basaloid/warty types comprise about a third of cases, are more common in younger women, tend to harbour VIN lesions, and are often associated with HPV DNA detection. These tumours appear to share the epidemiological factors of cervical cancer. On the contrary, keratinizing types, with older average age at diagnosis, apparently arise from chronic vulvar dermatoses or from squamous metaplasia, and are more rarely associated with HPV. See Table 2.6 available at <http://monographs.iarc.fr/ENG/Monographs/vol100B/100B-06-Table2.6.pdf>, Table 2.7 available at <http://monographs.iarc.fr/ENG/Monographs/vol100B/100B-06-Table2.7.pdf>, and Table 2.8 available at <http://monographs.iarc.fr/ENG/Monographs/vol100B/100B-06-Table2.8.pdf>.

#### (a) Case series

Tables 2.6 and 2.7 (on-line) present case series of more than ten cases of VIN3 or invasive cancer of the vulva.

A large proportion of VIN3 cases harbour HPV DNA with HPV 16 being the most common type detected in over 79% of positive samples (Table 2.6 on-line). More recent larger series confirm the presence also of HPV 33 and more

rarely, other cervical carcinogenic HPV types such as HPV 31 and HPV 18. HPV 6 is present in a small proportion, and HPV 11 is extremely rarely identified, pointing to a doubtful role of these condyloma types in VIN3.

Table 2.7 (on-line) describes those studies that provide HPV detection in cases of invasive, basaloid/warty tumours.

A meta-analysis by [De Vuyst et al. \(2009\)](#) estimated an HPV prevalence of 40.4% among 1873 vulvar carcinomas, and confirmed the difference in HPV detection by histological type (69.4% HPV positivity in warty/basaloid type and 13.2% in keratinizing type). The place of origin of the samples and the age of the women appeared both to relate to the prevalence of HPV overall: women below 60 years old, and cases from North America had significantly higher prevalence estimates. HPV 16 was, in all studies, the most common type detected (32.2% with a 50–100% range among positives), followed by HPV 33 (4.5%), HPV 18 (4.4%), HPV 6 (2.0%), HPV 45 (1.0%), HPV 31 (0.6%), and HPV 11 (0.1%).

This observation was also made by [Insinga et al. \(2008\)](#) in another meta-analysis restricted to studies carried out in the US population. The overall HPV detection estimate for squamous cell carcinoma of the vulva was higher for the US studies (65.3%) than in the [De Vuyst et al. \(2009\)](#) meta-analysis for other regions (range, 24.2–38.2%). The multitype-adjusted prevalence estimates reported by [Insinga et al. \(2008\)](#) were as follows: HPV 16 (49.5%), HPV 33 (6.0%), HPV 18 (4.2%), HPV 6 (3.6%), HPV 31 (1.7%), and HPV 52 (0.0%).

Low-risk HPV types have been suggested to be associated with a small subset of vulvar cancers, but their role is not yet clear. Vulvar skin is prone to genital condylomas that might be concomitant to other neoplastic lesions. In some circumstances, these types are present in combined lesions such as giant condyloma with an invasive lesion or in verrucous carcinoma.

HPV 6 was slightly more frequent in vulvar (2.0%) and anal (2.9%) carcinoma than in cervical carcinoma (0.5%) ([Smith et al. 2007](#); [De Vuyst et al. 2009](#)), but it was most often accompanied, among cases where this information was available, by multiple infections with high-risk types. [De Vuyst et al. \(2009\)](#) observed that HPV 6 and 11 were frequently detected in VIN1 and AIN1 (anal intraepithelial neoplasia, as in anogenital warts), but not in VAIN1 (vaginal intraepithelial neoplasia).

In [Insinga et al. \(2008\)](#), after multitype adjustment, HPV 6 was estimated to contribute to the largest fraction (29.2%) of VIN1 lesions, with the top two (HPV 6 and 11), four (HPV 6, 11, 68, and 16), and eight (HPV 6, 11, 68, 16, 58, 59, 31, and 66) reported HPV types accounting for 41.7%, 55.9%, and 67.8% of VIN1 lesions, respectively. The attribution of HPV 6 and 11 to VIN1 lesions (41.7%) was greater than that estimated for CIN1 (6.9%;  $P < 0.0001$ ).

### (b) Case-control studies

There are several case-control studies on invasive vulvar cancer using serology, but few have tested for HPV DNA ([Sagerman et al. 1996](#); [Madsen et al. 2008](#); see Table 2.9 available at <http://monographs.iarc.fr/ENG/Monographs/vol100B/100B-06-Table2.9.pdf>). Also, the design of these studies was considered inadequate (e.g. using endometrial cases as controls). Serological studies reported contradictory data on the association between type-specific epitopes and vulvar cancer. In [Bjørge et al. \(1997\)](#), [Madeleine et al. \(1997\)](#), and [Carter et al. \(2001\)](#), statistically significant increased risks associated with antibodies against HPV 16 were observed but not against HPV 18. Results did not reach statistical significance in [Hildesheim et al. \(1997\)](#).

Overall, the data indicate that HPV plays a role in vulvar cancer, in particular in tumours with basaloid/warty features, with HPV 16 having a predominant role. Based on case series,

HPV 18 and HPV 33 may also be involved in a small fraction of these tumours.

### 2.2.2 Cancer of the vagina

Tumours of the vagina are very rare but varied. They can be of different cell origin: epithelial, mesenchymal, melanocytic, lymphoid, and secondary. Epithelial tumours are the most common and include keratinizing, non-keratinizing, basaloid, verrucous, and warty squamous cell carcinoma types, and are strongly related to HPV. In the previous *IARC Monograph*, HPV 16 was reported in more than 50% of the cases, and the evidence of an association with HPV 18 was reported to be weaker.

The epidemiology of vaginal cancer is not clearly understood as few analytical studies are available, and it is difficult to disentangle these tumours with those that originate in the cervix. Approximately 30% of all cases report treatment for a prior anogenital tumour compared to 2% in controls, most often of the cervix ([Daling et al., 2002](#)).

A recognized independent risk factor for vaginal cancer is the exposure to diethylstilbestrol during pregnancy. Tumours arising after this exposure are clear cell adenocarcinoma of the vagina. Recurrent clear cell adenocarcinoma has been observed as long as 20 years after primary therapy ([Herbst & Anderson, 1990](#)).

#### (a) Case series

In the two recent studies on VAIN3 tissues, over 90% harboured HPV DNA. The meta-analyses by [De Vuyst et al. \(2009\)](#), including 298 cases of VAIN and 136 cases of invasive cancer, reported detection of HPV DNA in 93.6% of VAIN3 lesions, and in 69.9% of vaginal carcinomas. In both VAIN3 and vaginal carcinomas, HPV 16 was by far the most common type detected followed by HPV 18, 31, 33, and 6.

The most recent series of invasive cases found HPV DNA in 17/21 women [80.9%] ([Ferreira](#)

[et al., 2008](#)). HPV 16 was the predominant type but HPV 31 and HPV 33 were also relatively common. [Madsen et al. \(2008\)](#) detected HPV DNA in 24/27 [88.9%] squamous cell carcinomas of the vagina. HPV 16 was the most prevalent type followed by HPV 33. The meta-analysis by [De Vuyst et al. \(2008\)](#) estimated an overall HPV prevalence of 69.9% with a geographic range of 43.8% in Asia to 76.8% in Europe.

See Table 2.10 available at <http://monographs.iarc.fr/ENG/Monographs/vol100B/100B-06-Table2.10.pdf> and Table 2.11 available at <http://monographs.iarc.fr/ENG/Monographs/vol100B/100B-06-Table2.11.pdf>.

#### (b) Case-control studies

Since the previous *IARC Monograph*, only one case-control study of HPV and vaginal cancer has been published. [Madsen et al. \(2008\)](#) carried out a case-control study on cancer of the vagina in Denmark. [The Working Group considered this study as inadequate to provide analytical evidence of HPV and vaginal cancer due to problems with the selection of controls.]

In summary, recent data on HPV and vaginal cancer are few, and have not altered the evaluation of the role of genotypes other than HPV 16. The rarity of these tumours and their contiguity with cervical cancer remain difficulties for specific epidemiological research of this cancer.

See Table 2.12 available at <http://monographs.iarc.fr/ENG/Monographs/vol100B/100B-06-Table2.12.pdf> and Table 2.13 available at <http://monographs.iarc.fr/ENG/Monographs/vol100B/100B-06-Table2.13.pdf>.

### 2.2.3 Cancer of the penis

Cancer of the penis is a rare tumour whose incidence correlates with that of cervical cancer. Analogous to vulvar cancer, some risk factors vary by histology. The large majority of penile tumours are squamous cell carcinomas although making a histological subtype distinction can be

controversial. Preneoplastic intraepithelial lesions (PIN) are recognized precursor stages in which HPV is also generally identified. HPV-negative squamous carcinoma cases are suspected to relate to chronic inflammation with risk factors such as phimosis, lichen sclerosis, and lack of circumcision. Smoking and a history of genital warts have also been linked to cancer of the penis.

#### (a) Case series

The prevalence of HPV DNA in penile cancer varies by histological type. Case series tend to include a relatively small number of cases per histological subtype, ranging from one to over 170 cases (see Table 2.14 available at <http://monographs.iarc.fr/ENG/Monographs/vol100B/100B-06-Table2.14.pdf>).

Since the previous *IARC Monograph*, more than a dozen new case series on invasive penile cancer have been identified in which HPV DNA was assessed. No studies on PIN were identified. Among these studies, the largest series is that of [Lont et al. \(2006\)](#) with 171 paraffin-embedded penile tumours, in which the overall detection of HPV was 29.2%. The low prevalence of HPV among the warty or basaloid types (25% and 0%, respectively) in this study and in [Guerrero et al. \(2008\)](#) contrasts with some smaller series showing prevalence estimates over 60–70% ([Rubin et al., 2001](#); [Salazar et al., 2005](#); [Pascual et al., 2007](#)). Intermediate HPV prevalence estimates of < 50% were observed in another small series of warty carcinoma of the penis ([Bezerra et al., 2001](#)).

Although there is a wide variation of HPV prevalence in penile cancer studies, two recent meta-analyses provide a summary estimate that may be more meaningful ([Backes et al., 2009](#); [Miralles-Guri et al., 2009](#)). Taking into account over 1400 cases of penile cancer, HPV was detected in 47% of the cases with the highest HPV prevalence (76%) in penile tumours with a basaloid squamous cell carcinoma component, and the lowest (24.5%) in penile verrucous squamous

cell carcinoma. Basaloid/warty tumours were overall 3.5 times more likely to be HPV-positive.

Contrary to that observed for vulvar cancer, [Backes et al. \(2009\)](#) reported a 2.7 times higher presence of HPV-positive tumours in Asia as compared to cases derived from North America (OR, 2.7; 95%CI: 1.8–4.0) without any clear explanation of why this should be. Recent studies were more likely to report HPV-positive cases. Consistent with the previous *IARC Monograph*, HPV 16 was the most common type detected (60.2%), irrespective of histology, followed by HPV 18 (13.3%) ([Miralles-Guri et al., 2009](#)). Other cervical carcinogenic HPV types such as HPV 35, 45, 51, 52, 56, and 59 were detected sporadically as were HPV 68, 70, and 74. Types not established to be carcinogenic were observed in close to 10% of cases, but it was not clarified how many of these were found in combination with carcinogenic infections.

HPV 6 was detected in 39 (6.7%) of 580 cases that were tested for HPV 6, with a notable presence of multiple co-infections with high-risk types.

#### (b) Case-control studies

The data from case-control studies in which HPV was evaluated using serological markers is summarized in Table 2.15 (available at <http://monographs.iarc.fr/ENG/Monographs/vol100B/100B-06-Table2.15.pdf>). In these small studies, which were not included in the previous *IARC Monograph*, no detailed histological evaluation was provided. The risk for penile cancer, including in-situ cases, was associated with seropositivity to HPV 16 in particular.

In summary, studies of penile cancer suggest that HPV infection appears to play an important role in almost half of the cases. No additional validated information on the specific contribution of types other than HPV 16 or 18 can be derived from recent studies. HPV 6 and HPV 11 were detected in a small proportion of cases.

## 2.2.4 Cancer of the anus

Cancer of the anus is a relatively rare disease but increasing incidence is being reported in some countries with increases of >160% in men or 78% in women in the USA over a period of 20 years (Daling *et al.*, 2004). Established risk factors for anal cancer include a high number of sexual partners, receptive anal sex, history of venereal diseases; smoking has also been proposed (Frisch *et al.*, 1997; Daling *et al.*, 2004). Anal cancer is more common among HIV-infected subjects (for anal cancer in HIV patient see Section 2.8.3b).

The anal canal consists of a segment of approximately 4 cm of squamous mucosa limited distally by the anal verge or margin (transitional zone between the skin and mucosa) and proximally by the dentate line (transitional zone between the squamous and glandular mucosa). Malignant tumours of the anal canal are largely carcinomas of squamous cell origin. Most adenocarcinomas arising in the anal mucosa represent a downward spread from an adenocarcinoma in the rectum or arise in colorectal-type mucosa above the dentate line, and generally are excluded in the studies specific to the anal canal.

### (a) Case series

Case series of precursor lesions, AIN, published since the previous *IARC Monograph* have been included in Table 2.16 (available at <http://monographs.iarc.fr/ENG/Monographs/vol100B/100B-06-Table2.16.pdf>). The more recent studies (Hampl *et al.*, 2006; Varnai *et al.*, 2006) demonstrate that the great majority of cases are HPV-positive, mainly with HPV 16. Case series of invasive lesions have been included in Table 2.17 (available at <http://monographs.iarc.fr/ENG/Monographs/vol100B/100B-06-Table2.17.pdf>) (Kagawa *et al.*, 2006; Varnai *et al.*, 2006; Laytragoon-Lewin *et al.*, 2007; Tachezy *et al.*, 2007). All studies found high proportions of HPV in the tumours, predominantly HPV 16. Of interest is the lower proportion of HPV-related

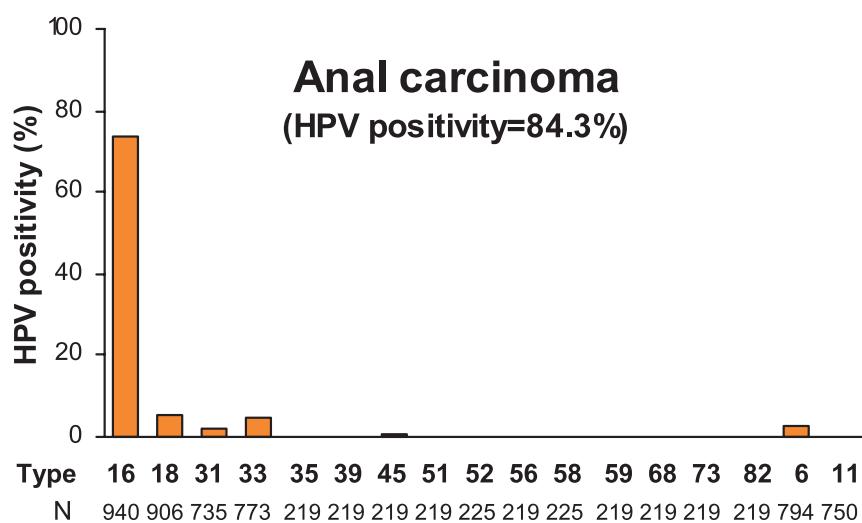
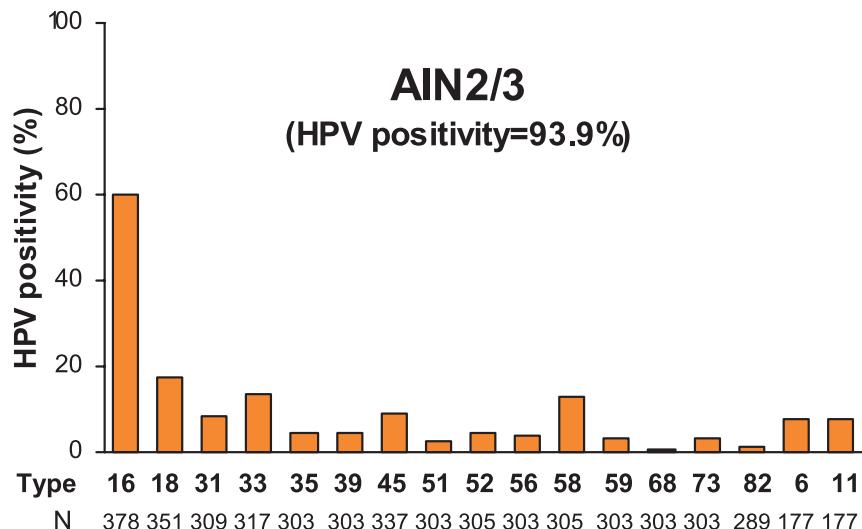
tumours in those cancers localized in the perianal skin as compared to other anal sites (Frisch *et al.*, 1999).

De Vuyst *et al.* (2009), in a meta-analysis of 1280 cases of AIN (671 AIN1 and 609 AIN2/3) of which 805 were HIV positive and 955 cases of invasive carcinomas, estimated that the large majority of AIN2/3 lesions (93.9%) and of invasive carcinomas (84.3%) were attributable to HPV. As seen in Fig. 2.2, the most frequent types detected for AIN2/3 were HPV 16 (59.8%), 18 (17.4%), 33 (13.6%), and 58 (13.1%). In anal carcinoma, the most frequent types were HPV 16 (73.4%), followed by HPV 18 (5.2%), and HPV 33 (4.8%). Although not studied extensively, HPV 45 was rarely identified, which probably reflects the squamous nature of these tumours. HPV 6 was detected in 2.9% of the anal carcinomas, but its presence could not be disentangled from concomitant infections with high-risk types.

### (b) Case control studies

One case-control study has compared the presence of HPV DNA in anal tumours of 417 individuals (349 squamous cell carcinomas and 68 in-situ squamous cell carcinomas) to 534 individuals with rectal adenocarcinomas (Frisch *et al.*, 1997). High-risk types of HPV were detected in 84% of the anal cancer specimens, but no HPV was identified in the rectal tumours. HPV 16 was the most commonly identified type, followed by HPV 33, 18, 6, and 31. HPV was more commonly identified in tumours of women with invasive or in-situ anal cancer (93%) as compared to men (69%). Table 2.18 (available at <http://monographs.iarc.fr/ENG/Monographs/vol100B/100B-06-Table2.18.pdf>) and Table 2.19 (available at <http://monographs.iarc.fr/ENG/Monographs/vol100B/100B-06-Table2.19.pdf>) summarize case-control studies of anal cancer that relied on HPV serology. The studies (Carter *et al.*, 2001; Bjørge *et al.*, 2002; Daling *et al.*, 2004) resulted in odds ratios of 3–5 for the association between HPV 16 and HPV 18 seropositivity and

**Figure 2.2 Prevalence of human papillomavirus types in case series of anal intraepithelial neoplasia (AIN) grades 2/3 and anal cancer**



Adapted from [De Vuyst et al. \(2009\)](#)

anal cancer, for both men and women. As an indication of type specificity, [Bjørge et al. \(2002\)](#) found neither association for HPV 33 nor HPV 73.

Overall, HPV is detected in over 80% of anal cancer cases suggesting a causal relationship. The large majority of tumours are related to HPV 16, and although consistently identified in this tumour type HPV 18 and HPV 33 are detected in smaller proportions.

## 2.3 Cancer of the upper aerodigestive tract

The fraction of cancers of the oral cavity and pharynx associated with HPV infection varies between studies according to: 1) the accuracy in the distinction of cancer of the oropharynx and tonsil from other subsites; 2) the competing effect of tobacco smoking or chewing; and 3) the quality of tissue biopsies and HPV-testing protocols used.

As a consequence of the above, especially high proportions of HPV positivity have been recently found in the USA, where early cancer cases restricted to the oropharynx and detected in a substantial proportion among non-smokers have been very carefully evaluated ([Andrews et al., 2009](#)).

### 2.3.1 Cancer of the oral cavity

Cancers of the oral cavity, including the tongue, floor of the mouth, gum, palate, and other sites of the mouth have a clearly established association with smoking or chewing tobacco, and with alcohol drinking. However, there is a subset of cancers that occurs among subjects not exposed to smoking or drinking. The previous *IARC Monograph* concluded that, in the oral cavity, there was sufficient evidence for the carcinogenicity of HPV 16, and limited evidence for the carcinogenicity of HPV 18.

Multiple case series have been reported with variable prevalence estimates of HPV – between 4–74% – including the series included in the previous *IARC Monograph*, and those reviewed for this current volume (see Table 2.20 available at <http://monographs.iarc.fr/ENG/Monographs/vol100B/100B-06-Table2.20.pdf> and Table 2.21 available at <http://monographs.iarc.fr/ENG/Monographs/vol100B/100B-06-Table2.21.pdf>).

The recent case series ([Koppikar et al., 2005](#), India; [Luo et al., 2007](#), Taiwan, China; [da Silva et al., 2007](#), Brazil; [Soares et al., 2007](#), Brazil; [Liang et al., 2008](#), USA) confirmed the same pattern of variability. In Taiwan, China, and one of the Brazilian studies, the reported prevalence was about 25%, but the other Brazilian study that included only tongue cancers reported a prevalence of 74%. In contrast, the study in India only detected mucosal HPV types in 6% of the tumours, and in the US study, the reported prevalence was only 2% in tongue cancers. Three of the case series included non-cancer cases as comparison groups. In all studies, HPV positivity was much lower in these control biopsy specimens than in tumour specimens. HPV 16 was the most common type in all studies, followed or equalled by HPV 18, with the exception of one of the Brazilian studies, where this pattern was reversed ([Soares et al., 2007](#)).

In a meta-analysis that included 2642 oral cancers tested with PCR methods, the overall prevalence of HPV was 23.5%, with predominance of HPV 16, which was detected in 16% of the cases (68% of the positives), followed by HPV 18, detected in 8% (34.1% of the positives). About 3% had HPV 6 and 1.6% had HPV 11. No other type was detected in more than 1% of the cases ([Kreimer et al., 2005](#)). [The Working Group noted a tendency for the largest and more carefully conducted studies to yield lower prevalence estimates than the smaller studies, suggesting the possibility of publication bias.]

Evidence from case-control studies reported until the previous *IARC Monograph* was mainly

based on serological studies. Two studies that had used exfoliated cells from cases and controls failed to demonstrate an association ([Schwartz et al., 1998](#); [Herrero et al., 2003](#)), and a third observed a doubling of the risk ([Smith et al., 2004](#)). A recent case-control study ([Hansson et al., 2005](#), Sweden) demonstrated positive associations using exfoliated cells of the oral cavity. Another study conducted in Canada ([Pintos et al., 2008](#)) reported a non-significant odds ratio of 1.3.

In the study by [Hansson et al., \(2005\)](#), swabs of tumours and tonsillar fossa and mouthwash of cases and controls were collected. The odds ratios adjusted for tobacco and alcohol were 24 (95%CI: 3.2–180) for the tongue, 51 (95%CI: 3.2–810) for the floor of the mouth, and 22 (95%CI: 2.8–170) for the other oral sites.

In summary, there is epidemiologicalevidence for the role of HPV 16 and possibly HPV 18 in the etiology of cancers of the oral cavity.

### *2.3.2 Cancers of the oropharynx and tonsil*

Cancers of the oropharynx and tonsil are also associated strongly with smoking and drinking, but extensive evidence has accumulated in recent years to support a causal role of HPV in a sizable fraction of those cancers, which have been increasing in incidence in some populations ([Hammarstedt et al., 2006](#)).

The fraction of cancers of the oropharynx that is HPV-related is larger than for the oral cavity, but as mentioned above, this fraction varies between studies. Many studies confirm this association, in particular for the tonsil. HPV 16 is present in about 90% of HPV-positive tumours (see Table 2.22 available at <http://monographs.iarc.fr/ENG/Monographs/vol100B/100B-06-Table2.22.pdf> and Table 2.23 available at <http://monographs.iarc.fr/ENG/Monographs/vol100B/100B-06-Table2.23.pdf>).

The original case series showed a high variability in the prevalence of HPV, although in

general, the prevalence reported was higher than for the oral cavity, in particular for the tonsil. In recent case series ([Tachezy et al., 2005](#), Czech Republic; [Hammarstedt et al., 2006](#), Sweden; [Ernster et al., 2007](#), USA; [Kim et al., 2007a](#), Republic of Korea; [Charfi et al., 2008](#), France), the prevalence of HPV in oropharyngeal tumours was close to 60% or more, with a clear predominance of HPV 16.

An interesting study from Sweden ([Hammarstedt et al., 2006](#)) retrieved tonsil cancer archival specimens from different time periods to determine if the observed increase in incidence of tonsil cancer in that area could be explained by increases in HPV-related tumours. They found that cases diagnosed in the 1970s had an HPV positivity of only 23.3%, and this increased to 68.0% in tumours diagnosed in 2000–02. HPV 16 was detected in 42% of cases (all years combined, 87.0% of the positives). Patients with HPV-positive cancers were younger at diagnosis.

In the meta-analysis by [Kreimer et al. \(2005\)](#), HPV positivity in cancer of the oropharynx was 35.6% with HPV 16 detected in 30.9% (86.8% of the positives) of oropharyngeal tumours. HPV 18 was detected in only 1.0% of the tumours, and HPV 6 in 2.5% of the tumours.

An important, well conducted case-control study was recently reported by [D'Souza et al. \(2007\)](#). They included 100 patients with newly diagnosed oropharyngeal cancer, and 200 gender- and age-matched controls. Sexual behaviours (vaginal and oral sex) were strongly associated with a risk of oropharyngeal cancer, an association that was stronger when the analysis was restricted to HPV-positive tumours. Strong associations with a risk of oropharyngeal cancer were detected for serological markers of HPV infection or progression (adjusted OR, 32.2; 95%CI: 14.6–71.3 for antibodies against HPV 16 L1 VLPs; adjusted OR, 58.4; 95%CI: 24.2–138.3 for antibodies against HPV 16 E6 or E7), and for detection of HPV DNA in oral exfoliated cells (OR, 12.3; 95%CI: 5.4–26.4). HPV 16 DNA was

detected in 72% (95%CI: 62–81) of tumour biopsies by in-situ hybridization.

Two other case-control studies ([Pintos et al., 2008](#), Canada; [Hansson et al., 2005](#), Sweden) also detected strong associations with risk.

In summary, there is strong epidemiological evidence for the causal role of HPV 16 in the etiology of cancers of the oropharynx and tonsil. HPV 18 is detected in 1% of the tumours.

### 2.3.3 Cancer of the oesophagus

Squamous cell carcinoma of the oesophagus is also associated with tobacco and alcohol consumption, but a potential role of HPV has been proposed ([Kamangar et al., 2006](#); [Lu et al., 2008](#)).

There have been many studies of HPV and oesophageal cancer, and findings have been very inconsistent geographically, with studies showing extreme variations in HPV detection probably in relation to the lack of standardized testing methods. There are areas, particularly in Asia, where HPV is more commonly detected in oesophageal cancer.

Recent case series have been reported from Egypt ([Bahnassy et al., 2005](#)), Colombia and Chile ([Castillo et al., 2006](#)), Brazil ([Souto Damin et al., 2006](#)), Germany ([Pantelis et al., 2007](#)), the Republic of Korea ([Koh et al., 2008](#)), the Islamic Republic of Iran ([Far et al., 2007](#)), and China ([Shuyama et al., 2007](#); [Lu et al., 2008](#)). HPV detection in these recent studies ranged from 0% (Republic of Korea) to 54% (Egypt). HPV 16 was the most common type in all studies, followed by HPV 18.

Several case-control studies based on serological measures of HPV have been reported, but the results have not been consistent. A recent study was reported from China ([Kamangar et al., 2006](#)) in which prediagnostic serum was tested by enzyme-linked immunosorbent assay (ELISA) for antibodies against HPV 16, 18, and 73 viral capsids; only HPV 16 was (weakly)

associated. In Australia, another seroepidemiological study ([Sitas et al., 2007](#)) showed increasing risk of oesophageal cancer with increasing levels of antibodies against HPV 16.

See Table 2.24 available at <http://monographs.iarc.fr/ENG/Monographs/vol100B/100B-06-Table2.24.pdf> and Table 2.25 available at <http://monographs.iarc.fr/ENG/Monographs/vol100B/100B-06-Table2.25.pdf>.

In view of the inconsistency of results, the Working Group considered that the epidemiological evidence for a role of HPV in oesophageal cancer is inadequate.

### 2.3.4 Cancer of the larynx

HPVs (usually types 6 and 11) cause recurrent respiratory papillomatosis, and it has long been suggested that some of these viruses could be related to cancer of the larynx ([IARC, 2007](#)).

Three recent case series ([de Oliveira et al., 2006](#), Brazil; [Gungor et al., 2007](#), Turkey; [Koskinen et al., 2007](#), Finland, Norway and Sweden) reported HPV positivity of 37.3%, 7.4%, and 4.4%, respectively. In Brazil, HPV 18 was predominant, and in Turkey, HPV 11 was the most common type.

The meta-analysis by [Kreimer et al. \(2005\)](#) reported on 1435 cases of cancer of the larynx. Overall HPV positivity was 24.0%, with HPV 16 detected in 16.6% of laryngeal tumours (69.2% of the positives), followed by HPV 6 in 5.1%, and HPV 18 in 3.9% (see Table 2.26 available at <http://monographs.iarc.fr/ENG/Monographs/vol100B/100B-06-Table2.26.pdf>).

The Working Group considered that the epidemiological evidence is not conclusive to confirm the role of HPV 16 or 18 in cancer of the larynx. Similarly, as discussed in the previous *IARC Monograph*, there is some evidence for a role of HPV 6 and 11, which cause laryngeal papillomatosis that can occasionally become malignant.

## 2.4 Cancer of the skin

As summarized in the previous *IARC Monograph*, multiple case series have demonstrated that HPV DNA is frequently found both in skin lesions and in healthy skin. At present, it is known that cutaneous HPVs are found in five genera: alpha (species 2, 4, 8), beta, gamma, mu, and nu ([Michael et al., 2008](#)). The cutaneous HPV types 1 (genus mu); 2, 27, 3, 10, 57 (genus alpha); and, HPV 4 (gamma) belong to different genera but are all associated with benign plantar, common, and flat skin warts. Certain HPV types in the beta-1 species (HPV 5 and 8 in particular) are found in the rare hereditary disease *epidermodysplasia verruciformis* (EV). It is these beta-1 HPV types, the other beta species and, more recently, the gamma genus HPVs, which have elicited the most interest with regard to risk of skin cancer. The other motivation for study has been the known increased prevalence of skin and genital warts and squamous cell cancers among individual with immunosuppressive treatments related to organ transplantation. These associations have suggested the importance of immune surveillance, and a possible direct role for HPV that has, nonetheless, proven difficult to demonstrate.

Improving test methods continue to detect an expanding variety of HPV types that infect the non-genital skin; these are largely types not found in the anogenital region or in the oral cavity. More types in addition to HPV 5 and 8 have now been found in the lesions of patients with EV, albeit at lower viral loads ([Dell'Oste et al., 2009](#)). With an expanding number of characterized types, the taxonomy of cutaneous HPV types has been clarified; in this section, the current terminology will be used ([de Villiers et al., 2004](#); [Forslund, 2007](#)) (e.g., “beta papillomaviruses” replaces “EV types”). Fully-characterized types are shown in Fig. 2.1, but more are already known to exist.

Case-control studies of skin cancer have been complicated by methodological issues including:

the heterogeneity of skin lesions, the multiplicity of HPV types including many novel types found in skin, evolving methods for sampling lesions and healthy control skin, variable and incompletely validated measurement techniques for DNA and serology, potential confounding by UV light exposure, and the possibility of “reverse causality” in which the development of a skin lesion could lead to increased HPV positivity thereby creating the false impression of a causal association.

Skin neoplasia comprises a diverse set of invasive and preinvasive lesions. Most recent studies have focused on non-melanoma skin cancers, especially squamous cell carcinoma rather than nodular or superficial focal basal cell carcinoma. The precursors to squamous cell carcinoma, called actinic keratoses or solar keratoses have also been included in several studies.

One measurement problem that is unique to skin cancer studies is the possible confounding role of UV light, which might enhance HPV replication, HPV detection and/or seropositivity through a local immune-modulating effect. The scant data are contradictory, and do not permit a conclusion. Because UV exposure and sunburn are strong risk factors for squamous cell carcinoma, it is possible that some of the association between HPV detection and squamous cell carcinoma could be due to confounding by the extent to which the skin under study is exposed to UV light. A fundamental question is whether the increased prevalence of HPV DNA in squamous cell carcinoma and actinic keratoses compared with that in healthy skin proves a causal role for HPV in these tumours ([Pfister, 2003](#); [Majewski & Jablonska, 2006](#); [Bouwes Bavinck et al., 2008](#); [Feltkamp et al., 2008](#); see Table 2.27 available at <http://monographs.iarc.fr/ENG/Monographs/vol100B/100B-06-Table2.27.pdf>).

### 2.4.1 Review of case-control studies measuring HPV DNA

To date, the studies have reported HPV DNA detection in lesions or healthy skin from persons with skin cancers compared with various sorts of controls. Only one very small study of RNA, not large enough to alter conclusions, was found in this review ([Purdie et al., 2005](#)).

In the previous *IARC Monograph*, a few case-control studies were summarized that in aggregate supported a possible association between the detection of DNA from beta-species papillomaviruses and a risk of squamous cell carcinoma. Additional studies reported since then have not provided consistent evidence of an etiological role for any one viral type or groups of types including anogenital HPV types ([Gustafsson et al., 2004](#)), beta-1 species ([Patel et al., 2008](#)), beta-2 species ([Forslund, 2007](#)), and novel types ([Alotaibi et al., 2006](#)).

[Forslund et al. \(2007\)](#) observed that heavily light-exposed areas of the skin were much more likely to test positive for HPV (OR, 4.4), raising the important possibility of confounding of the more modest positive associations found between HPV prevalence and squamous cell carcinoma (OR, 2.1), particularly with beta-species such as HPV 38.

### 2.4.2 Review of case-control studies measuring HPV seroreactivity

Two serological studies were reviewed in the previous *IARC Monograph*, with data on only a few beta-species papillomaviruses. Overall, the data from [Feltkamp et al. \(2003\)](#) and [Masini et al. \(2003\)](#) suggested an association of skin cancer with both beta-1 and beta-2 papillomaviruses, with the most consistent evidence for HPV 8.

Since, the advent of multiplex serology has permitted the assessment of many HPV types at once, although it is not known whether the assays are equally accurate for all of the types detected.

In a population-based case-control study of 252 squamous cell carcinoma case patients, 525 basal cell carcinoma case patients, and 461 control subjects, [Karagas et al. \(2006\)](#) used multiplex serology to detect antibodies in plasma samples against HPV types from phylogenetic genera alpha, beta, and mu. They observed an association between squamous cell carcinoma and seropositivity to HPV types in genus beta as a group, and particularly the beta-1 HPV type 5, but no associations with basal cell carcinoma. Individuals with tumours on chronically sun-exposed sites were more likely to be seropositive for beta HPV types than individuals with squamous cell carcinoma at other anatomical sites. [Waterboer et al. \(2008\)](#) also used multiplex serology but observed associations with squamous cell carcinoma for other types, namely beta-2 species combined, and all gamma species combined (43 cases, 77 controls).

[Casabonne et al. \(2007\)](#) conducted a small but potentially revealing study of HPV seropositivity in stored plasma, comparing prevalent cases of squamous cell carcinoma and incident cases of squamous cell carcinoma to controls. This is the closest to a cohort study available to the Working Group in published form. Using a multiplex method, they assayed 38 HPV types. There were no differences between the 80 controls and 39 incident cases that developed squamous cell carcinoma subsequently to blood draw. However, the 15 cases with prevalent squamous cell carcinoma detected before blood draw tended to have non-significantly elevated seropositivity to multiple HPV types. Moreover, the incident cases diagnosed closest to blood draw were more likely than those diagnosed later to be seropositive (again non-significantly). Although too small to be more than suggestive, these data would indicate that antibodies could be produced during the process or as a consequence of tumour formation.

[Casabonne et al. \(2009\)](#) recently reported a null study among organ transplant recipients.

They studied 140 transplant recipients and 454 controls with multiplex serology, and found expected strong associations of HPV 16 antibodies in subjects with a history of abnormal Pap smears, and HPV 6 antibodies in those with a history of genital warts. However, they observed no associations between any HPVs in the beta genus and squamous cell carcinoma of the skin, by history of abnormal Pap smears or genital warts, or by examination.

#### *2.4.3 Case-control studies combining both HPV DNA measurements and serology*

Since the previous *IARC Monograph*, [Termorshuizen et al. \(2004\)](#) investigated 156 patients with squamous cell carcinoma and 320 controls from outpatient ophthalmology clinics. They tested beta HPV types from plucked eyebrow hairs, tested sera using ELISA, and administered an epidemiological questionnaire focused on sunlight exposure and sunburn. The results were complex, which probably reflects accurately the possible role of HPV in skin cancer etiology. Both the DNA and serological measures of HPV were associated with case status, but the two measurements were not associated with each other. HPV DNA was decreased in controls reporting more lifetime sun exposure, but elevated in controls reporting painful sunburn at specific ages. The type-specific results appeared to show elevations for virtually all types tested.

[Struijk et al. \(2006\)](#) compared 64 squamous cell carcinoma cases, 126 actinic keratosis cases, and 57 tumour-free ophthalmology clinic patients from the same catchment area in Brisbane, Australia. They employed type-specific PCR to study EV-type DNA in plucked eyebrow hairs, and assessed seropositivity using both L1 VLP and E6 fusion protein ELISA. The E6 assays were not associated with DNA or risk. However, HPV DNA prevalence was elevated in the actinic keratosis group compared with either the squamous cell carcinoma group or the healthy

controls, and the results for HPV 20 achieved statistical significance. In this study, seropositivity to L1 VLPs was significantly associated with HPV DNA positivity among the controls and the actinic keratosis group. L1 VLP seropositivity was non-significantly associated with actinic keratosis and squamous cell carcinoma for all types combined, and for squamous cell carcinoma, the odds ratios were elevated (often significantly) for all the individual HPV types.

[Andersson et al. \(2008\)](#) collected serum and biopsy samples from both lesions and healthy skin from 434 non-immunosuppressed patients (72 squamous cell carcinomas, 160 basal cell carcinomas, 81 actinic keratoses, and 121 benign lesions). The presence of HPV DNA and of antibodies to the same HPV type was not significantly correlated. However, seropositivity to any HPV type was significantly more common among patients positive for HPV DNA of any HPV type. The seroprevalence tended to be somewhat higher among squamous cell carcinoma patients than among basal cell carcinoma patients.

#### *2.4.4 Conclusion*

Epidemiological data do not yet support that any single HPV type causes skin cancer in the general population owing to a lack of consistency of the associations described. At present, a positive association has been observed between HPV infection and both squamous cell carcinoma and its precursor actinic keratosis. The evidence is derived from a tendency, not type-specific, of increased odds ratios of DNA detection and seroreactivity compared to control study participants and/or paired healthy skin. The role of HPV in skin cancer may be complex, non-causal, and/or linked to other factors like UV damage, immunosuppression, and genetics.

## 2.5 Cancer at other sites

The evidence for the carcinogenicity of HPV in the following cancers was evaluated in the previous *IARC Monograph*: cancers of the nose and nasal sinuses, cancer of the lung, cancer of the colon and rectum, cancer of the breast, cancer of the ovary, cancer of the prostate, and cancer of the urinary bladder and urethra.

### 2.5.1 Cancer of the nose and nasal sinuses

Inverted papillomas of the nasal cavity and paranasal sinuses are frequently positive for HPV 6, 11 and 57, and a small percentage of rare carcinomas arising at those sites sometimes have HPV 16, 18, 11, 6 and 57 in decreasing frequency ([IARC, 2007](#)). The rarity of these tumours makes it difficult to evaluate the role of HPV, and no case-control studies have been reported.

A recent study by [El Mofty & Lu \(2005\)](#) conducted in the USA correlated HPV detection by PCR with histological subtypes of carcinoma of the sinonasal tract. They studied paraffin blocks of a small series ( $n = 39$ ) of keratinizing, non-keratinizing, and undifferentiated tumours. HPV was detected more often in the small number of keratinizing tumours, with a predominance of HPV 16. On the other hand, another recent study ([Kim et al., 2007b](#)) studied 57 paraffin-embedded biopsies from inverted papillomas of different grades (I – IV, with grade IV being carcinoma originating in inverted papilloma). Only 12.3% of cases (restricted to grade I and II) had detectable HPV DNA, mainly of the mucosal carcinogenic type. [Alos et al. \(2009\)](#) recently reported a prevalence of HPV in 20% of a series of 60 sinonasal tumours, with HPV 16 detected in 11/12 cancers.

### 2.5.2 Cancer of the lung

Multiple groups have tested for HPV in lung cancer samples, with variable results. The geographic areas where the highest prevalence has been reported are predominantly in Asia.

The rare occurrence of lung cancer in patients with recurrent laryngeal papillomatosis has been documented, and some studies have reported HPV-associated lung cancers among women with a history of CIN3.

Since the last *IARC Monograph*, there have been seven new case series reported including at least 40 cases of lung cancer, from various locations including France ([Coissard et al., 2005](#)), China ([Fei et al., 2006](#)), the Islamic Republic of Iran ([Nadji et al., 2007](#)), the Republic of Korea ([Park et al., 2007](#)), Chile ([Aguayo et al., 2007](#)), Taiwan, China ([Cheng et al., 2007](#)), and India ([Jain et al., 2005](#)). HPV detection was variable, from 2% in France to 46% in Taiwan, China, with a predominance of HPV 16 in all studies. In the study from China, a non-cancer group was included without detection of HPV 16. In the Korean study, HPV 16 was more common in biopsies of younger subjects.

See Table 2.28 available at <http://monographs.iarc.fr/ENG/Monographs/vol100B/100B-06-Table2.28.pdf>.

### 2.5.3 Cancer of the colon and rectum

The discussion of the association of HPV and cancer of the colon and rectum (usually adenocarcinoma) requires a clear distinction of the histology and anatomical site, to distinguish cases from cancers of the anus (Section 2.2.4), which are clearly HPV-related. Several small case series have investigated the prevalence of HPV in biopsies of colorectal cancer, with variable results. Many of the studies were completely negative. However, one study ([Bodaghi et al., 2005](#)) reported 42% HPV positivity in cases, 29% in biopsies from adjacent tissues, and no HPV in control subjects. HPV 16 was the predominant type in tumours.

A recent case series from Brazil ([Damin et al., 2007](#)) investigated 72 cases of primary colorectal adenocarcinoma. For each patient, two specimens were collected: one from the tumour and

one from the normal colorectal mucosa at least 15 cm apart from the tumour. In addition, biopsies from 30 individuals without cancer were also evaluated. HPV DNA was detected in 83.3% of cancer patients but in none of the tissues from the non-malignant control group ( $P < 0.001$ ). HPV was present only in the tumour of 32% of the cases, in the tumour and adjacent tissue of 32% of cases, and only in adjacent tissue in 19.5% of cases. HPV 16 was the predominant type (68.3% of positive cases), followed by HPV 18 (50% of positives). More than 30% of cancer patients were infected by multiple HPV types. HPV genotyping was confirmed by sequencing; there were no epidemiological differences between positive and negative cases.

#### 2.5.4 Cancer of the breast

There is contradictory evidence for the role of HPV in breast cancer. Several small negative studies were reported in the previous *IARC Monograph*, as well as a few studies in which HPV was detected in different fractions of breast tumours. HPV 16 and 18 were the most common types. One of the studies ([Hennig et al., 1999](#)) reported HPV-type concordance between CIN3 cases and subsequent breast cancers.

A recent study from Switzerland ([Lindel et al., 2007](#)) analysed paraffin-embedded sections of 81 patients with breast cancer using the well validated SPF10 PCR system; all samples were negative. Another study conducted in the Syrian Arab Republic ([Akil et al., 2008](#)) found extremely high HPV positivity in 113 blocks. [Cazzaniga et al. \(2009\)](#) investigated the presence of cutaneous and cervical carcinogenic HPV types in ductal lavages, colostrum, and milk of 90 women at risk of breast cancer. A total of 14% (10/70 analysed) of the specimens contained cutaneous types, and only one had a mucosal type (HPV 16). Removal of the superficial epidermal cells significantly reduced prevalence, with HPV detection in only 2/45 specimens (beta HPV types).

#### 2.5.5 Cancer of the ovary

There is limited information about the potential role of HPV in cancer of the ovary, and several small negative case series have been reported as well as a few studies with positive findings. The presence of HPV in the ovary may be related to HPV-associated disease ascending from the cervix.

A study by [Quirk et al. \(2006\)](#) from the USA reported the analysis of fresh frozen biopsies from 20 women with ovarian carcinomas. Using commercial PCR amplification kits, no HPV DNA was detected in any of the tumours. Another study in Turkey ([Atalay et al., 2007](#)) observed HPV in 8.5% of 94 patients. All HPV-positive patients had serous papillary tumours and advanced stage disease. A third study reported from Italy ([Giordano et al., 2008](#)) included 71 women with borderline ( $n, 21$ ) and malignant ( $n, 50$ ) ovarian tumours. Three cases (4.2%) of epithelial ovarian neoplasm had detectable HPV DNA.

#### 2.5.6 Cancer of the prostate

Prostate cancer is associated with sexual behaviour in some studies ([Damber & Aus, 2008](#)), suggesting a possible role for HPV. A variety of studies have been conducted, including case series and case-control studies mainly based on serological measures of exposure. The results are inconclusive although most studies are negative.

Two case series of cancer of the prostate ([Leiros et al., 2005](#), Argentina; [Balis et al., 2007](#), Greece) have been reported recently. The study from Argentina detected HPV in 41.5% of tumours. Most of the tumours where HPV was identified harboured HPV 16. All prostatic hyperplasias were negative. In contrast, the study in Greece only found HPV DNA in 2/42 cases of prostatic carcinoma (4.8%).

Several case-control studies, some of them nested in prospective cohorts and using serological measures, have been reported on the

association of HPV and prostate cancer ([Adami et al., 2003](#), Sweden; [Rosenblatt et al., 2003](#), USA; [Korodi et al., 2005](#), Nordic Countries; [Bergh et al., 2007](#), Sweden; [Sutcliffe et al., 2007](#), Sweden). All of the serology-based studies, some of them using pre-diagnostic and some post-diagnostic specimens, were basically negative, with no association with antibodies against L1 VLPs of HPV 16, 18 and 33 as determined by ELISA, except for a minor elevation in risk for HPV 33 in one of the studies. The study by [Bergh et al. \(2007\)](#) also studied paraffin-embedded biopsies without detection of HPV in either cases in controls.

See Table 2.29 available at <http://monographs.iarc.fr/ENG/Monographs/vol100B/100B-06-Table2.29.pdf> and Table 2.30 available at <http://monographs.iarc.fr/ENG/Monographs/vol100B/100B-06-Table2.30.pdf>.

### 2.5.7 Cancer of the urinary bladder and urethra

Bladder cancers are predominantly transitional cell carcinomas, with the exception of areas where schistosomiasis is highly prevalent where a predominance of squamous cell carcinomas is observed. Similarly to other cancer sites, case series have reported a variable prevalence of HPV across studies.

Since the previous *IARC Monograph*, a case series was reported from the Islamic Republic of Iran ([Barghi et al., 2005](#)), which included paraffin-embedded biopsies of 59 transitional cell carcinomas, and detected HPV in 35.6% of the tumours. The most common type was HPV 18 but HPV 16 was not detected. [Moonen et al. \(2007\)](#) reported on 107 cases from the Netherlands, testing bladder wash samples. Overall, the prevalence of any HPV type was 13.7%. HPV 18 was the most common type detected, followed by HPV 16.

## 2.6 Cofactors of HPV in cervical cancer

HPV is a necessary cause of cervical cancer. However, only a small fraction of women infected develops cervical cancer or its high-grade precursors, indicating that there must be additional viral, host or environmental factors conditioning viral persistence and progression. Cofactors were reviewed extensively in the previous *IARC Monograph*.

The study of cofactors has been difficult because many of the exposures involved are correlated with sexual behaviour and therefore with HPV acquisition. Analyses restricted to HPV-positive subjects have been considered to be the best analytical alternative for control of this type of confounding. However, in the context of almost universal exposure, women with current infection are not necessarily the most appropriate control group, because they are a combination of both persistent and recently acquired infections that vary with the population and age of the control group, and there is currently no adequate measure of past exposure to HPV. Other exposures have the analytical difficulty of being associated with screening behaviour, and most suffer some degree of measurement error.

One of the most studied cofactors is tobacco smoking, a well established carcinogen that is known to exert its effect in organs not directly exposed to smoke. Most studies, using different methods to adjust for confounding including the restriction to HPV-positive cases and controls, have found an approximately 2-fold increase in risk of cervical cancer or high-grade precursors ([Kjær et al., 1996](#); [Ho et al., 1998a](#); [Krüger-Kjaer et al., 1998](#); [Olsen et al., 1998a](#); [Ylitalo et al., 1999](#); [Kjellberg et al., 2000](#); [Hildesheim et al., 2001](#); [Plummer et al., 2003](#); [Giuliano et al., 2004](#)), some of them with dose responses for the amount of cigarettes smoked and duration of smoking ([Ho et al., 1998a](#); [Kjellberg et al., 2000](#); [Hildesheim et al., 2001](#); [Harris et al.,](#)

2004). In a large meta-analysis including more than 13000 cases and 23000 controls from 23 epidemiological studies, smokers were at an increased risk (pooled OR, 1.6; 95%CI: 1.5–1.7) of squamous cell but not of adenocarcinoma of the cervix. The risk of squamous cell carcinoma increased in current smokers with the number of cigarettes smoked per day, and with younger age at starting smoking (Appleby *et al.*, 2006a). Several cohort studies have clearly shown the association, particularly for CIN3 (Deacon *et al.*, 2000; Castle *et al.*, 2002). Cigarette smoking is an established cofactor of HPV for cervical cancer, although it is still unclear if its effect is mediated by genotoxicity or immunomodulation. The role of other forms of tobacco exposure, including chewing and passive smoking, as well as the role of tobacco as a cofactor of HPV in other organs requires further research.

Multiple studies have also suggested a potential role of hormonal contraceptives as HPV cofactors. The agents investigated most extensively are oral contraceptives, and several case-control studies (Ylitalo *et al.*, 1999), but not all (Kjær *et al.*, 1996; Lacey *et al.*, 2000; Shapiro *et al.*, 2003; Giuliano *et al.*, 2004) detected increases in risk, particularly among users of oral contraceptives for more than 5 or 10 years (Berrington *et al.*, 2002; Moreno *et al.*, 2002). One of the studies (Lacey *et al.*, 1999) reported an association restricted to glandular lesions. A very large pooled re-analysis confirmed the association between oral contraceptives and cervical cancer and its precursors, and the observed reduction in risk after cessation of use (Appleby *et al.*, 2007). These findings were not replicated in cohort studies of precursor lesions (Deacon *et al.*, 2000; Castle *et al.*, 2002), possibly reflecting the protective effect of screening among oral contraceptive users. Injectable contraceptives have also been shown to increase the risk of cervical cancer (Herrero *et al.*, 1990, Appleby *et al.*, 2007), and also to increase CIN3 among HPV-positive women (Castle *et al.*, 2005b). An important

consideration when interpreting the role of hormonal contraceptives is that most studies and systematic reviews evaluated the risk associated with the contraceptive products used in previous decades. Information is needed about the potential association of the newer contraceptive agents that have different hormonal formulations.

The number of pregnancies is also an established cofactor of HPV in cervical cancer, with most studies showing increases in risk associated with increasing number of pregnancies (Kjær *et al.*, 1996; Hildesheim *et al.*, 2001; Muñoz *et al.*, 2002; Appleby *et al.*, 2006b). A few prospective studies of precursor lesions have not confirmed this association (Deacon *et al.*, 2000; Castle *et al.*, 2002), possibly because they had limited numbers of highly multiparous women. The possible role of a hormonal, nutritional or immune status change during pregnancy or potential cervical trauma during delivery as a cofactor of HPV remains to be determined. It is possible that part of the decline in cervical cancer incidence and mortality observed in some populations is related to declines in fertility rates.

The likely role of nutrients as preventive agents in several epithelial cancers is generally accepted. However, despite numerous studies using different methods for ascertaining the impact of dietary exposures, the investigation of the role of nutrients as cofactors of HPV in cervical cancer and its precursors has yielded inconsistent results, with some studies suggesting potential protective effects of fruits and vegetables (Rajkumar *et al.*, 2003), folate (Weinstein *et al.*, 2001), vitamin C (Giuliano *et al.*, 2003), carotenoids (Giuliano *et al.*, 1997), tocopherols (Giuliano *et al.*, 1997), and vitamin B12 (Sedjo *et al.*, 2002), but other studies have shown no significant associations (Ho *et al.*, 1998b; Wideroff *et al.*, 1998). Notably, a recent study (Piyathilake *et al.*, 2007) suggested that an increase in risk of CIN2+ was associated with a strong interaction between HPV 16 and low red-blood cell folate levels. The assessment of dietary exposures is difficult, and subject to

variation in different populations. In addition, it is correlated with sociodemographic and behavioural variables, requiring the consideration of multiple confounders. However, further studies are needed, particularly for nutrients in the one-carbon metabolic pathways like folate, given the potential for intervention.

There is some evidence of familial aggregation in cervical cancer ([Amundadottir et al., 2004](#); [Zelmanowicz et al., 2005](#); [Couto & Hemminki 2006](#)), suggesting the possibility of genetic factors that could act as cofactors of HPV infection, although additional studies are needed to confirm these associations, to define possible mechanisms, and to rule out confounding by environmental exposures shared by families.

Immunosuppression, both in transplant recipients and in HIV-infected subjects, is an established cofactor of HPV infection in the development of precursor lesions and invasive cancer at most anatomical sites investigated (see *HIV-Monograph* in this volume). Immunosuppression was reviewed extensively in the previous *IARC Monograph*. In this context, the genetics of the immune response is also likely to play an important role in the fate of HPV infections. Some HLA (human leukocyte antigen) polymorphisms, related with antigen recognition, have been proposed as determinants of risk of progression, while others have been associated with protection. Many studies conducted in different populations have reported associations in both directions, particularly for DR and DQ class II genes ([Apple et al., 1994, 1995](#); [Duggan-Keen et al., 1996](#); [Cuzick et al., 2000](#); [Madeleine et al., 2002](#)). Some of the difficulties of these analyses include variable ethnic compositions of the populations and multiple comparisons.

Other infectious agents, including *Chlamydia trachomatis* ([Koutsky et al., 1992](#); [Jha et al., 1993](#); [de Sanjosé et al., 1994](#); [Bosch et al., 1996](#); [Giuliano et al., 2001](#); [Smith et al., 2002a](#); [da Silva et al., 2004](#)) and herpes simplex virus ([Hildesheim et al., 1991](#); [Peng et al., 1991](#); [Koutsky et al., 1992](#);

[Jha et al., 1993](#); [Becker et al., 1994](#); [Olsen et al., 1998b](#); [Smith et al., 2002b](#)), as well as inflammation associated with various infections have been shown in some studies to be associated with a risk of cervical cancer or its precursors. Most studies of these agents are seroepidemiological, and some have used markers of bacterial or viral DNA. The most abundant and consistent evidence is for *C. trachomatis*, but the correlation is so high between sexually transmitted agents that it is difficult to completely rule out residual confounding, and the mechanism for its interaction with HPV, if there is one, remains unknown. Cervical inflammation, as ascertained in cytological specimens has been associated with an increase in risk ([Schiff et al., 2000](#); [Castle et al., 2001](#)), and genotoxic damage associated with the inflammatory response is a likely mechanism for the interaction between HPV and these infectious agents.

Most cofactors appear to act as determinants of persistence and progression to advanced precursors, but no risk factor has been established for progression to invasion, except probably age.

### 3. Cancer in Experimental Animals

In this volume, the Working Group decided not to include a separate section on “Cancer in Experimental Animals” in the *Monographs* on viruses but rather to include description of such studies under Section 4 (below). The reasoning for this decision is explained in the General Remarks.

## 4. Other Relevant Data

### 4.1 Mechanisms of HPV-associated carcinogenesis

There is compelling evidence that certain HPVs encode oncoproteins that directly contribute to the development and maintenance of cervical cancer. This is supported by extensive biochemical and biological studies performed with animal cells, primary human cells, and with cells derived from the cervical cancers.

#### 4.1.1 Rodent cells

The earliest assays to confirm that HPV encodes proteins with cell-transforming capacity were carried out with established cell lines of murine origin, such as NIH3T3, Rat-1, and Rat3Y1. These tests can be considered to be the simplest and least rigorous for establishing the oncogenic potential of a given agent, in that the cells used in these assays are already immortal. The cells are transfected with entire HPV genomes or individual gene products, and their transforming capacity is assessed on the basis of either growth in an anchorage-independent manner, or tumour formation in nude mice. These early studies established that HPV 16, in particular, had transforming potential in established rodent cells ([Tsunokawa et al., 1986](#); [Yasumoto et al., 1986](#)) and that the principal activity resided in the E7 oncoprotein ([Kanda et al., 1988](#); [Vousden et al., 1988](#)), although transforming potential in such assays has also been reported for the E5 and E6 proteins of both HPV 16 and HPV 18 ([Bedell et al., 1989](#); [Sedman et al., 1991](#); [Pim et al., 1992](#)).

Oncogene-cooperation assays were next used to demonstrate the transforming potential of different HPV types. These tests most commonly involved primary baby rat kidney cells or primary baby mouse kidney cells. The cooperating oncogene was typically activated *Ras* ([Matlashewski et al., 1987](#)), although co-transforming activity

has also been reported with activated *Fos* ([Crook et al., 1988](#)). In all of these cases, transformation was assessed on the basis of the appearance of morphologically transformed immortalized cells, or the ability of these cells to form tumours in syngeneic animals. In baby rat kidney cells, the majority of the transforming activity of HPV 16 appears to be carried by the E7 oncoprotein ([Storey et al., 1988](#)), but some transforming activity is also detectable from the E6 oncoprotein in baby mouse kidney cells ([Storey & Banks, 1993](#)).

#### 4.1.2 Human cells

Human keratinocytes are the cells of choice to study HPV oncoproteins, as these cells are the natural target for the virus *in vivo*. In these cells, some mucosal and cutaneous HPVs can readily induce immortalization. This property has been demonstrated in cervical, foreskin and oral keratinocytes as well as tonsil epithelial cells ([Dürst et al., 1987](#); [Pirisi et al., 1987](#); [Woodworth et al., 1989](#); [Klingelhutz et al., 1996](#); [Caldeira et al., 2003](#); [Spanos et al., 2008](#)). In the majority of cases, these activities required both E6 and E7 oncoproteins ([Barbosa & Schlegel, 1989](#); [Hawley-Nelson et al., 1989](#)).

Although these assays measure only immortalizing capacity, full transformation, i.e. tumour growth, can be attained by prolonged passage in tissue culture or by transfection of additional activated oncogenes, such as *RAS* ([Dürst et al., 1989](#); [Spanos et al., 2008](#)).

Other primary human cells that are susceptible to the transforming potential of HPVs include mammary epithelial cells, which appear to be particularly susceptible to the effects of E6 ([Band et al., 1991](#)).

#### 4.1.3 Cells derived from cervical tumours

The clearest demonstration that E6 and E7 are necessary for the development of cervical cancer comes from studies where expression of either protein is blocked or inhibited in cells derived from cervical tumours. The expression of both viral proteins continues many years after the establishment of these cell lines *in vitro* ([Schwarz et al., 1985](#); [Smotkin & Wettstein, 1986](#); [Androphy et al., 1987](#); [Banks et al., 1987](#)), suggesting a requirement for their continued expression for the maintenance of cell proliferation. This has in fact been confirmed in many studies using a range of techniques to ablate the expression or activity of E6 and E7. This includes selective alteration of viral gene expression ([von Knebel Doeberitz et al., 1988](#); [Goodwin & DiMaio, 2000](#)), anti-sense RNA ([Steele et al., 1992](#); [von Knebel Doeberitz et al., 1992](#)), RNAi ([Butz et al., 2003](#); [Yoshinouchi et al., 2003](#)), and blocking peptides ([Butz et al., 2000](#)). In all of these examples, cervical cancer cells cease to proliferate, and enter into either senescent or apoptotic states. This is perhaps the single most important evidence of the role of HPV in the maintenance of the malignant phenotype of cervical cancer.

#### 4.1.4 Transforming capacity of HPVs

HPV 16 and HPV 18 display transforming potential in all of the assays listed above. The other mucosal types that have been shown to possess transforming activity in keratinocyte immortalization assays are HPV types 31, 33, 45, 53, 56, 58, 66, and 82 ([Woodworth et al., 1989](#); [Hiller et al., 2006](#); [Morandell et al., 2008](#)). It should be stressed, however, that except for HPV types 31 and 33, this has only been reported once.

Cutaneous beta HPV types also exhibit transforming activity under the conditions of some of the above assays. HPV 5, 8, and 47 are active in established rodent cell transformation assays ([Kiyono et al., 1992](#); [Schmitt et al., 1994](#));

HPV types 12, 14, 15, 24, 36, and 49 are active in primary rodent cell co-transformation assays (oncogene cooperation assays with activated *Ras*) ([Massimi et al., 2008](#)); HPV 38 is active in primary human keratinocyte assays ([Caldeira et al., 2003](#)), and a weak keratinocyte immortalizing activity has been reported for HPV 8 ([Schmitt et al., 1994](#)).

### 4.2 Biochemical properties of HPV proteins

#### 4.2.1 The E5 protein

The E5 protein is a small hydrophobic polypeptide (10 kD) whose localization is believed to be in membrane compartments including the Golgi apparatus, and the endoplasmatic reticulum ([Conrad et al., 1993](#)). A possible role for the E5 protein of the mucosal high-risk HPV types may be at an early stage of carcinogenesis given that E5 is not expressed in many invasive cancers due to the integration of the viral DNA into the host genome, which frequently leads to the loss of the E5 ORF or at least loss of its expression. Surprisingly, there is still very little known about the biochemical mode of action of the E5 protein. There is some evidence to suggest that it can alter the processing of epidermal growth factor receptors ([Straight et al., 1993](#)), most likely through interaction with the 16-kD component of the vacuolar proton-ATPase, and endosomal processing ([Conrad et al., 1993](#); [Straight et al., 1995](#)). However, there is also evidence that E5 can affect antigen presentation by interaction with, and downregulation of, class 1 HLA ([Ashrafi et al., 2005, 2006](#)), thereby contributing to immune evasion. It should be stressed that all the information on biochemical properties of the E5 proteins are derived from studies of HPV 16 and the low-risk HPV type 6. Finally, an anti-apoptotic function for E5 has also been described in keratinocytes, although the molecular basis

behind this activity is unknown ([Kabsch & Alonso, 2002](#)).

It should be noted that many beta HPV types do not possess an E5 ORF, so this protein would not seem to be relevant in these virus types. Whether other viral gene products have evolved to carry out some of the functions of the E5 protein described above, remains to be determined.

#### 4.2.2 The E6 protein

The E6 protein is approximately 158 amino acids in length, and is one of the most extensively studied HPV proteins. A major feature of its activity is directed at the degradation of cellular binding partners through the combined activity of a ubiquitin ligase, E6AP (E6-associated protein), and the cellular ubiquitin proteasome pathway ([Scheffner et al., 1993](#)). More than a dozen cellular binding partners of the E6 protein have been described ([Mantovani & Banks, 2001](#); [Thomas et al., 2008](#)). Because of the complex nature of the E6 structure, it has been extremely difficult to determine which particular binding partner is responsible for any given function ([Nominé et al., 2006](#)). Clearly, interaction with the tumour-suppressor protein p53 is fundamental to the activity of the E6 protein of transforming mucosal HPV types. Indeed, E6 proteins from multiple mucosal types (HPV 16, 18, 33, 35, 39, 45, 51, 52, 53, 56, 58, 66, 70, and 82) target p53 for degradation at the 26S proteasome ([Hiller et al. 2006](#); see also [Scheffner et al., 1990](#)). Similarly, the ability to induce telomerase activity is a common theme in HPV-induced malignancy, although the precise mechanism by which this occurs is subject to considerable debate ([James et al., 2006](#); [Liu et al., 2008](#); [Sekaric et al., 2008](#)). An important feature of these E6 proteins is the presence of a PDZ-binding motif at the C-terminal. At present, multiple binding partners have been described, all of which are degraded by proteasomes. It remains to be

determined, however, which of these are functionally important *in vivo* ([Thomas et al., 2008](#)).

The discussion above relates primarily to E6 proteins of the mucosal HPV types. Much less is known about the biochemical activity of the beta HPV E6 proteins. They all lack the capacity to degrade p53 and none of them, whether they are associated with cancer or not, possesses PDZ-binding motifs. This suggests major differences in how the beta HPV types interact with p53, and in the pathways that involve PDZ-domain-containing substrates. Intriguingly, in the case of HPV 38, p53-responsive pathways appear to be abrogated through the induction of the p53 repressor, ΔNp73, although it remains to be determined whether this is due to E6 or E7 ([Accardi et al., 2006](#)). The E6 protein of HPV 20 displays a unique characteristic in that it is actually targeted for degradation by p53 in a caspase-dependent manner. However, this does not occur if p53 is mutated as is frequently the case in non-melanoma skin cancer ([Fei & de Villiers, 2008](#)). A common feature of E6 proteins from cutaneous and mucosal HPV types is their ability to interact with and inhibit the activity of the pro-apoptotic protein Bak. This has now been reported for E6 proteins of HPV 16, 18, 5, 8, 11, 20, 22, 38, 76, 92, and 96 ([Thomas & Banks, 1998, 1999](#); [Jackson et al., 2000](#); [Dong et al., 2005](#); [Underbrink et al., 2008](#)). This appears to be an important evolutionarily conserved activity that may also play a role in the development of HPV-induced malignancy. Likewise, telomerase activation has also been described for the oncoproteins E6 and E7 of several beta HPV types, in particular HPV 38 ([Bedard et al., 2008](#); [Gabet et al., 2008](#)), with other types such as HPV 5, 8, 20 and 22 exhibiting only low-to-background levels of telomerase activation ([Bedard et al., 2008](#)).

#### 4.2.3 The E7 protein

The E7 protein is approximately 100 amino acids in length and, like E6, targets many of its cellular substrates for proteasome-mediated degradation. To achieve this, E7 makes use of a multiprotein cullin-2 ubiquitin-ligase complex ([Huh et al., 2007](#)). Again, numerous cellular targets have been described for E7 ([Münger et al., 2001](#)). As for E6, a subset of these targets has been clearly shown to be important for the biological activity of E7. Perhaps the most prominent are pRb and the related pocket proteins, p107 and p130, all of which are degraded by E7, thereby allowing the induction of S-phase progression. Paradoxically, E7 can also cause an increase in p53 protein levels by a mechanism that is not yet understood ([Blanton et al. 1992; Seavey et al. 1999](#)). E6 can overcome E7's induction of p53, which can be at multiple levels, both by directly targeting p53 for degradation, and by inhibiting p53 acetylation ([Thomas & Chiang, 2005; Shamanin et al., 2008](#)). Other key regulators of the cell cycle that are known interacting partners of E7 include the p21/p27 cdk inhibitors, and a subset of cyclins ([Münger et al., 2001](#)).

E7 also has a profound impact on transcriptional regulation, being found in association with AP1 transcription factors ([Antinore et al., 1996](#)), histone deacetylases ([Longworth et al., 2005](#)), and MPP2 ([Lüscher-Firzlaff et al., 1999](#)). Interestingly, one of the consequences of E7 activity is the strong upregulation of p16<sup>INK4a</sup> in cervical cancers, a feature that can be used as a surrogate marker for the presence of cancer-associated HPV types ([Vinokurova et al., 2005](#)).

As is the case with the E6 protein, the function of the beta HPV E7 protein is also poorly understood at the biochemical level. However, the ability to target pRb is a feature of the E7 protein of some beta HPV types, as has been shown for HPV 38 ([Caldeira et al., 2003](#)).

Certain biomarkers that reflect the expression and function of E6 and E7, such as the

overexpression of p16 induced by E7, and the lack of mutation in p53 due to its inactivation by E6, have been useful indicators of the involvement of HPV in cervical cancer. In several studies, the correlation between the presence of HPV, an increased level of p16, and the absence of p53 mutation has been investigated in cancers other than cervical cancer. The results are summarized in [Table 4.1](#).

### 4.3 Biological Properties of HPV

#### 4.3.1 Immortalization

As discussed above, for many different HPV types, effective immortalization of keratinocytes requires the combined presence of the viral proteins E6 and E7, although there are reports of a weak immortalizing activity with E7 alone.

#### 4.3.2 DNA-damage responses

HPVs have the capacity to abrogate normal DNA-damage responses, which is likely to contribute to the accumulation of genetic alterations in HPV-positive cells, including those that contribute to HPV-associated cancers (see Section 4.3.3).

Because E6 can inactivate p53 by stimulating its proteasome-mediated degradation ([Scheffner et al., 1990](#)), it is not surprising that many studies have confirmed that E6 can abrogate cell-cycle arrest induced by a variety of DNA-damaging agents ([Kessis et al., 1993; Foster et al., 1994](#)). *In vivo*, this activity of E6 correlates at least in part with its ability to inactivate p53, as was shown in mice ([Song et al., 1998](#)).

Cell-cycle arrest induced by p53 may arise through the induction of p21, and the consequent inhibition of cdk-mediated phosphorylation of pRb and the related proteins p107 and p130 ([el-Deiry et al., 1993; Harper et al., 1993; Slebos et al., 1994](#)). E7 is able to inhibit the activity of p21 ([Funk et al., 1997; Jones et al., 1997](#)) as well as that of the pRb family of proteins ([Münger et al.,](#)

**Table 4.1 Assessment of the correlations between p16 positivity and HPV status**

Site	Reference	p16/p53 status	HPV status	p16 <sup>+</sup> :HPV <sup>+</sup> correlation
Lung	<a href="#">Carlson et al. (2007)</a>	All p16 <sup>+</sup>	All negative	None
	<a href="#">Aguayo et al. (2007)</a>	Most p16 <sup>+</sup>	Some positive	None
Colorectum	<a href="#">Kong et al. (2007)</a>	All p16 <sup>+</sup>	All positive	Yes but no HPV- samples
	<a href="#">Lu et al. (2003)</a>	All p16 <sup>+</sup>	All positive	Yes but no HPV
Sinus	<a href="#">El-Mofty &amp; Lu (2005)</a>	9/39 p16 <sup>+</sup>	9/39 positive, all 9 were p16 <sup>+</sup> and p53 <sup>-</sup>	Yes
Oesophagus	<a href="#">Castillo et al. (2006)</a>	Some p16 <sup>+</sup>	Some positive	Incomplete: some p16 <sup>-</sup> were HPV <sup>+</sup> , some p16 <sup>+</sup> were HPV <sup>-</sup>
Tonsil	<a href="#">Klussmann et al. (2003)</a>	16/34 p16 <sup>+</sup>	18/34 HPV <sup>+</sup>	Near complete (one HPV <sup>-</sup> cancer was p16 <sup>+</sup> )
Larynx	<a href="#">Laco et al. (2008)</a>	14/24 p16 <sup>+</sup>	14/24 HPV16 <sup>+</sup> same 14 that were p16 <sup>+</sup>	Yes <sup>a</sup>
Oropharynx	<a href="#">O'Regan et al. (2008)</a>	Some p16 <sup>+</sup>	Some positive (10/24)	Incomplete Some p16 <sup>-</sup> were HPV <sup>+</sup> , some p16 <sup>+</sup> were HPV <sup>-</sup>
	<a href="#">Shuyama et al. (2007)</a>	Some positive	Some positive (19/59)	None
	<a href="#">Reimers et al. (2007)</a>	25/29 p16 <sup>+</sup> and HPV <sup>+</sup>	27/96 HPV <sup>+</sup> in total	High <sup>b</sup> : 4 HPV p16 <sup>+</sup> ; 2 p16 <sup>-</sup> HPV <sup>+</sup>

<sup>a</sup> Data for laryngeal carcinoma<sup>b</sup> Data for tonsil/base of tongue

Compiled by the Working Group

1989; [Slebos et al., 1994](#)). The inactivation of pRb and p107 by E7 is mediated in part by its ability to induce their proteasome-mediated degradation ([Münger et al., 2001](#)). Given the properties of E7, and the role of these targets in mediating the cellular response to DNA damage, it is not surprising that E7 was found to abrogate these responses both in tissue culture ([Slebos et al., 1994](#)), and *in vivo* ([Song et al., 1998](#)).

#### 4.3.3 Genomic instability

As noted above, HPV E6 and E7 proteins can abrogate the cellular response to DNA damage, which under normal conditions would help prevent the accumulation of genetic changes in host chromosomes. It is, therefore, not unexpected that a hallmark of E6- and E7-expressing keratinocytes is genomic instability ([Smith et al., 1989](#); [Popescu & DiPaolo, 1990](#); [Hashida & Yasumoto, 1991](#); [Steenbergen et al., 1998](#)). Abnormalities observed include monosomies

and trisomies, chromatid gaps and breaks, double minutes, and aberrant chromosomes. Structural changes are most commonly detected in chromosomes 1, 3 and 5, and less frequently in chromosomes 6, 7, 8, 10, 12, 13, 16 and 22. Some of the allelic losses have been associated with particular genes that could be involved in malignant conversion and/or progression. Allelic imbalance on 6q has been associated with telomerase activation ([van Duin et al., 2003](#)), which is a crucial step for cell immortalization mediated by high-risk HPVs ([Klingelhutz et al., 1996](#); [Anderson et al., 1997](#)). Mitotic abnormalities can also be induced by E6 and E7 through direct subversion of the mitotic spindle checkpoint ([Thomas & Laimins 1998](#); [Duensing et al., 2000](#)).

The ability of E6 to induce genomic instability most likely reflects its capacity to inhibit the function of p53 (see above), leading to the disruption of normal DNA-repair processes and the consequent accumulation of genetic damage.

In the case of E7, genomic instability may also reflect its effect on centrosome biogenesis, and the consequent defects in segregation of daughter chromosomes during cell division ([Duensing & Münger, 2002](#)). How E7 induces genomic instability remains unclear. Although studies in mice suggest that the inactivation of pRb in tissues that express E7 is sufficient to induce centrosome abnormalities ([Balsitis et al., 2003](#)), another study demonstrates that E7 can induce such abnormalities through a pRb-independent mechanism ([Duensing & Münger, 2003](#)). Furthermore, this may occur through cell-cycle perturbations that involve cyclin/CDK2 activity ([Duensing et al., 2004](#)).

Chromosomal instability has been associated with the integration of the viral genome upon prolonged culture of the keratinocyte cell line W12 harbouring episomal HPV, when grown as monolayers ([Pett et al., 2004](#)). However, the contrary has been shown in raft cultures of keratinocytes, in which genomic instability was observed in the absence of viral DNA integration ([Duensing et al., 2001](#)). Clearly more work is required in this area to fully understand the possible effects of viral integration upon genomic integrity of the host cell.

#### 4.3.4 Cell proliferation and differentiation

The most pronounced effects on cell proliferation and differentiation are exerted by the E7 oncoprotein. When expressed in immortalized rodent cells, HPV 16 E7 can stimulate DNA-synthesis and cell proliferation ([Sato et al., 1989](#)). As E7 enhances proliferation of keratinocytes, it reactivates the host DNA-replication machinery in suprabasal differentiated, non-cycling cells ([Blanton et al., 1992](#); [Cheng et al., 1995](#)). Thus, while differentiation still takes place, E7 also induces cell proliferation in cells that have already migrated away from the basal cell layer, suggesting that differentiation occurs, but in a modified state ([Blanton et al., 1992](#); [Cheng et al., 1995](#); [Jones et al., 1997](#)).

#### 4.3.5 Inhibition of apoptosis

E6 has long been known to be a potent inhibitor of the apoptotic response. This has a major impact on the survival of cells that are being driven to proliferation by E7. This activity of E6 can occur in both a p53-dependent and -independent manner ([Pan & Griep, 1994, 1995](#)), the latter probably being related to the ability of E6 to induce the degradation of the pro-apoptotic factor Bak ([Thomas & Banks, 1998](#)), which is a common feature of both cutaneous and mucosal HPV E6 proteins. Recent studies also suggest the ability of HPV 16 E6 to inhibit receptor-mediated pathways of apoptosis, such as the TNF- and Fas-induced pathways ([Filippova et al., 2002, 2004](#)).

### 4.4 Role of HPVs in malignant conversion

#### 4.4.1 Requirement of HPV genome expression for cell growth

As previously described for HPV 16 and HPV 18, E6 and E7 need to be continually expressed in cervical-cancer-derived cell lines to maintain cell proliferation ([von Knebel Doeberitz et al., 1988, 1992](#); [Butz et al., 2003](#); [Yoshinouchi et al., 2003](#)). Without either protein, the cells cease to proliferate and, depending on the experimental conditions, will either enter apoptosis or senesce.

#### 4.4.2 Integration of the HPV genome

The integration of the viral genome into the genome of the host cell is not a pre-requisite for the development of malignancy. Although integrated HPV genomes are still the most prevalent form of the viral DNA in tumours, it is apparent that cancers can arise, in a significant number of cases, before genome integration. It is worth noting that there are differences depending upon the genotype involved, with HPV 16, 18 and 45

being more frequently found in an integrated state than HPV 31 and 33 ([Vinokurova et al., 2008](#)). Integration has not been found to be a common characteristic of beta HPV types.

#### *4.4.3 Alteration of specific cellular proto-oncogenes*

The etiological association of high-risk HPVs with anogenital cancers and a subset of head and neck cancers is well established. It is also clear, however, that other genetic or epigenetic changes are required for these HPV-associated cancers to develop. This conclusion is based upon the long latency period between infection and the onset of cancer, and the fact that only a small percentage of infected individuals will develop cancer. Several studies have looked for alterations in cellular proto-oncogenes, particularly the *MYC* and *RAS* gene families, as possible cofactors for the development of cervical cancer, but the results are inconsistent. Some investigators have reported that *MYC* is amplified at the gene level or increased in its expression in cervical cancers and sometimes in CIN lesions, but others have not observed this. Likewise, some studies have reported upregulation of, and mutation in, genes of the *RAS* family, while others have not. At this stage, the overall conclusion is that the activation of proto-oncogenes can occur in cervical cancers and precursor lesions, but none of those reported so far is an absolute pre-requisite for the development of the cancer, nor is there evidence that any of these alterations have prognostic value (for reviews, see [Whang & Lee, 1997](#); [Spandidos et al., 2000](#)).

#### *4.4.4 Effect on the host immune response*

Evasion of the host immune system by the virus is a likely key event in HPV-mediated carcinogenesis. Persistence of the viral infection is a necessary condition for the development of cancer. Innate immunity is considered as a first

line of cellular defence against pathogens. HPVs activate Toll-Like Receptors (TLRs) in antigen-presenting cells ([Fausch et al., 2003](#); [Yang et al., 2004](#); [Yan et al., 2005](#)). The high-risk HPV oncogenes interfere with the innate immune responses at multiple levels. Both high-risk HPV E6 and E7 can interfere with factors induced by innate immune responses including those mediated by interferon ([Ronco et al., 1998](#); [Li et al., 1999](#); [Park et al., 2000](#); [Perea et al., 2000](#); [Nees et al., 2001](#)). HPV 16 E6 and E7 together can cause a reduction in the expression of TLR9 ([Hasan et al., 2007](#)).

Effects of HPV genes on acquired immune responses have also been noted. The E6 and E7 proteins of high-risk HPV can inactivate components of the biosynthetic pathway of the major histocompatibility class I (MHC I) complex such as class I heavy chain, transporter associated with antigen processing subunit 1 (TAP1), and low molecular weight protein 2 (LMP2) ([Georgopoulos et al., 2000](#)). HPV 16 E5 down-regulates surface MHC class I ([Ashrafi et al., 2005, 2006](#)) as well as class II ([Zhang et al., 2003](#)) expression. There is also evidence that beta HPV types, specifically HPV 38, can inhibit innate immune responses ([Cordano et al., 2008](#)). These findings may have important implications in predicting the overall efficacy of immunotherapeutic strategies for treating HPV infections.

### **4.5 Transgenic models for HPV-associated cancers**

The advent of the technology to generate germline-transgenic mice has provided investigators with the ability to assess the *in vivo* properties of HPV oncogenes implicated in human cancers. This section summarizes advances made in the assessment of the role of HPV oncogenes in anogenital and head and neck cancers in the case of high-risk anogenital HPVs, and skin cancers in the case of beta HPVs (i.e. EV-associated HPVs).

#### 4.5.1 Cancer of the cervix

Studies on the role of HPV oncogenes in anogenital cancers were greatly facilitated by the use of the human keratin 14 (K14) transcriptional promoter, which directs the expression of HPV genes to the stratified epithelium of the lower female reproductive tract and of the oral cavity. The first germline-transgenic mouse model for HPV-associated cervical cancer was developed by the use of a K14HPV 16 transgenic mouse line in which the early genes of HPV 16 were placed under the control of the K14 promoter ([Arbeit et al., 1994](#)). Although these mice did not develop cervical cancers spontaneously, treatment with exogenous estrogen, sufficient to induce continuous estrus, led to a highly penetrant cervical cancer phenotype ([Arbeit et al., 1996](#)). The cervical cancers occurred in the context of a progressive disease much like that seen in women, being preceded by the onset of CIN grades 1–3. As in women, the cancers preferentially arose in the transformation zone ([Arbeit et al., 1996](#)). The individual contributions of the two HPV 16 oncogenes E6 and E7 have been assessed ([Riley et al., 2003](#)). From these studies, it has become clear that HPV 16 E7 is the dominant oncogene in the context of cervical carcinogenesis in the mouse. Whereas 100% of K14E7 mice treated with exogenous estrogen for 6 months developed high-grade dysplasia and/or cervical cancers, K14E6 mice only developed cervical cancer when estrogen treatment was extended to 9 months ([Shai et al., 2007](#)). Synergy between E6 and E7 was also observed ([Riley et al., 2003; Shai et al., 2007, 2008](#)).

The mechanism of action of E6 and E7 in cervical cancer has also been studied in the context of transgenic mouse models. Although it has long been thought that the capacity of E7 to inactivate the tumour-suppressor pRB is critical in the context of HPV-associated cancers, studies in mice indicate that this inactivation is not

sufficient to account for the oncogenic properties of the E7 protein ([Balsitis et al., 2003](#)).

Similarly to HPV 16 E7, HPV 16 E6 appears to contribute to cervical carcinogenesis through multiple activities. A mouse strain expressing a mutant form of HPV 16 E6 that is unable to inactivate p53, displayed a reduced tumorigenic phenotype in the cervix when treated with estrogen, compared with mice expressing the wild-type HPV 16 E6; however, this mutant E6-expressing strain also displayed an enhanced susceptibility to cervical carcinogenesis compared with estrogen-treated non-transgenic mice ([Shai et al., 2007](#)). These results are consistent with the hypothesis that the inactivation of p53 partially contributes to the oncogenic potential of the E6 protein. Consistent with this observation, mice conditionally null for p53 displayed an increased susceptibility to cervical cancers when treated with estrogen compared with treated p53-proficient mice ([Shai et al., 2008](#)).

E6 is also known to bind to several cellular proteins that contain PDZ domains (see above). Transgenic mice expressing a mutant form of HPV 16 E6 unable to bind to PDZ-domain proteins ([Nguyen et al., 2003](#)) had a reduced susceptibility to cervical cancers compared with mice expressing the wild-type E6 protein ([Shai et al., 2007](#)). It remains unclear which of the interactions with PDZ-domain proteins contribute to E6-mediated carcinogenesis *in vivo*, and how E7 contributes to this carcinogenic process ([Simonson et al., 2005; Shai et al., 2007](#)).

The mechanism by which E5 may contribute to cervical carcinogenesis *in vivo* has yet to be determined. In mouse skin, E5 can induce epithelial hyperplasia, and this is dependent upon the presence of a functional epidermal growth-factor receptor (EGFR) ([Genther Williams et al., 2005](#)). E5 has been implicated in two steps in skin carcinogenesis, promotion and progression ([Maufort et al., 2007](#)). It remains to be seen whether the activation of EGFR contributes to

the oncogenic potential of E5 *in vivo*, in the skin or the cervix.

The importance of the continued expression of E6 and E7 for maintenance of the tumour phenotype *in vitro* is well documented (see above). This has now been evaluated in HPV-transgenic mice through the use of a tet-regulated HPV 16 E7 transgene. In these mice, the continued expression of E7 was found to be critical for the maintenance not only of cervical cancers but also the dysplastic neoplasia that is recognized as the precursor lesion to cervical cancer ([Jabbar et al., 2009](#)). These data support the development of therapies aimed at inhibiting the expression or function of HPV oncogenes implicated in cervical cancers.

Estrogen is an important cofactor in the development of cervical cancers in HPV-transgenic mouse models ([Arbeit et al., 1996](#)). Estrogen was found to be necessary not only for the development of cervical cancers in HPV-transgenic mice, but also for their persistence ([Brake & Lambert, 2005](#)). Estrogen receptor α (ERα) was found to be necessary for cervical carcinogenesis in K14E7-transgenic mice ([Chung et al., 2008](#)). Indirect evidence potentially supporting a role of estrogen in human cervical cancers has come from studies demonstrating that women who have used oral contraceptives for at least 5 years or have had multiple pregnancies are at increased risk for cervical cancer, although there are also conflicting reports ([Beral et al., 1999](#); [Hannaford et al., 2007](#); [IARC, 2011](#)). Furthermore, a subset of human cervical cancers has been found to overexpress aromatase, a key enzyme in estrogen biosynthesis, suggesting a role of estrogen in these cancers ([Nair et al., 2005](#)). However, women on estrogen replacement therapy have not been found to be at increased risk for cervical cancer ([Archer, 2004](#)). [The Working Group noted that this study did not control for HPV status.] The fact that tamoxifen treatment did not reduce cervical disease in women ([Bigler et al. 2004](#)) could be explained by the fact that tamoxifen acts as an

ER-agonist, not antagonist, in the human cervix ([Senkus-Konefka et al., 2004](#)). It remains to be determined whether anti-estrogens (specifically, drugs that act as ER-antagonists in the human cervix, e.g., fulvestrant) and/or aromatase inhibitors will be effective in treating women with HPV-associated cervical disease.

#### **4.5.2 High-risk HPVs and cancers of the head and neck**

There is a growing appreciation that the same high-risk HPV types etiologically associated with anogenital cancers, particularly HPV 16, are also associated with a subset of human head and neck squamous cell carcinomas (HNSCC), most notably of the oropharynx (e.g., tonsils) and the base of the tongue. The role of HPV 16 E6 and E7 oncogenes in HNSCC has been evaluated in HPV-transgenic mice that express these viral oncogenes in the relevant tissues. These mice do not spontaneously develop HNSCC, but when treated with the synthetic carcinogen 4-nitroquinoline-N-oxide (4-NQO), they become more susceptible to head and neck cancers ([Strati et al., 2006](#)). The progressive disease observed in the 4-NQO-treated HPV-transgenic mice was similar to that seen in humans. Also, the cancers that occurred were primarily high-grade HNSCC as observed in HPV-positive HNSCC in humans. As in cervical cancer, E7 proved to be the more potent oncogene and the inactivation of pRb could not fully account for the role of the E7 protein in HNSCC ([Strati & Lambert, 2007](#)).

#### **4.5.3 Carcinogenic potential of EV-associated beta HPVs in the skin**

HPVs of the genus beta are associated with a rare, familial benign disease termed *epidermodysplasia verruciformis* (EV). EV patients are at an increased risk for squamous cell carcinomas of their skin at sites exposed to the sun. Transgenic mouse models have been developed to investigate

the role of EV-associated HPVs (specifically HPV 8 and HPV 38) in skin carcinogenesis. K14HPV-8-transgenic mice expressing the early genes of HPV 8 in the epidermis were found to be susceptible to the spontaneous development of both benign and malignant skin cancers ([Schaper et al., 2005](#)). Likewise, K10-HPV38 E6/E7 transgenic mice were highly susceptible to multistage skin carcinogenesis, specifically when treated with ultraviolet light (UVB) or chemical carcinogens ([Dong et al., 2005](#)). Interestingly, these mice express high levels of ΔNp73, which appears to be a primary mechanism for these mice to overcome the growth-suppressive activities of p53 ([Accardi et al., 2006](#); [Dong et al., 2008](#)).

The synergy between cutaneous HPVs and UV in the development of squamous cell carcinomas in the skin has also been studied in transgenic mice expressing in their epidermis the *E6* and *E7* genes of HPV 20, which is commonly associated with squamous cell carcinoma arising in renal transplant patients, or HPV 27, which is only associated with benign papillomas. Upon UV irradiation, both HPV-20- and HPV-27-transgenic mice are more susceptible to tumours than non-transgenic mice, however, the HPV-20-transgenic mice displayed an increased incidence of malignant tumours. Alterations in the expression of both p53 and p63 were noted in the UV-exposed transgenic mice ([Michel et al., 2006](#)).

## 4.6 Synthesis

The characterization of the mechanisms of action of the HPV oncoproteins in assays both *in vitro* and *in vivo* provides compelling evidence for a direct role of high-risk mucosotropic HPVs in the development of cervical cancer. The mechanisms involve immortalization, transformation, inhibition of apoptosis, induction of genomic instability, and deregulation of the immune response. The mechanistic evidence for

individual mucosotropic HPV types found in cancers is as follows:

- A common feature of mucosotropic HPV-associated cancers is the expression of the viral genes *E6* and *E7*.
- The *E6* and *E7* genes of HPV 16 and HPV 18 have been the most extensively studied and were found to confer a similar set of biological phenotypes (e.g., immortalization, inhibition of DNA-damage response, genomic instability, inhibition of differentiation) on epithelial cells from multiple human tissues in which HPV-associated cancers are found, including the cervix, penis, and tonsil.
- The *E6* and *E7* proteins of the same HPVs (16 and 18) share similar sets of biochemical properties (e.g., for *E6*: inactivation of p53, induction of hTERT, binding to PDZ; for *E7*: inactivation of pRb and related pocket proteins, activation of E2Fs) that correlate with their transforming, immortalizing and tumorigenic properties both *in vitro* and *in vivo*.
- Suppression of HPV 16/18 *E6* and *E7* gene expression in cell lines derived from human cervical cancers leads to senescence or apoptosis.

Given these observations, there is strong mechanistic evidence that HPV 16 and HPV 18 act directly to cause cancers in those tissues in which they are found.

For other mucosotropic HPVs (31, 33) that are found in human cancers, there is moderate mechanistic evidence from available experimental data (biochemical and/or biological) that they are directly acting in causing cancers in those tissues in which they are found.

For a third set of mucosotropic HPVs (35, 39, 45, 51, 52, 53, 56, 58, 66, 68, 82) found in human cancers, there is positive mechanistic evidence from one or a few studies (biochemical and/or biological) that they directly act to cause cancers in those tissues in which they are found.

For HPV 6 and 11, there is little to no mechanistic evidence that they can contribute to carcinogenesis (weak inactivation of p53, no activation of hTERT, no binding to PDZ, weak inactivation of pRB, no immortalization, no transformation).

With respect to most of the cutaneous HPV types of the genus beta, there is a general paucity of experimental studies assessing their role in cancer development. For a subset, HPV 8, and 38, which have been analysed in both *in vitro* and *in vivo* models, there is strong evidence of their potential capacity to cause cancer. However, unlike what has been found for mucosotropic HPVs associated with human cancers, the limited data available indicate that the majority of, if not all, cells within skin cancers potentially associated with beta HPV types do not contain the viral genome. Thus, novel mechanisms for HPV-induced carcinogenesis would need to be invoked, for which there is currently little evidence.

## 5. Evaluation

There is *sufficient evidence* in humans for the carcinogenicity of HPV 16. HPV 16 causes cancer of the cervix, vulva, vagina, penis, anus, oral cavity, oropharynx, and tonsil. Also, a positive association has been observed between infection with HPV 16 and cancer of the larynx.

There is *sufficient evidence* in humans for the carcinogenicity of HPV 18. HPV 18 causes cancer of the cervix. Also, a positive association has been observed between infection with HPV 18 and cancer of the vulva, penis, anus, oral cavity, and larynx.

There is *sufficient evidence* in humans for the carcinogenicity of HPV types 31, 33, 35, 39, 45, 51, 52, 56, 58 and 59. HPV types 31, 33, 35, 39, 45, 51, 52, 56, 58 and 59 cause cancer of the cervix. Also, a positive association has been observed

between infection with HPV 33 and cancer of the vulva and of the anus.

There is *limited evidence* in humans for the carcinogenicity of HPV types 26, 53, 66, 67, 68, 70, 73, and 82. A positive association has been observed between infection with HPV types 26, 53, 66, 67, 68, 70, 73, and 82 and cancer of the cervix.

There is *inadequate evidence* in humans for the carcinogenicity of HPV types 30, 34, 69, 85 and 97. Nonetheless, their phylogenetic analogy to HPV types with sufficient or limited evidence in humans suggests that these types may be potentially carcinogenic.

There is *inadequate evidence* in humans for the carcinogenicity of HPV 6 and HPV 11 in the larynx.

There is *inadequate evidence* in humans for the carcinogenicity of HPV genera beta and gamma types in the skin. In the rare case of patients with *epidermodysplasia verruciformis*, there is *limited evidence* for the carcinogenicity of HPV genus-beta types 5 and 8 in the skin.

HPV types 16, 18, 31, 33, 35, 39, 45, 51, 52, 56, 58 and 59 are *carcinogenic to humans* (Group 1).

HPV 68 is *probably carcinogenic to humans* (Group 2A).

HPV types 26, 53, 66, 67, 70, 73, 82 are *possibly carcinogenic to humans* (Group 2B).

HPV types 30, 34, 69, 85 and 97 are *possibly carcinogenic to humans* (Group 2B) based on their phylogenetic analogy to HPV types with sufficient or limited evidence in humans.

HPV types 6 and 11 are *not classifiable as to their carcinogenicity to humans* (Group 3).

Some types of HPV genera beta and gamma are *not classifiable as to their carcinogenicity to humans* (Group 3), with the notable exception that HPV 5 and HPV 8 are *possibly carcinogenic to patients with epidermodysplasia verruciformis* (Group 2B).

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# HUMAN T-CELL LYMPHOTROPIC VIRUS TYPE 1

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Human T-cell lymphotropic virus Type 1 was considered by a previous IARC Working Group in 1996 ([IARC, 1996](#)). Since that time, new data have become available, these have been incorporated in the *Monograph*, and taken into consideration in the present evaluation.

## 1. Exposure Data

### 1.1 Taxonomy, structure, and biology

#### 1.1.1 Taxonomy

Retroviruses can be classified according to the morphology of their virion core or according to sequence homologies that become evident after phylogenetic analyses. Human T-lymphotropic virus type 1 (HTLV-1) is a member of the delta-type retrovirus group, other members of which include HTLV types 2, 3 and 4, bovine leukaemia virus (BLV), and simian T-cell leukaemia virus (STLV) types 1, 2 and 3 ([Matsuoka & Jeang, 2007](#)). STLV-1 is found in Old World monkeys and great apes. HTLV-1 and STLV-1 are thought to originate from common ancestors ([Vandamme et al., 1998](#)). Together with the STLVs, HTLVs form the primate T-cell lymphotropic viruses (PTLV) group. The PTLVs belong to the complex retrovirus family since, in addition to the structural *gag*, *pol* and *env* genes, their genome also contains regulatory and accessory genes. Among these retroviruses, HTLV-1 and STLV-1 induce T-cell neoplasms ([Tsujimoto et al., 1987](#); [Gallo, 2002](#)), BLV causes a B-cell neoplastic disease in cattle

and in sheep, and HTLV-3 and HTLV-4 have not been clearly associated with any haematological disease ([Mahieux & Gessain, 2009](#)). A recent report demonstrated that HTLV-2 infection is linked with higher lymphocyte and platelet counts, although it has not been yet associated with oncogenesis ([Bartman et al., 2008](#)).

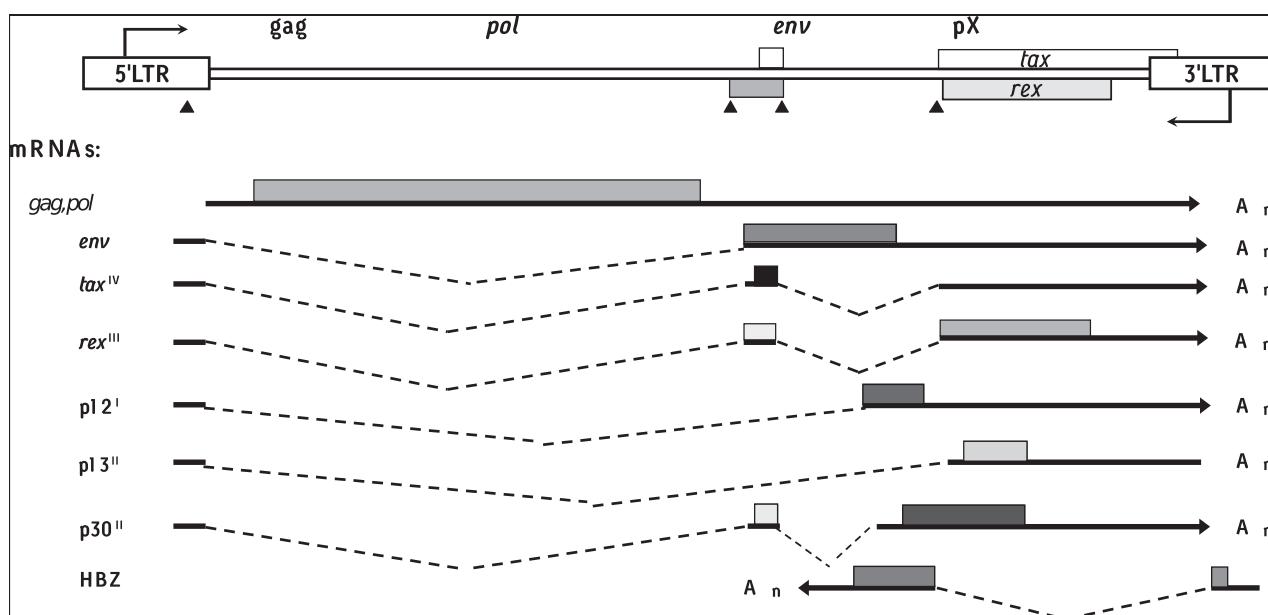
#### 1.1.2 Structure of the virion

The structure of retroviruses is reviewed in the *Monograph* on HIV-1 in this volume. HTLVs are enveloped viruses with a diameter of approximately 80–100 nm. The HTLV virions contain two covalently bound genomic RNA strands, which are complexed with the viral enzymes reverse transcriptase (RT; with associated RNase H activity), integrase and protease, and the capsid proteins. The outer part of the virions consists of a membrane-associated matrix protein and a lipid layer intersected by the envelope proteins ([IARC, 1996](#)).

#### 1.1.3 Structure of the viral genome

As stated above, HTLV-1 is a complex retrovirus that contains regulatory genes (*tax* and *rex*) and accessory genes (*p12*, *p13*, *p30* and *HBZ*), in

**Fig. 1.1 Scheme of the HTLV-1 genome: alternatively spliced mRNAs and putative proteins encoded by each mRNA are shown**



Prepared by the Working Group

addition to structural genes (*gag*, *pol* and *env*) (see Fig. 1.1). The Gag precursor protein (53 kD) is translated from unspliced genomic RNA. This protein is cleaved into p19 (matrix), p24 (capsid), and p15 (nucleocapsid) by the viral protease. The protease–polymerase products are generated by two frameshifts, which produce protease, reverse transcriptase, and integrase. The Env precursor protein is translated from a single-spliced mRNA, and is cleaved by a cellular protease into the extracellular protein, gp46, and the transmembrane protein, gp21 ([Seiki et al., 1983](#); [Sakalian & Hunter, 1998](#); [Matsuoka & Jeang, 2007](#); [Verdonck et al., 2007](#)).

#### 1.1.4 Host range

HTLV-1 naturally infects humans. However, several publications have clearly demonstrated that HTLV-1 can experimentally be inoculated to

different animals, including rabbits, rats, mice, and New World monkeys ([Lairmore et al., 2005](#)).

#### 1.1.5 Target cells

HTLV-1 can infect different cell types (T cells, B cells, dendritic cells, fibroblasts, etc.) in tissue culture. However, it can transform only T cells both *in vitro* and *in vivo*. HTLV-1 induces the clonal proliferation of T lymphocytes, mainly CD4-positive T cells, and to a lesser extent, CD8-positive T cells ([Etoh et al., 1997](#); [Cavrois et al., 1998](#); [Yasunaga et al., 2001](#)). Proliferation is thought to be mediated by one or several viral genes, such as *tax*, *rex*, *p12*, *p13*, *p30*, or *HBZ*.

HTLV-1 infection of dendritic cells has been recently shown to play a major role in HTLV-1 cell-to-cell transmission ([Jones et al., 2008](#)). In experimentally infected squirrel monkeys (*S. Sciureus*), HTLV-1 was mainly detected in the lymphoid organs, which were therefore suggested

to be a major reservoir of the virus ([Kazanji et al., 2000](#)).

### 1.1.6 Life cycle, replication, and regulation of gene expression

The glucose transporter 1 (GLUT1), neuropilin 1, and heparan sulfate proteoglycan form the HTLV-1 receptor complex ([Manel et al., 2003; Ghez et al., 2006](#)). These proteins are ubiquitously expressed in cultured cells, therefore allowing HTLV-1 to infect a variety of cell types *in vitro*.

The life cycle of HTLV-1 is similar to that of other retroviruses. A characteristic of HTLV-1 is that it is mainly spread through cell-to-cell contact, although the exact mechanism is still a matter of debate ([Igakura et al., 2003](#)). After reverse transcription and integration into the genome, HTLV-1 propagates through clonal expansion of infected cells ([Etoh et al., 1997; Cavrois et al., 1998](#)). The limited use of the viral reverse transcriptase explains the remarkable genetic stability of HTLV-1. This is why the administration of reverse transcriptase inhibitors *in vivo* does not influence provirus load ([Miyazato et al., 2006; Taylor et al., 2006](#)). Consequently, the HTLV-1 provirus sequence variability is very low ([Gessain et al., 1992; Van Dooren et al., 2004](#)). This striking genetic stability is used as a molecular tool to follow the migration of infected populations in the recent or distant past to gain new insights into the origin, evolution, and modes of transmission of such retroviruses and their hosts. The few nucleotide substitutions observed among virus strains are indeed specific to the geographic origin of the patients rather than being linked to the pathology.

Three modes of transmission are known for HTLV-1, and for each of these routes, cell-to-cell contact is required. The transmission is discussed in Section 1.2.

#### (a) Regulation of gene expression

Viral gene expression initiates from the 5'Long Terminal Repeat (LTR), and is highly dependent on the Tax protein. The details on the regulation of the viral LTR are described in Section 4. However, Tax is a major target of cytotoxic T cells *in vivo* ([Koenig et al., 1993](#)), and Tax-expressing cells are, therefore, rapidly eliminated by cytotoxic T cells ([Hanon et al., 2000; Asquith et al., 2007; Asquith & Bangham, 2008](#)). Despite a strong cytotoxic T-cell response, proliferation of HTLV-1-infected cells *in vivo* is likely to depend on viral gene expression ([Asquith et al., 2007](#)). Epigenetic changes to the 5'LTR may control viral gene expression *in vivo*, enabling escape from cytotoxic T cells. A recent report demonstrated that the administration of valproate, a histone deacetylase inhibitor, to tropical spastic paraparesis/HTLV-1-associated myelopathy (TSP/HAM) patients decreased the provirus load *in vivo* without, however, improving the clinical symptoms ([Lezin et al., 2007](#)). The mechanism by which the infected cells are eliminated is unknown, but these results indicate that increasing viral expression may represent a potential approach to decreasing the proviral load.

## 1.2 Epidemiology of infection

In 1977, Takatsuki *et al.* described regions in Japan with high frequencies of T-cell-associated lymphoproliferative disorders and proposed that these diseases shared a viral etiology ([Takatsuki et al., 1977](#)). This led to the discovery of HTLV-1 just a few years later, and it became the first human retrovirus to be implicated as a causative agent for human malignancy ([Poiesz et al., 1980; Hinuma et al., 1981](#)). Its discovery paved the way to a greater understanding of retroviruses, notably HIV, and their effects on humans. It is now apparent that HTLVs have been infecting humans for thousands of years. An estimated

15–20 million persons worldwide are infected with HTLV-1 ([Gessain & de Thé, 1996](#)) [The Working Group noted that the accuracy of this estimate is unknown], and a vaccine is not yet available.

All HTLV types have simian counterparts, and the viral strains found are predominantly related to geography rather than pathology ([Slattery et al., 1999](#)). These are all believed to have originated from Africa, the only continent where all PTLVs have been found. From there, PTLV migrated to Asia, where it evolved into STLV-1. This Asian STLV-1 virus type diffused through India, Japan, and Indonesia before returning to Africa, where phylogenetic analyses and anthropological studies place PTLV-1 spread among non-human primates at approximately 27300 years ago (95% confidence interval [CI]: 19100–35500) ([Van Dooren et al., 2001](#)).

Interspersed patterns of STLV-1 and HTLV-1 strains suggest frequent interspecies transmissions between humans and primates in Africa. Evidence of these frequent crossings are distinguished by the four major geographic subtypes: cosmopolitan HTLV-1 subtype A, Central African subtype B, Melanesian subtype C, and subtype D, also found in Central Africa ([Cassar et al., 2007](#)). The slave trade and an increase in human immigration and mobility facilitated the expansion of HTLV-1 into the New World, Japan, the Middle East, and North Africa ([Verdonck et al., 2007](#)). The majority of infected individuals from these regions are infected with cosmopolitan subtype A (HTLV-1A) ([Proietti et al., 2005](#)).

### 1.2.1 Prevalence, geographic distribution

Even though the global geographic distribution of HTLV-1 has been well defined in the literature, fine-scale variations in HTLV-1 prevalence are less well understood. HTLV-1 is often found in micro-epidemic regions surrounded by regions with low prevalence (Fig. 1.2). For example, regions of Kyushu and Okinawa, Japan,

have rates as high as 20%; whereas, neighbouring the People's Republic of China and the Republic of Korea have rates of less than 0.1% ([Proietti et al., 2005](#)). In general, regions of high endemicity include south-western Japan, parts of sub-Saharan Africa, the Caribbean Islands, and South America ([IARC, 1996](#)). Infection has also been detected in Melanesia, the Solomon Islands, and among Australian Aborigines; there is only low prevalence in Europe and North America.

There is a characteristic age- and gender-dependence of HTLV-1 seroprevalence in many populations. HTLV-1 prevalence increases with age and is higher in women ([Beilke & Murphy, 2006](#)). Published studies from several countries including Jamaica, Japan, Brazil, and the United States of America ([Beilke & Murphy, 2006](#)) have demonstrated similar trends, in addition to a significant increase of seropositivity in low socio-economic strata, and in those with a history of blood transfusions. HTLV-1 is most prevalent in populations that have a low geographic mobility, and correspondingly higher rates of vertical and sexual transmission.

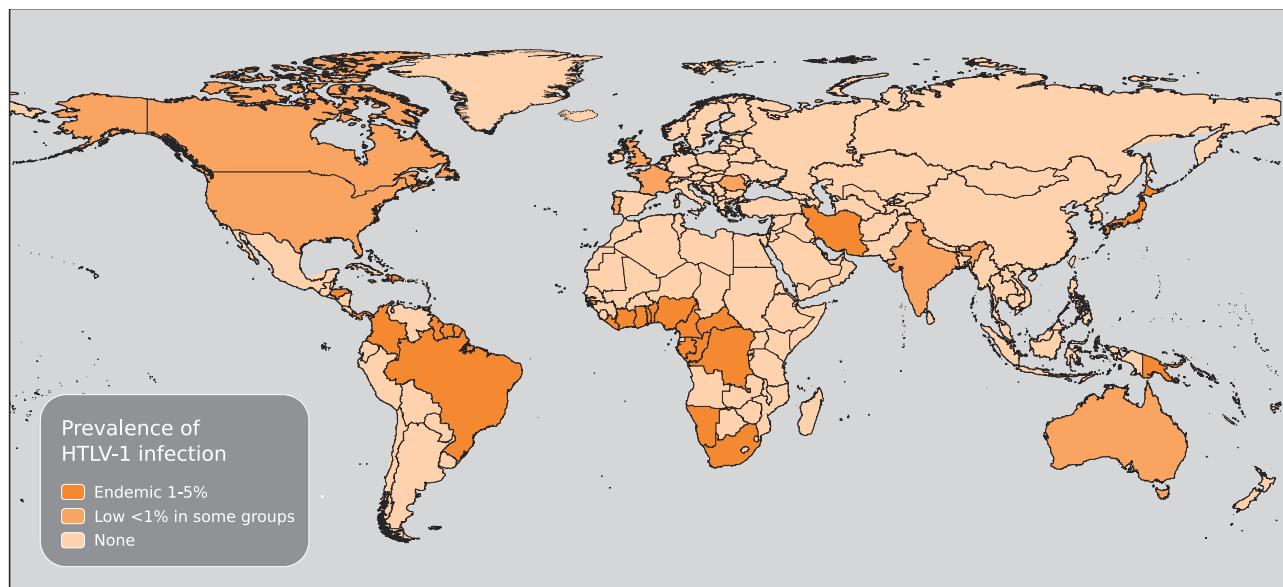
### 1.2.2 Transmission, and risk factors for infection

There are three main modes of transmission in HTLV-1 infection: vertical transmission, sexual transmission, and parenteral transmission. Each has its respective risk factors: prolonged breastfeeding; unprotected sex with an infected partner, multiple lifetime sexual partners, infection with sexually transmitted diseases (STDs); blood transfusion before institution of HTLV-antibody screening of donors, and injection drug use.

#### (a) Vertical transmission

The highest rates of HTLV-1 transmission are due to breastfeeding, and in southern Japan, the overall infection rate of breastfed children by HTLV-1-carrier mothers is estimated at 10–30%

**Fig. 1.2 Global geographic distribution of HTLV-1 infection:** It should be noted that HTLV-1 endemic areas do not correspond exactly to the country boundaries shown in the map, for example, Brazil, Japan and Iran, where HTLV-1 is limited to residents of certain areas of each country



Adapted from and reprinted by permission from Macmillan Publishers Ltd: Oncogene, [Proietti et al. \(2005\)](#). <http://www.nature.com/onc/index.html>

([Tajima & Hinuma, 1992](#)). There may be a higher rate of transmission of mother-to-female compared to mother-to-male infants for HTLV in French Guyana ([Ureta-Vidal et al., 1999](#)). In Japan, there does not appear to be gender differences in HTLV-1 before the age of 20 years ([Hino, 1990](#)). In a longitudinal study of Japanese children born to carrier mothers, those who did not seroconvert by the age of 3 years remained seronegative until the age of 18 years ([Kusuhara et al., 1987](#)). In-utero infectivity is much lower, probably because of limited trafficking of HTLV-1-infected lymphocytes across the placenta. The risk of infection in children has been shown to correspond to the proviral load in the mother's breast milk, and to the duration of breastfeeding ([Wiktor et al., 1997](#)). In Japan, the avoidance of breastfeeding by an HTLV-1-infected mother reduced the transmission from 20% to 3%, and efforts to eliminate breastfeeding or to at least reduce its duration to less than 12 months by

seropositive mothers, significantly reduced HTLV-1 transmission to children. In a subset of children with HTLV-1 positive cord blood, none had seroconverted ([Hino et al., 1996](#)).

#### (b) Sexual transmission

The second main type of transmission is sexual. One prospective study of heterosexual discordant couples (one partner seropositive, one partner seronegative) suggested higher male- to-female transmission ([Stuver et al., 1993](#)), but another did not ([Roucoux et al., 2005](#)). In Latin America, gender differences in sexual practices and the seroprevalence of STDs between populations might partially explain these discrepancies ([Plancoulaine et al., 1998](#); [Sanchez-Palacios et al., 2003](#)). HTLV-1 carriers infect their spouses at low rates (1–2 per 100 person-years); however, within long-term sexual relationships, HTLV-1 proviral load and lower rates of condom use were shown

to increase transmission efficiency ([Kaplan et al., 1996](#); [Iga et al., 2002](#); [Roucoux et al., 2005](#)).

### (c) Parenteral transmission

HTLV-1 transmission is also known to occur by the transfusion of cellular blood components, requiring testing of blood products by blood banks in high prevalence regions. The “residual risk” of transfusion-transmitted HTLV-1 infection after serological testing has been estimated as 1 in 641000 blood units transfused in the USA (95%CI: 256000–2000000) ([Schreiber et al., 1996](#)). Transmission through this route has been reduced by improvements in the sensitivity of serological assays for HTLV-1 and leukoreduction of blood products, so the current residual risk is probably less than 1 per million blood units transfused. Although nucleic-acid testing of blood products has been introduced for HIV, hepatitis C and hepatitis B viruses (HCV, HBV), it has not yet been developed for HTLV-1, because HTLV-1 would require a cell-based rather than a plasma-based assay ([Murphy et al., 1999](#)). HTLV-1 is also transmitted via needle-sharing associated with injection drug use. However, compared to HTLV-2, the prevalence of HTLV-1 is relatively low among injection drug users and their sexual partners in the USA, Italy, Spain, Brazil, and Argentina ([Gotuzzo, 2000](#)), perhaps because injection drug use is less common among HTLV-1- as opposed to HTLV-2-risk groups ([Roucoux & Murphy, 2004](#)). However, increasing human mobility and cultural interaction create the opportunity for increased HTLV-1 transmission by this route.

#### 1.2.3 Persistence, latency and natural history of infection

The lifetime risk of developing adult T-cell leukaemia/lymphoma (ATLL) has been estimated at 2–4% among HTLV-1 carriers, and the latency period from primary infection until ATLL onset is about 60 years in Japan, and 40 years

in Jamaica ([Tajima, 1988](#); [Murphy et al., 1989](#); [Takatsuki et al., 1994](#); [Hanchard, 1996](#); [Yasunaga & Matsuoka, 2007](#)). The incubation period for HTLV-associated myelopathy is thought to be shorter: 10–20 years after sexual transmission but as little as 6 months after transfusion-transmitted HTLV-1 infection ([Gout et al., 1990](#)). Several viral and immunological markers have been proposed as markers for predicting which infected subjects will progress from latency to disease (see Section 2.1), but prospective validation of these markers is lacking. HTLV-1 may also be associated with increased overall mortality ([Arisawa et al., 2003](#); [Orland et al., 2004](#)).

Compared to HIV, the HTLV-1’s genome is very stable, with proviral integration predominating over production of viral RNA particles ([Mortreux et al., 2003](#)). Occasionally, abnormal, multilobulated lymphocytes “flower cells” can be observed in the peripheral blood ([Hisada et al., 1998](#); [Sacher et al., 1999](#)). Aside from detection in the peripheral blood, infected cells have also been detected in the cerebrospinal fluid, an indication of HTLV-1 ability to cross the blood–brain barrier ([Mortreux et al., 2003](#)). In an assessment of the patterns of HTLV-1 proviral DNA and antibody titre levels among transfusion recipients, in early infection, proviral loads are initially elevated with corresponding low antibody titres, and as proviral load begins to decrease, antibody titres increase, and later remain stable within each of the cases ([Manns et al., 1999](#)). Proviral load may also be related to the route of infection, with transfusion-transmitted HTLV associated with a higher proviral load ([Murphy et al., 2004](#)).

Platelet and lymphocyte counts may be chronically elevated in HTLV-1 ([Glynn et al., 2000](#)). Both higher platelet counts and lower eosinophils counts were found to be significantly associated with HTLV-1 status among blood donors from the USA ([Bartman et al., 2008](#)). HTLV-1 participants also had a small increase in absolute lymphocyte count compared with controls, which was not statistically significant.

HTLV-1 preferentially targets CD4-positive T cells, and infection is transmitted through direct cell-to-cell contact. Recent reports also support a role for dendritic cells in HTLV-1 transmission ([Jones et al., 2008](#)). Once inside the cell, the HTLV-1 provirus integrates itself into the host genome. A study demonstrated a significant rate of viral integration within genes (as opposed to non-coding regions of the genome), which suggests an HTLV-1 preference to insert in growth-related genes over random integration ([Hanai et al., 2004](#)). Because of clonal expansion, T cells with identical integration sites are considered to have originated from the same infected cell. More sensitive assays with the ability to amplify regions of the host genome adjacent to the integration site, such as inverse polymerase chain reaction (PCR), may be used to identify clones of infected T lymphocytes in asymptomatic carriers ([Okayama et al., 2004](#); [Tanaka et al., 2005](#)).

In contrast to HIV, which produces a large amount of cell-free virions in plasma, HTLV-1 increases its copy number through the proliferation of infected cells, and infection is maintained through this expansion. Early expression of viral tax protein and HTLV-1 accessory proteins induce and maintain initial replication ([Manns et al., 1999](#)). An increased proviral load derives from this persistent clonal expansion of virus-infected cells ([Wattel et al., 1995](#)). After initial infection, individuals are asymptomatic with proviral loads ranging from  $10^2$ – $10^5$  per million peripheral blood mononuclear cells ([Wattel et al., 1992](#); [Etoh et al., 1999](#); [Mortreux et al., 2003](#)). The proviral load remains relatively stable within a single infected individual over several years ([Mortreux et al., 2003](#); [Kwaan et al., 2006](#)).

## 2. Cancer in Humans

### 2.1 T-cell malignancies

#### 2.1.1 HTLV-1 infection and ATLL

As described in the previous *IARC Monograph* ([IARC, 1996](#)), ATLL occurs almost exclusively in areas where HTLV-1 infection is endemic, such as Japan, the Caribbean, and West Africa. In other areas, cases are usually found among immigrants from endemic populations. Evidence of HTLV-1 infection was initially found in at least 90% of ATLL cases and subsequently, HTLV-1 infection became part of the diagnostic criteria for ATLL. In ATLL, the virus is monoclonally integrated into the tumour cells. The previous IARC Working Group concluded that HTLV-1 infection was a necessary cause of ATLL ([IARC, 1996](#)). The current *Monograph* will focus primarily on recent evidence on predictors or risk of ATLL in HTLV-1 carriers. To date, no other malignancies have been convincingly linked to HTLV-1.

Since 1996, several case series have been published on ATLL occurring in diverse HTLV-1-endemic and –non-endemic populations. These include reports from Japan ([Tsukasaki et al., 1999](#)); Brazil ([Farias de Carvalho et al., 1997](#); [Barbosa et al., 1999](#); [Pombo De Oliveira et al., 1999](#)); Argentina ([Marin et al., 2002](#)); Chile ([Cabrera et al., 2003](#)); the Commonwealth of Dominica ([Adedayo & Shehu, 2004](#)); and Hong Kong Special Administrative Region ([Chan & Liang, 1996](#); [Au & Lo, 2005](#); see Table 2.1 available at <http://monographs.iarc.fr/ENG/Monographs/vol100B/100B-07-Table2.1.pdf>). One report from the Hong Kong Special Administrative Region estimated an HTLV-1 prevalence of only  $4 \times 10^{-5}$  but identified six cases of apparent ATLL by systematically screening all non-Hodgkin lymphoma cases for HTLV-1 antibody ([Au & Lo, 2005](#)).

To date, there are a total of six cohort analyses based in Japan that document the incidence

of ATLL among HTLV-1 carriers, and confirm that male carriers have about a 3–5 fold higher risk of developing ATLL than female carriers ([Tokudome et al. 1991](#); [Iwata et al., 1994](#); [Arisawa et al., 2000, 2003, 2006](#); [Hisada et al., 2001](#)). It is worth noting that because HTLV-1-seropositivity is part of the diagnosis of ATLL, relative risks for ATLL can not be calculated (see Table 2.2 available at <http://monographs.iarc.fr/ENG/Monographs/vol100B/100B-07-Table2.2.pdf>). These cohort studies confirm the causal relationship between HTLV-1 infection and the incidence of ATLL, and also underline the higher risk among infected men. However, the reason for the apparently higher disease penetrance in HTLV-1-infected men than HTLV-1-infected women seen in Japan, and whether it exists elsewhere, is unknown. Modelling data from the Caribbean have not identified such male excesses ([Murphy et al., 1989](#); see Table 2.3 available at <http://monographs.iarc.fr/ENG/Monographs/vol100B/100B-07-Table2.3.pdf>).

There are four case-control analyses nested within prospective HTLV-1 cohorts in Japan that have compared incident ATLL cases with matched HTLV-1 carriers. These have focused on viral or serological predictors of either ATLL or the prevalence of circulating abnormal lymphocytes that resemble ATLL cells ([Hisada et al., 1998a, b](#); [Arisawa et al., 2002](#); [Okayama et al., 2004](#); see Table 2.4 available at <http://monographs.iarc.fr/ENG/Monographs/vol100B/100B-07-Table2.4.pdf>). Pre-diagnosis predictors of ATLL included a higher proviral load, higher antibody titres, and a higher prevalence of soluble interleukin-2 receptor- $\alpha$  (sIL2-R). The predictors for higher levels of abnormal lymphocytes were a higher proviral load and male gender. However, these studies are based on a small number of incident cases, and have not been repeated in other HTLV-1-infected populations.

### 2.1.2 Host susceptibility

As noted above, there is consistent evidence from cohorts in Japan that male carriers have a higher risk compared to female carriers for this malignancy.

It has long been proposed that the risk of ATLL is strongly related to very early HTLV-1 infection. Testing this hypothesis would be difficult, requiring prospective follow-up of large birth cohorts. However, in a recent update of an ongoing study in the Caribbean of mothers of ATLL- and of HTLV-1-associated TSP/HAM patients, 35/36 mothers (97.2%) of ATLL cases were seropositive versus 5/15 mothers (33%) of TSP/HAM patients ( $P<0.001$ ; [Bartholomew et al., 1998](#)). The cells of the sole seronegative mother of an ATLL case were also negative on PCR. However, the father and one older sister of this ATLL case were both HTLV-1 seropositive. All patients in the study were breastfed, and none of the patients or their mothers had a history of a blood transfusion. These findings support the proposal that early and/or mother-to-child infection with HTLV-1 plays an important role in the genesis of ATLL.

Finally, much of the notion of the epidemiology of ATLL is based on epidemiological studies conducted in Japan. It is important to note that geographically defined social environments may alter the natural history of this infection. Specifically, the peak incidence of ATLL occurs earlier and at a reduced frequency among HTLV-1 carriers in the Caribbean in comparison to Japan. Several studies compared viral and immune markers between HTLV-1 carriers and uninfected controls, and between Jamaican and Japanese HTLV-1 carriers as an approach to determine why the penetrance of ATLL may differ between populations.

[Hisada et al. \(2004\)](#) analysed viral markers between a matched set of Jamaican ( $n = 51$ ) and Japanese carriers ( $n = 51$ ) ([Hisada et al., 2004](#)). They found that the anti-HTLV-1 titres were

higher among the Jamaicans ( $P=0.03$ ), as was the prevalence of antibodies against the tax protein (anti-tax) ( $P=0.002$ ). There was no significant difference in proviral load between the Jamaican and Japanese carriers.

[Birnbaum et al. \(2009\)](#) further added a matched HTLV-1 seronegative subject to each carrier within each population reported in [Hisada et al. \(2004\)](#) to evaluate a set of serum immune markers ([Birnbaum et al., 2009](#)). HTLV-1 infection was associated with activated T-cell immunity among the Jamaican subjects as indicated by a higher prevalence of a low EBV nuclear antigens (EBNA-1:EBNA-2) ratio, higher serum levels of sIL2R, and of soluble CD30. The results among the Japanese subjects indicated diminished T-cell immunity among the carriers, as indicated by lower C-reactive protein levels. [The Working Group noted that the observed population differences in non-carriers between the two populations and the impact of HTLV-1 infection within the population in immune profiles may begin to explain the divergent natural history of HTLV-1 infection in the two sentinel populations, and highlight the importance of social environments. Factors that may contribute to these findings include differences in age-specific infection rates, co-existing infections and nutritional status.] See Table 2.5 available at <http://monographs.iarc.fr/ENG/Monographs/vol100B/100B-07-Table2.5.pdf>.

## 2.2 Other malignancies

### 2.2.1 Cutaneous T-cell lymphoma

One study reported that 60 patients with mycosis fungoides had detectable tax-related proteins in peripheral blood mononuclear cell samples; of these, they reported that 83% also had antibodies to HTLV-1 tax proteins ([Pancake et al., 1996](#)). However, several large studies on patients with cutaneous T-cell malignancies, including many cases of mycosis fungoides in the

USA, Republic of Korea, Japan, Spain, Europe, Mali, and Taiwan (China) could not replicate these results, and did not confirm an association with HTLV-1 ([Wood et al., 1996, 1997; Bazarbachi et al., 1997; Kikuchi et al., 1997a, b; Chang et al., 1998; Fouchard et al., 1998; Kim et al., 1998](#); see Table 2.6 available at <http://monographs.iarc.fr/ENG/Monographs/vol100B/100B-07-Table2.6.pdf>). Overall, there is no consistent evidence that HTLV-1 is associated with cutaneous T-cell malignancies, with the exception of ATLL presenting in the skin.

### 2.2.2 B- and T-cell lymphomas

Since the previous IARC Monograph ([IARC, 1996](#)), several case series have examined HTLV-1 infection in B- and T-cell lymphomas ([Farias de Carvalho et al., 1997; Marin et al., 2002; Cabrera et al., 2003; Suefuji et al., 2003; Adedayo & Shehu, 2004](#)). With the exception of some T-cell lymphomas which may, in retrospect, have been cases of ATLL ([Marin et al., 2002](#)), there was no evidence that HTLV-1 infection played a role in these lymphomas (see Table 2.7 available at <http://monographs.iarc.fr/ENG/Monographs/vol100B/100B-07-Table2.7.pdf>).

The majority of primary gastric lymphomas are of B-cell origin, and only rarely of T-cell phenotype ([Isaacson et al., 2008](#)). However, a study reported that 18/58 lymphoma-type ATLL patients had gastric involvement of ATLL cells; and of these, three had primary gastric lymphoma ([Sakata et al., 2001](#)). Another report described 67 cases of surgically resected primary gastric lymphoma, of which five were found to have T-cell lymphoma: two were HTLV-1 positive and three were HTLV-1 negative ([Shimada-Hiratsuka et al., 1997](#)). A final report described 14 T-cell lymphomas in 233 cases of primary gastric lymphoma ([Nakamura & Tsuneyoshi, 1998](#)). [The Working Group remarked that although further research may be useful, it is not clear if gastric lymphoma represents a separate

entity, or simply ATLL involving the stomach or adjacent lymphoid tissue.] See Table 2.8 available at <http://monographs.iarc.fr/ENG/Monographs/vol100B/100B-07-Table2.8.pdf>

Except for a few cases of ATLL, two studies were unable to find HTLV-1 in post-transplant lymphoproliferative disorders. [Gentile et al. \(1998\)](#) reported three patients who developed T-cell lymphoproliferative disorders after renal transplantation, but none had evidence of HTLV-1 infection. The second study reported on 24 cases of post-transplant lymphoid proliferation in Japan: 12 B-cell, ten T-cell, and two natural killer phenotype. A total of 5/10 T-cell tumours were classified as ATLL ([Hoshida et al., 2001](#)). See Table 2.9 available at <http://monographs.iarc.fr/ENG/Monographs/vol100B/100B-07-Table2.9.pdf>.

In one study, 12 cases of leukaemia of large granular lymphocytes with and tested for IgG antibodies to proteins related to HTLV-1 were reported ([Starkebaum et al., 1987](#)). Sera from 6/12 cases reacted with the HTLV-1 proteins, while none of the ‘control’ serum (from healthy persons and patients with other disorders) reacted. By analogy, an initial report of HTLV-2 and large granular lymphocytes ([Loughran et al., 1992](#)) was not confirmed in a controlled study ([Loughran et al., 1994](#)). A set of 27 specimens from cases of splenic lymphoma on Kyushu Island, Japan – an HTLV-1-endemic area – were tested for anti-HTLV-1 antibody, and all were negative ([Kumagawa et al., 2001](#)).

### 2.2.3 Non-lymphomatous tumours

DNA from HTLV-1 and human foamy virus has been detected in recent studies of thymoma, and in 2002, a study reported that 11/12 samples from thymoma patients (91.6%) from Italy tested positive for the *tax* gene of HTLV-1/2 and 9/12 samples (75%) positive for the *tax* and *pol* genes of HTLV-1 ([Manca et al., 2002](#)). However, a later study did not find any evidence for HTLV-1 or

human foamy virus in 21 thymoma patients from the USA ([Li et al., 2004](#)).

Whether or not HTLV-1 is linked to an increased risk of solid malignancies has been studied with generally negative results. However, there is intriguing evidence for a possible protective effect of HTLV-1 on gastric carcinoma. In a prospective cohort study, 4136 adults living in four towns in the Nagasaki Prefecture in south-western Japan were followed for 6 years ([Arisawa et al., 2003](#)). A total of 1063 were seropositive for anti-HTLV-1 antibodies at baseline, including 439 (22.9%) men and 624 (26.2%) women. There were a total of 290 deaths in the cohort, with increased all-cause mortality for HTLV-1 both when ATLL was counted among the deaths (RR, 1.5; 95%CI: 1.2–1.9), and when ATLL deaths were excluded (RR, 1.3; 95%CI: 1.0–1.7). HTLV-1 was not associated with an increased risk of all-site cancer mortality after excluding cases of ATLL (RR, 1.1; 95%CI: 0.77–1.7). Other interesting findings from the study included a decreased incidence of gastric cancer (RR, 0.42; 95%CI: 0.17–0.99). These negative results for HTLV-1 and solid tumours are in contrast to previous cross-sectional studies, which reported a higher HTLV-1 seroprevalence among 394 non-transfused patients with non-ATLL malignancy than in age- and sex-matched healthy controls ([Asou et al., 1986](#)), and a higher prevalence of all malignant neoplasms among siblings of ATLL patients compared to siblings of HTLV-1 seronegative non-Hodgkin lymphoma cases ([Kozuru et al., 1996](#)).

In a recent retrospective cohort study in the Nagasaki Prefecture, Japan, 497 HTLV-1-positive and 497 HTLV-1-negative persons who did not have gastric cancer at baseline were followed with serial endoscopy of the stomach ([Matsumoto et al., 2008](#)). *Helicobacter pylori* antibodies were found in 61.7% of HTLV-1-positive cases compared to 71.6% of the HTLV-1-negative cases. There were 14 cases (2.8%) of gastric cancer in the HTLV-1-positive subjects compared to 35

cases (7%) in the age- and sex-matched HTLV-1-seronegatives (OR, 0.38; 95%CI: 0.21–0.70). [The Working Group noted that these data suggest that HTLV-1 infection may reduce the inflammation usually associated with *H. pylori* infection, and thereby reduce the risk of gastric carcinoma.]

[The Working Group noted that further investigation is warranted on the subject of persistent *H. pylori* infection among HTLV-1 seropositives to determine whether HTLV-1 infection may reduce the inflammatory response to *H. pylori* and reduce the risk of gastric carcinoma, as mentioned above ([Arisawa et al., 2003](#); [Matsumoto et al., 2008](#)).]

Finally, one study of 85 cases of oesophageal squamous cell carcinoma in the Islamic Republic of Iran found no increase in HTLV-1 antibody prevalence compared to non-cancer controls ([Mirsadraee et al., 2007](#)).

See Table 2.10 available at <http://monographs.iarc.fr/ENG/Monographs/vol100B/100B-07-Table2.10.pdf>.

## 2.3 Cofactors

In addition to the roles of being of masculine gender and infected at a younger age (described above), undernutrition and repeated exposure to filariasis in childhood were proposed as potential risk factors for ATLL ([Tajima & Hinuma, 1984](#)).

Since then, there is some evidence that co-infection with *Strongyloides stercoralis* is detrimental to HTLV-1 carriers. Among 38 ATLL cases, those who were positive for *S. stercoralis* were younger at diagnosis than those uninfected ([Plumelle et al., 1997](#)).

[Gabet et al. \(2000\)](#) reported that among HTLV-1 carriers from West Guyana and Martinique, those who were co-infected with *S. stercoralis* had a substantially higher HTLV-1 proviral load, and substantially more oligoclonal expansion of HTLV-1-infected lymphocytes than carriers who were negative for *S. stercoralis*. These observations were substantiated in a

Japanese population of HTLV-1 carriers ([Satoh et al., 2002](#)). [The Working Group noted that, together, these papers suggest that co-infection with *S. stercoralis* may increase the risk of ATLL in HTLV-1 carriers, and presents a potential for risk reduction with parasite treatment and control.]

One case series reported three cases of ATLL among eight HTLV-1 carriers within 2 years after immunosuppression with tacrolimus for liver transplantation ([Kawano et al., 2006](#)). In another study, five cases of ATLL were reported following immunosuppression for kidney transplantation ([Hoshida et al., 2001](#)).

See Table 2.11 available at <http://monographs.iarc.fr/ENG/Monographs/vol100B/100B-07-Table2.11.pdf>.

## 3. Cancer in Experimental Animals

In this volume, the Working Group decided not to include a separate section on “Cancer in Experimental Animals” in the *Monographs* on viruses but rather to include description of such studies under Section 4 (below). The reasoning for this decision is explained in the General Remarks.

## 4. Other Relevant Data

### 4.1 Mechanism of HTLV-1-linked carcinogenesis

#### 4.1.1 Transforming capacity of HTLV-1

*In vivo*, HTLV-1 infection has been reported not only in T lymphocytes, but also in B lymphocytes, myeloid cells, monocytes, and dendritic cells ([Koyanagi et al., 1993](#); [Manel et al., 2005](#); [Jones et al., 2008](#)). However, HTLV-1 immortalizes only T lymphocytes *in vitro* through the action of the Tax viral protein.

#### 4.1.2 Biochemical and biological properties of HTLV-1 proteins

HTLV-1 belongs to the complex retroviruses family. The pX region, which is localized between the *env* gene and the 3'LTR, encodes regulatory genes (*tax* and *rex*), and accessory genes (*p12*, *p13*, *p30* and *HBZ*) (Matsuoka & Jeang, 2007). These proteins not only control viral gene transcription, but also modulate the proliferation of infected cells. Indeed, the fact that HTLV-1 induces the proliferation of infected cells facilitates its transmission through cell-to-cell contact rather than through the release of viral particles (see also Section 1 and Fig.1.1).

##### (a) *Rex*

The Rex protein binds to Rex-responsive elements (RxRE), a highly stable stem-loop structure in the R/U3 region of the 3'LTR (Hanly et al., 1989). Rex regulates viral gene expression at the post-transcriptional level, by increasing the level of unspliced RNA in the nucleus, and by enhancing the nuclear export and the expression of the unspliced *gag/pol* and single-spliced *env* transcripts (Inoue et al., 1991).

##### (b) *p12*

The open reading frame I of the pX region of HTLV-1 encodes the protein p12, which is located in the endoplasmic reticulum and the Golgi. In quiescent primary lymphocytes and *in vivo*, p12 is important for establishing HTLV-1 infection and optimal viral infectivity. The p12 protein therefore facilitates host-cell activation, and the establishment of persistent infection (Collins et al., 1998; Albrecht et al., 2000; Nicot et al., 2005).

##### (c) *p13*

The protein p13 contains a mitochondrial-targeting signal, and exists in the nucleus and mitochondria. Mutation of the *p13* gene impairs viral proliferation *in vivo*, indicating that p13

is critical for viral replication (Hiraragi et al., 2006). In addition, p13 expression is associated with a suppressed cell proliferation *in vitro* (Silic-Benussi et al., 2004).

##### (d) *p30*

The protein p30 is a nuclear and nucleolar protein (Koralnik et al., 1993) that binds to and retains the *tax/rex* mRNA within the nucleus. Therefore, p30 is a post-transcriptional negative regulator of viral replication and viral gene expression (Nicot et al., 2004).

##### (e) *Tax*

Tax, a 40-kD phosphoprotein, encoded from a spliced mRNA, is found mainly in the nucleus but also in the cytoplasm (Meertens et al., 2004). Tax interacts with several host factors (Boxus et al., 2008), which results in *trans*-activation of some genes, *trans*-repression of others, modulation of the cell cycle, and dysregulation of apoptosis (Matsuoka & Jeang, 2007).

The transduction of a pX-containing sequence into primary human T Lymphocytes by use of a defective simian herpesvirus is sufficient to immortalize these cells (Grassmann et al., 1989). However, since this vector could express not only *tax* but also the genes *p12*, *p13*, *p30* and *HBZ*, it was difficult to conclude whether Tax was the only responsible viral protein for cell transformation. Subsequently, immortalization (IL-2-dependent growth) of human CD4-positive T cells was demonstrated *in vitro* by the use of a retroviral vector expressing only the *tax* gene (Akagi et al., 1995). In addition, the transforming ability of Tax was demonstrated in the Rat-1 fibroblast cell line *in vitro* in a soft-agar assay, and *in vivo* in nude mice (Tanaka et al., 1990). These findings clearly showed that Tax is oncogenic. In addition, several studies with animals transgenic for Tax have clearly demonstrated that Tax expression leads to the induction of tumours, confirming that Tax is oncogenic *in vivo* (see Section 4.1.6).

Transcription pathways activated by Tax include those of NF-κB, CREB, SRF and AP-1 (Azran *et al.*, 2004). To turn on the NF-κB pathway, Tax binds to IKK $\gamma$ , and activates the IKK complex, leading to phosphorylation of I $\kappa$ B (Jin *et al.*, 1999). For survival, the Akt/PI3K pathway is also implicated in addition to the NF-κB pathway. Tax also activates this pathway by binding to the p85 $\alpha$  subunit of PI3K, leading to activation of AP-1 (Peloponese & Jeang, 2006).

The transcription factor p53 is a crucial element in the cellular defence against tumour development. Mutations in the *TP53* gene are frequently found in human cancers (IARC *p53* database available online at <http://www.p53.iarc.fr>). Mutations in *TP53* occur in less than 30% of adult T-cell leukaemia (ATLL) cells, depending on the clinical stage, which indicates that other mechanisms are involved. The precise mechanism that leads Tax to inhibit the function of p53 is still a matter of debate: some authors suggested that competition with the transcription co-activators CBP/p300 plays a major role (Mulloy *et al.*, 1998; Suzuki *et al.*, 1999), whereas others reported that activation of the NF-κB pathway was needed (Pise-Masison & Brady, 2005).

Tax activates the transcription of viral genes through three imperfect 21 base-pair repeat elements, the Tax-responsive element (TRE) (Fujisawa *et al.*, 1986). The neighbouring GC-rich sequences of TRE are required for the binding of Tax (Paca-Uccaralertkun *et al.*, 1994). The TRE contains sequences that are similar to that of the cyclic adenosine monophosphate (cAMP)-responsive element (CRE). CRE-binding protein/activating transcription factor (CREB/ATF) family members bind to TRE in a Tax-dependent manner (Franklin *et al.*, 1993). Tax can interact with transcriptional co-activators, CREB-binding protein (CBP) and p300, that acetylate histones in the promoter region. In addition, CREB co-activators – termed transducers of regulated CREB activity (TORCs) – activate Tax-mediated viral gene transcription through

the LTR. Tax interacts with TORCs (Siu *et al.*, 2006). Thus, co-activators, TORCs, and CBP/p300 are necessary for the Tax-mediated activation of viral gene transcription.

Tax also inhibits transforming growth factor  $\beta$  (TGF- $\beta$ )-mediated signals. It is likely that the inhibition of TGF- $\beta$ -signalling enables HTLV-1-infected cells to escape TGF- $\beta$ -mediated growth inhibition (Mori *et al.*, 2001; Lee *et al.*, 2002).

The Tax sequence contains several important domains that are involved in CREB and NF-κB activation. Recently, the C-terminal sequence of Tax was shown to contain a PDZ-binding motif. This PDZ-binding motif, which is absent from HTLV-2 Tax, seems critical for the ability of HTLV-1 Tax to transform cells *in vitro* (Rousset *et al.*, 1998; Endo *et al.*, 2002). It should be noted that Tax is not expressed in 60% of ATLL cases, due to deletions, epigenetic changes of the 5'LTR, and genetic changes in the Tax sequence (Matsuoka, 2005, 2010; Giam & Jeang, 2007).

#### (f) HBZ

The HTLV-1 bZIP factor (HBZ) is transcribed from the complementary strand of the proviral genome (Larocca *et al.*, 1989; Gaudray *et al.*, 2002). Viral transcription from the 5'LTR is highly dependent upon Tax expression and this is due to the presence of three TREs, as indicated above. Conversely, the transcription from the 3'LTR is dependent on Sp1 (Yoshida *et al.*, 2008). Therefore, HBZ gene transcription is relatively constant, and is correlated with proviral load (Usui *et al.*, 2008). Interestingly, and in contrast to the finding that the *tax* gene has not been frequently detected in ATLL cells, the HBZ mRNA could be detected in all ATLL cases. Even if defective proviruses are commonly detected in ATLL cells, the HBZ gene always remains intact (Miyazaki *et al.*, 2007). Importantly, HBZ expression is associated with the proliferation of ATLL cells since the knock-down of HBZ in ATLL cells decreases the growth of the leukaemic cells (Satou *et al.*, 2006), which further indicates that

HBZ is critical and essential for the growth of these cells. Several transcription factors bind HBZ, including c-Jun, JunD, JunB, RelA/p65, p300, and CREB ([Basbous et al., 2003](#); [Hivin et al., 2007](#); [Clerc et al., 2008](#)).

#### 4.1.3 Biological properties of HTLV-1 proteins relevant to carcinogenesis

##### (a) Immortalization

HTLV-1 can transform CD4-positive T lymphocytes *in vitro*. Among all the viral proteins, only Tax has the ability to immortalize CD4-positive T cells *in vitro* (see above).

##### (b) Genetic instability

Cytogenetic abnormalities that are specific to ATLL have not been found, but trisomies, deletions, and structural rearrangements are frequently reported in two of the four ATLL subtypes (acute leukaemia and lymphoma ATLL) ([Kamada et al., 1992](#)). This is therefore indicative of chromosomal instability in ATLL cells where the altered functions of several centrosome-associated proteins seem also to be involved in the Tax-driven aneuploidy ([Afonso et al., 2007](#)). As an example, the functions of HsMAD1 (also known as TXBP181) functions are impaired in Tax-expressing cells. HsMAD1 acts at the G2/M-checkpoint and has been found on the centrosome during metaphase. It is tempting to speculate that the loss of HsMAD1 function could be linked to the loss or modification of the centrosomal activity ([Jin et al., 1998](#)).

Tax has also been reported to interact with the anaphase-promoting complex/cyclosome (APC/C). This interaction leads to a premature mitotic exit, and may contribute to aneuploidy ([Liu et al., 2005](#)).

Recently, another partner of Tax, the centrosome-associated TAX1BP2 protein (also known as TXBP121) was also implicated in the Tax-dependent initiation of aneuploidy ([Ching et al., 2006](#)). By the use of *in-situ* fluorescence

microscopy the authors demonstrated that Tax binds to and co-localizes with endogenous TAX1BP2, forming peri-nuclear dots. In the absence of Tax, overexpression of TAX1BP2 leads to a reduction in the number of cells that contain supernumerary centrosomes. In contrast, depletion of endogenous TAX1BP2 induces centrosome amplification. Therefore, Tax and TAX1BP2 have opposite effects. Besides, a Tax mutant that does not interact with TAX1BP2 can no longer induce centrosome duplication. This suggests that Tax targets TAX1BP2 to cause aneuploidy. In addition, during mitosis, Tax binds to Ran and RanBP1, which fragments spindle poles, and induces multipolar segregation ([Peloponese et al., 2005](#)).

##### (c) DNA-damage responses

Tax has been reported to suppress the expression of DNA polymerase β ([Jeang et al., 1990](#)), which is implicated in DNA repair. This suppression is associated with impaired DNA repair in HTLV-1-infected cells. In addition, it has been reported that Tax inhibits ATM-mediated DNA-damage response, resulting in premature DNA replication in the presence of genomic lesions ([Chandhasin et al., 2008](#)).

##### (d) Cell proliferation and differentiation

As mentioned above, the HTLV-1 provirus can be detected not only in CD4-positive T cells, but also in CD8-positive T cells as well as in dendritic cells *in vivo*. Among CD4-positive T cells, the HTLV-1 provirus was detected in memory/effector T cells. After infection followed by a couple of cycles during which HTLV-1 uses its reverse transcriptase, the virus is amplified via clonal proliferation of the infected cells ([Takemoto et al., 1994](#); [Wattel et al., 1995](#)). The same infected clones survive *in vivo*, indicating that clonal proliferation is persistent ([Etoh et al., 1997](#); [Cavrois et al., 1998](#)). A prospective study has shown that this clonal proliferation is associated with the onset of ATLL in some cases ([Okayama](#)

*et al.*, 2004), although oligoclonal proliferation without ATLL occurs in most asymptomatic HTLV-1 carriers. Of note, the proviral load ranges from less than 0.1% up to 30% (of total peripheral blood mononuclear cells) in asymptomatic carriers. It is likely that a high proviral load is associated with a higher risk of developing ATLL ([Tachibana et al., 1992](#)).

#### 4.1.4 Role of HTLV-1 in malignant conversion

##### (a) Requirement of HTLV-1 expression for cell growth

Viral gene expression differs between in-vitro-transformed cell lines and primary ATLL cells in a manner that is similar to the relation between EBV-transformed cells and Burkitt lymphoma cells. Tax expression is usually high in transformed cells *in vitro* but TAX gene transcription is detected in only about 40% of ATLL cells cultured *ex vivo* ([Matsuoka & Jeang, 2007](#)). Analyses of the HTLV-1 provirus identified three mechanisms that inactivate Tax expression: 1) genetic changes in the TAX gene sequence that lead to a premature stop codon or to insertions/deletions; 2) DNA methylation of the provirus; and, 3) deletion of the proviral 5'LTR ([Matsuoka & Jeang, 2007](#)). Deletion or DNA methylation of the 5'LTR silenced transcription of the viral genes, including TAX, REX, P12, P13, and P30. The 3'LTR, on the other hand, was intact and unmethylated in all ATLL cases examined, and HBZ was shown to be expressed in all ATLL cases tested, and to induce lymphocyte proliferation ([Satou et al., 2006](#)). Interestingly, there is a correlation between the proviral load and HBZ mRNA levels ([Li et al., 2009](#)).

##### (b) Persistence of the HTLV-1 genome

HTLV-1 induces ATLL in a subset of carriers after a long latency period. As an example, the cumulative lifetime risk of developing ATLL was estimated to be 6.6% for men and 2.1% for women among Japanese HTLV-1 carriers ([Arisawa et al.,](#)

[2000](#)). ATLL cells retain the HTLV-1 provirus in the genome, but as stated above, defective proviruses are frequently detected, which are classified into two types. A type-1 defective provirus was found in 43% of all defective viruses; it lacks internal sequences such as *gag*, *pol* and *env* but retains both LTRs. A type-2 defective provirus lacks the 5'LTR and internal sequences. It is frequently observed in acute and lymphoma-type ATLLs whereas it is quite rare in chronic ATLL, indicating that this defective provirus is likely to be associated with disease progression ([Tamiya et al., 1996](#)). Detailed analyses show that the type-2 defective provirus can be generated before and after integration. A defective provirus formed after integration suggests that the deletion of the 5'LTR may block Tax expression, enabling ATLL cells to escape the host immune system. The frequency of type-2 defective proviruses is low in carriers, indicating that these defective proviruses were selected during leukaemogenesis. Another possibility is that infected cells with the type-2 defective provirus tend to transform into ATLL cells. ATLL cells with the type-2 defective provirus frequently cannot produce Tax as a result of the deletion of the promoter or the deletion of the second exon. However, all cases with the type-2 defective provirus maintain an intact HBZ gene sequence ([Miyazaki et al., 2007](#)).

As a mechanism of retroviral oncogenesis, the integrated LTR activates the transcription of cellular oncogenes, flanking integration sites. However, there are no common integration sites of HTLV-1 provirus in ATLL cells ([Doi et al., 2005](#)).

##### (c) Alterations of oncogenes and tumour-suppressor genes

Several ways by which tumour-suppressor genes can be inactivated have been demonstrated in cancer cells. Mutations of the TP53 gene occur in up to 40% of all ATLL patients ([Sakashita et al., 1992](#); [Yasunaga & Matsuoka,](#)

[2003](#)). Deletion or mutation of the *p16<sup>INK4A</sup>* gene has also been reported. Such genetic changes in the *p53* and *p16<sup>INK4A</sup>* gene sequence and/or function are detected in the more aggressive disease states, indicating that somatic DNA changes in these two genes are associated with the progression of ATLL ([Yasunaga & Matsuoka, 2003](#)).

Cytogenetic analysis of ATLL cells showed a common breakpoint cluster region in chromosome 10p11.2. Further analyses have shown that the transcription factor 8 (TCF8) is frequently disrupted by several mechanisms, including epigenetic silencing. Suppressed expression of TCF8 is associated with resistance to TGF-β. Mice carrying a mutation in *TCF8* frequently developed thymic T-cell lymphoma, indicating that *TCF8* is a tumour-suppressor gene ([Hidaka et al., 2008](#)).

There have been a few reports of cellular oncogenes in ATLL cells. By screening cDNA expression-libraries derived from leukaemic cells of ATLL patients for the potential to transform NIH3T3 mouse fibroblasts, a novel transforming gene, *Tgat*, was identified. Expression of *Tgat* in NIH3T3 cells resulted in cell transformation, indicated by anchorage-independent growth in semisolid medium, and tumorigenicity in nude mice ([Yoshizuka et al., 2004](#)).

#### 4.1.5 Interaction between HTLV-1 and environmental agents

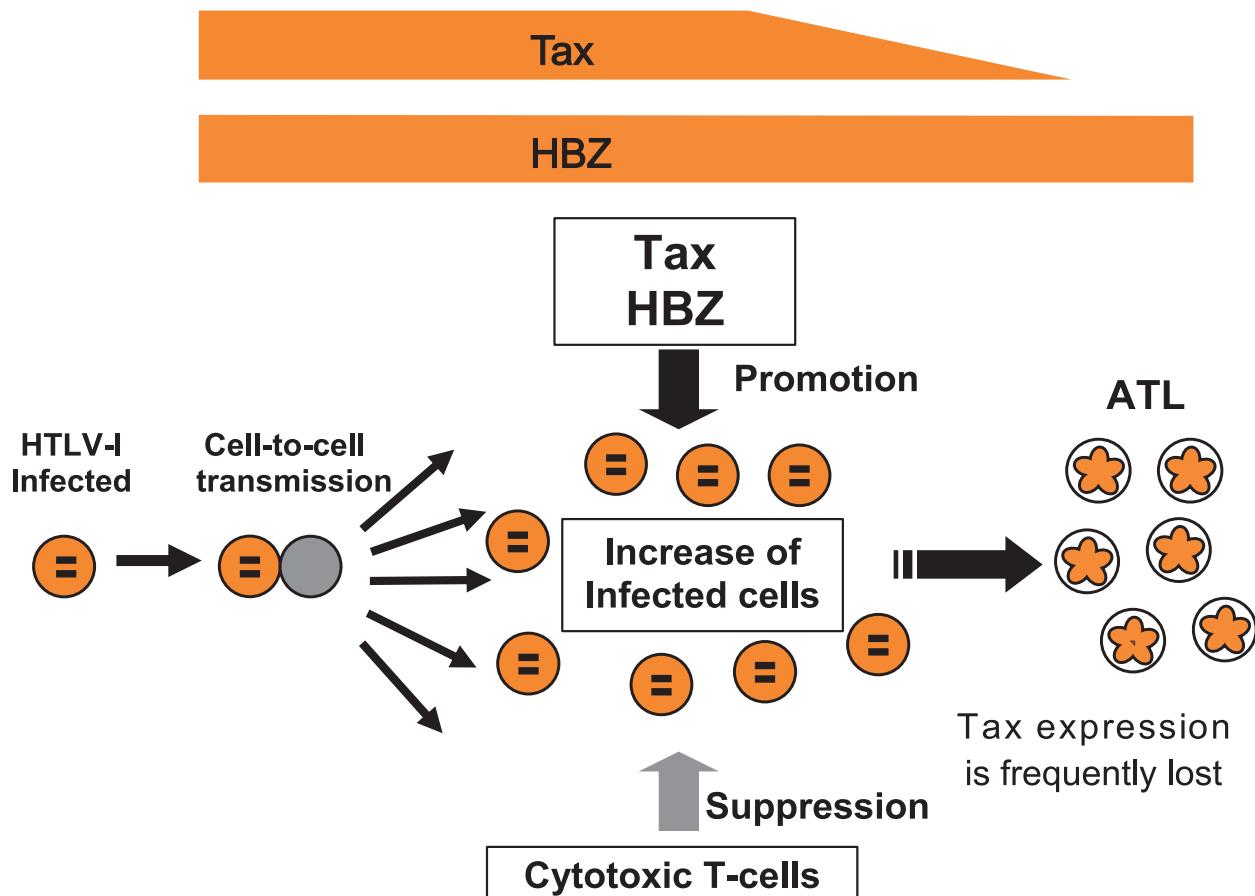
The frequency of opportunistic infections is fairly high among ATLL patients, indicating that T-cell-mediated immunity is severely impaired in such patients. The presence of the parasite *S. stercoralis* is commonly seen in immunosuppressed patients. In a study in the Japanese districts of Kyushu and Okinawa, where strongyloidiasis is endemic, 36 patients were identified as seropositive for HTLV-1. Fourteen of these patients (39%) had HTLV-1 DNA monoclonally integrated in their blood lymphocytes. It has been suggested that the parasitic infestation with

*S. stercoralis* may act as a cofactor for HTLV-1-induced leukaemogenesis ([Nakada et al., 1987](#)).

#### 4.1.6 Animal models for HTLV-1-associated cancers

Animals, including rabbits, rats, and monkeys can be experimentally infected with HTLV-1 ([Lairmore et al., 2005](#)). In rabbits and rats, HTLV-1 infection is persistent, and induces host immune response. However, HTLV-1 does not lead to definite diseases in these two species. A large number of Old World monkeys are naturally infected with STLV-1. This virus is almost identical at the nucleotide level with HTLV-1, and several cases of ATLL have been described in monkeys ([Tsujimoto et al., 1987](#); [Akari et al., 1998](#)). Experimental infection with HTLV-1 of squirrel monkeys (*S. Sciureus*) led to a substantial decrease in the proliferation rate of the CD4-positive T-cell population in those infected animals that were affected by a pathology similar to ATLL in humans ([Debacq et al., 2005](#)). Co-infection of rhesus macaques (*Macaca mulatta*) with HTLV-1 and simian immunodeficiency virus 1 (SIV-1) increased the number of multilobulated lymphocytes in the circulation. The study showed that SIV-1 may have the potential to upregulate HTLV-1 and disease expression ([Traina-Dorge et al., 2007](#)). So far, non-human primates represent the only suitable animal model to study human ATLL.

Several groups have shown that HTLV-1 can infect immunocompetent mice, although in most of these studies, no viral mRNA production or HTLV-1 antibody response were detected. In addition, these mice did not show progression to ATLL ([Lairmore et al., 2005](#)). Several transgenic animal models have been established to study HTLV-1; the Tax protein has been shown to be oncogenic in several of these models. The type of tumour depends on the promoter used in each study: transgenic mice expressing Tax using the granzyme B promoter developed tumours of

**Fig. 4.1 Natural history of HTLV-1 infection leading to onset of ATL**

After infection, HTLV-1 is transmitted mostly through cell-to-cell contacts. Tax and HBZ viral proteins promote oligoclonal proliferation of HTLV-1-infected cells. Tax expression is suppressed by cytotoxic T-lymphocytes *in vivo*. A fraction of carriers develop ATL after a long period. At the leukaemic stage, about 60% of ATL cases do not express Tax. ATLL, adult-T-cell leukaemia  
Prepared by the Working Group

natural killer cells ([Grossman et al., 1995](#)), and transgenic mice with Tax expression via the lck promoter developed a disease that resembles ATLL ([Hasegawa et al., 2006](#)). The major difference between most of these animal tumours and ATLL is the fact that, as stated above, a subset of human ATLL cells do not express Tax. HBZ-transgenic mice have also been shown to display increased T-cell proliferation ([Satou et al., 2006](#)).

## 4.2 HTLV-1, host immune system, and genetic susceptibility

The host immune system influences the condition of viral infection, and the diseases induced by it. Large interindividual variations in proviral load are commonly observed between HTLV-1 carriers, but the amount of provirus is relatively constant in HTLV-1-infected individuals over time ([Kwaan et al., 2006](#)), suggesting that host factors, including the immune system,

determine the provirus load. In spite of eliciting a strong immune response, HTLV-1 infection persists *in vivo*, mainly in CD4-positive T cells, and part of the CD8-positive T cells are infected by HTLV-1 ([Yasunaga et al., 2001](#)). An *in vivo* study of HTLV-1-infected cells used diuterium-labelled glucose to investigate lymphocyte kinetics, and showed that CD4+CD45RO+ and CD8+CD45RO+ T-lymphocyte proliferation was elevated in HTLV-1-infected subjects ([Asquith et al., 2007](#)). This was associated with viral gene expression, and indicates that active proliferation induced by viral infection induces the host immune response, and that the proviral load is determined by a balance between Cytotoxic T Lymphocytes activity and viral gene expression. The host immune system probably prevents the development of ATLL *in vivo* as suggested by a study where 3/8 HTLV-1-positive carriers, who were immunosuppressed during the course of a liver transplantation, developed ATLL ([Kawano et al., 2006](#)).

### 4.3 Synthesis

There is strong mechanistic evidence supporting the role of HTLV-1 in human carcinogenesis. The viral protein Tax has the ability to immortalize and to transform human T cells. At the leukaemic stage, the expression of Tax is often not maintained, but the viral protein HBZ continues to be expressed, and supports the sustained growth of the leukaemic cells (see Fig. 4.1).

### 5. Evaluation

There is *sufficient evidence* in humans for the carcinogenicity of HTLV-1. HTLV-1 causes adult T-cell leukaemia/lymphoma.

HTLV-1 is *carcinogenic to humans (Group 1)*.

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# **OPISTHORCHIS VIVERRINI AND CLONORCHIS SINENSIS**

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*Opisthorchis viverrini* and *Clonorchis sinensis* were considered by a previous IARC Working Group in 1994 ([IARC, 1994](#)). Since that time, new data have become available, these have been incorporated in the *Monograph*, and taken into consideration in the present evaluation.

## **1. Exposure Data**

### **1.1 Taxonomy, structure and biology**

#### *1.1.1 Taxonomy*

*Opisthorchis viverrini* (*O. viverrini*) and *Clonorchis sinensis* (*C. sinensis*) are pathologically important foodborne members of the genus *Opisthorchis*; family, Opisthorchiidae; order, Digenea; class, Trematoda; phylum, Platyhelminths; and kingdom, Animalia. They belong to the same genus (*Opisthorchis*) but to different species based on morphology; nonetheless, the genus *Clonorchis* is so well established in the medical literature that the term is retained here.

#### *1.1.2 Structure*

The adult of *O. viverrini* and *C. sinensis* are usually about 10–25 mm in length and 3–5 mm in width ([Liu & Chen, 1998](#); [Sripa et al., 2007](#)).

The yellowish-brown, ovoid eggs have a distinct operculum, which opens to release the miracidium – a fully formed larva. Eggs are on average 29 µm long by 17 µm wide for *C. sinensis* ([Liu & Chen, 1998](#)), and 27 µm by 15 µm for

*O. viverrini* ([Sadun, 1955](#)), and are difficult to differentiate between these two species ([Kaewkes et al., 1991](#)).

#### *1.1.3 Structure of the genome*

The genomic structures of *O. viverrini* and *C. sinensis* have not been reported.

*O. viverrini* is reported to have six pairs of chromosomes, i.e.  $2n = 12$  ([Rim, 2005](#)), to have neither CpG nor A methylations, but to contain a highly repeated DNA element that is very specific to the organism ([Wongratanacheewin et al., 2003](#)). Intra- and inter-specific variations in the gene sequences of 18S, the second internally transcribed spacer region ITS2, 28S nuclear rDNA, and of the mitochondrial cytochrome C oxidase subunit I (mtCOI) DNA are low and nearly identical ([Ando et al., 2001](#)). A comparison of the ITS2 region sequences of *O. viverrini* versus *C. sinensis* show a 95% match; the sequences differ at 28 nucleotide positions ([Park, 2007](#)).

The chromosome number of *C. sinensis* is  $2n = 56$ , and the chromosomes can be divided into two groups based on their sizes, consisting of eight pairs of large and 20 pairs of small chromosomes. The mean total length of the diploid

complements of liver flukes collected in the People's Democratic Republic of China is slightly longer than that of those collected in the Republic of Korea ([Park et al., 2000](#)).

#### 1.1.4 Host range

Three families of freshwater snails (Hydrobiidae, Bithyniidae, and Melaniidae) are first intermediate hosts ([Harinasuta & Harinasuta, 1984](#); [Liu & Chen, 1998](#)). Of these, *Parafossarulus striatulus*, *Alocinma longicornis* (Hydrobiidae), *Bithynia fuchsianus* (Bithyniidae) are currently considered to be of greatest importance in China in the life cycle of *C. sinensis* ([Lun et al., 2005](#)).

Over 130 species of fish (belonging to 16 families) are secondary intermediate hosts ([Komiya, 1966](#); [Vichasri et al., 1982](#); [Rim, 1986](#); [Joo, 1988](#); [Liu & Chen, 1998](#)). Fish in the family Cypriniidae are the major intermediate hosts ([Lun et al., 2005](#)).

In addition to human beings, other fish-eating mammals, for example dogs, cats, pigs, minks, weasels, civets, and house rats can be definite hosts, and some may act as reservoir hosts ([Wang, 1983](#); [Lun et al., 2005](#); [Rim, 2005](#)). There is also evidence that rabbits, guinea-pigs, hamsters, gerbils, mice, and rats are susceptible to the parasite in a laboratory setting ([Bhamarapravati et al., 1978](#); [Wang, 1983](#); [Boonmars et al., 2009](#)). Cats and dogs are considered to be the most important animal hosts in the endemic regions of China ([Lun et al., 2005](#)). In contrast with many other countries, most cats and dogs are not kept as pets in rural China but roam freely in villages, and thus have easy access to the remains of raw or undercooked fish in household waste ([Wang, 1983](#); [Jiang, 2001](#)).

#### 1.1.5 Target organs

The adult liver flukes usually reside in the medium-sized or small intrahepatic bile ducts. In heavy infections, adult parasites may be found

in the gallbladder, the extrahepatic bile duct, and the pancreatic duct ([Pungpak et al., 1985](#); [Rim, 1986, 2005](#); [Lim, 1990](#); [Sripa, 2003](#)). Over 100 flukes were recovered from the gallbladder of one patient ([Evans et al., 1971](#)), and 5140 and 1348 flukes of *C. sinensis* were found, respectively, in the bile ducts and in pancreatic ducts of a child patient who died of clonorchiiasis sinensis ([Chen et al., 1963](#)).

The pathophysiology and clinical manifestations for *O. viverrini* and *C. sinensis* and infection are very similar ([Lun et al., 2005](#); [Sripa, 2008](#)).

#### 1.1.6 Life cycle

The eggs produced by the mature adult worms pass down the bile duct and are excreted in the faeces. If the eggs reach a freshwater body (small ponds, streams and rivers, flooded rice fields, and reservoirs), they are ingested by snails, which act as the primary intermediate hosts. Asexual reproduction in the snail results in daily release of thousands of cercariae, 1–2 months after infection of the snail. The free-swimming cercariae penetrate the tissue of freshwater fish, which act as the secondary intermediate host, and encyst to become fully infective metacercariae under the fish's skin or in muscle after 21 days.

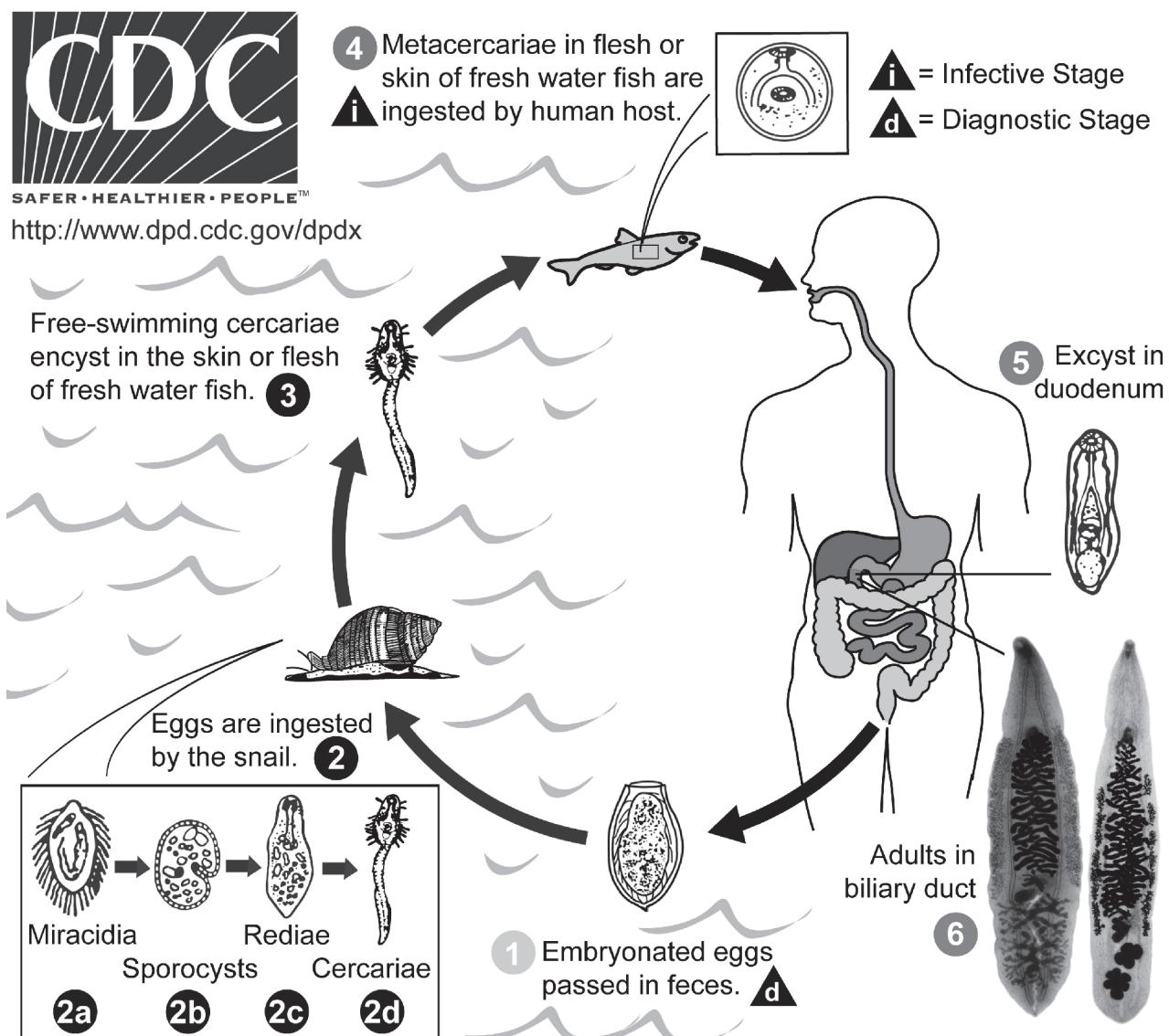
Humans or other fish-eating animals are infected through the ingestion of raw or undercooked (salted, pickled, or smoked) freshwater fish that contains metacercariae. After ingestion, the metacercaria excysts in the duodenum and ascends the biliary tract through the ampulla of Vater. Maturation to adulthood takes approximately 1 month.

The life cycle of the liver flukes is shown in Fig. 1.1 (for a review, see [Rim, 1986](#); [Sripa et al., 2007](#)).

#### 1.1.7 Genes and gene products

[Laha et al. \(2007\)](#) constructed an *O. viverrini* cDNA library that covers ~14% of the entire transcriptome. About 20% of contigs were assigned

Figure 1.1 Life cycle of *Clonorchis sinensis* and *Opisthorchis viverrini*



Adapted from <http://www.dpd.cdc.gov/DPDx/HTML/Opisthorchiasis.htm>

Gene Ontology classifications. Frequently represented protein families included those involved in physiological functions that are essential to parasitism, such as anaerobic respiration, reproduction, detoxification, surface maintenance, and feeding. An assessment of evolutionary relationships showed that *O. viverrini* was similar to other parasitic flukes such as *C. sinensis* and *Schistosoma japonicum*. A total of 164 *O. viverrini* contigs contained open reading frames (ORFs) with signal sequences, many of which were platyhelminth-specific. Moreover, ORFs representing secreted proteins with known roles in tumorigenesis were identified such as granulin, kallikrein-like serine proteases, phospholipase A2 (PLA-2), saponin-like protein, and thioredoxin peroxidase. These proteins might play a role in the pathogenesis of *O. viverrini*-induced cholangiocarcinoma ([Laha et al., 2007](#)). Gene expression profiling of adult *O. viverrini* was also constructed by the first 5' serial analysis of gene expression (5' SAGE) library, and vitelline B precursor protein and myoglobin were found to be the most abundant proteins ([Chutiwitoonchai et al., 2008](#)).

By using the expressed sequence tag (EST) approach, [Lee et al. \(2003\)](#) constructed the *C. sinensis* adult cDNA library. A total of 220 genes were sorted into seven functional categories including: energy metabolism (38), gene expression/RNA metabolism (21), regulatory/signalling components (14), protein metabolism/sorting (98), the structure/cytoskeleton (29), membrane transporters (10), and antigenic proteins (10). The high frequency of cysteine protease expression (30/415 randomly selected clones) suggests an important role of this protein in the metabolism and/or pathogenesis of clonorchiasis. Also identified were Cu/Zn-superoxide dismutase and glutathione-S-transferase, which are believed to play a crucial role in protecting the parasite from the host immune effector mechanisms, and are being pursued as drug targets in other parasitic infections ([Lee et al., 2003](#)). [Cho et al. \(2008\)](#)

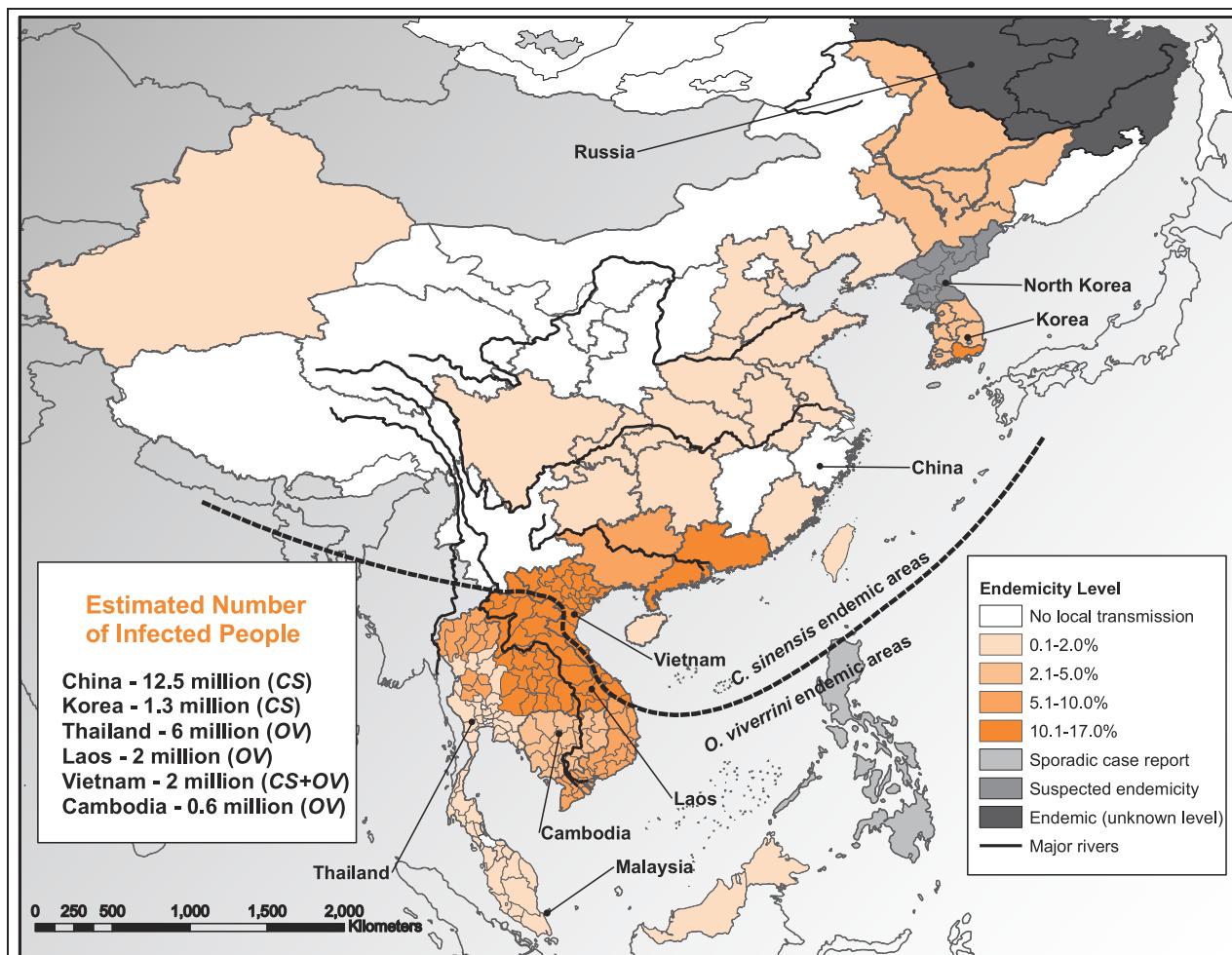
reported gene expression profiles in *C. sinensis* metacercariae compared to those of adult worms. The genes expressed more abundantly in the metacercariae were a group of structural and cytoskeletal proteins, followed by transcription and translation machinery proteins, and a group of energy metabolism proteins. In contrast, adult *C. sinensis* has abundant mRNA clusters encoding for regulatory and signal proteins, other metabolic proteins and enzymes, and structural and cytoskeletal proteins, in decreasing order ([Cho et al., 2008](#)). This may be explained by the fact that metacercariae in the muscles of freshwater fish are in a resting stage wherein they simply maintain a basal metabolic status, and adult *C. sinensis* have a high metabolic rate and produce a large numbers of eggs in mammalian hosts ([Rim, 2005](#)).

## 1.2 Epidemiology of infection

### 1.2.1 Prevalence, geographic distribution

Human liver fluke infection is endemic in China, Thailand, the Republic of Korea, the Democratic People's Republic of Korea, Viet Nam, Lao People's Democratic Republic, and Cambodia. Endemicity for *C. sinensis* is also suspected in the Russian Federation long the Amur River. Persons from Singapore and Malaysia with *C. sinensis* infection have been reported infrequently; many of them may be infected during travelling in other countries or through eating imported fish.

A very crude estimate of the global number of infected people is of the order of 45 million, comprising 35 million infected with *C. sinensis* ([Korea Association of Health Promotion, 2004](#); [Lun et al., 2005](#); [Fang et al., 2008](#)), and 10 million with *O. viverrini* ([WHO, 1995](#); [Jongsuksuntigul & Imsomboon, 2003](#)). The geographic distribution of *O. viverrini* and *C. sinensis* is shown in Fig. 1.2.

**Figure 1.2 Distribution of Liver fluke infection in Asia**

*C. sinensis* is distributed in China, Republic of Korea, Democratic People's Republic of Korea, the Russian Federation, and northern parts of Viet Nam, and *O. viverrini* in Thailand, Laos, Cambodia, Malaysia, and southern part of Viet Nam. The data used for the map were derived from most recent national surveys and published literature.

Note that in the legend, "no local transmission" stands for "no reported local transmission".

A courtesy of Dr Song Liang, College of Public Health, The Ohio state University, USA, who did the art work based on data provided by the Working Group.

### (a) *Opisthorchis viverrini*

Thailand is the most endemic country for opisthorchiasis due to *O. viverrini*. In Thailand and neighbouring countries, human opisthorchiasis is caused by *O. viverrini*. In 1980–81, the prevalence in the north, north-eastern, centre and the south of Thailand was 5.6%, 34.6%, 6.3%, and 0.01%, respectively, with an overall prevalence of 14% or 7 million people infected. As a

result of intensive and continuous control activities, the prevalence of infection in north-eastern Thailand declined to 15.7% in 2001, and the rates in other areas were as follows: the north (19.3%), the centre (3.8%) and the south (0%), with an average prevalence of 9.6% or 6 million people infected ([Jongsuksuntigul & Imsomboon, 2003](#)).

It was estimated that 1.7 million people were infected with *O. viverrini* in Laos in 1992 ([WHO](#),

[1995](#)), mainly along the Mekong River, and as far as in the lowlands among people with close ethnic ties to the majority of the north-eastern Thai population. Based on a national survey of primary schoolchildren conducted in 2000–02 that included 17 provinces and the Vientiane Municipality, the prevalence of *O. viverrini* was 10.9% (29846 participants). Again, the regions along the Mekong River such as Khammuane, Saravane or Savannakhet Province showed a higher prevalence of *O. viverrini* (32.2%, 21.5%, 25.9%, respectively) ([Rim et al., 2003](#)). More recently, a survey in the Saravane district revealed a high prevalence of *O. viverrini* infection (58.5%) among 814 persons from 13 villages ([Sayasone et al., 2007](#)).

A few official reports or published data on *O. viverrini* infection in Cambodia are available. A small survey in primary schoolchildren from Kampongcham province showed a prevalence of *Opisthorchis* spp. of 4.0% from 251 fecal specimens in 2002 ([Lee et al., 2002](#)).

Viet Nam has been reported to be endemic for *C. sinensis* in the northern part, and *O. viverrini* in the southern region ([De et al., 2003](#)).

#### (b) *Clonorchis sinensis*

*C. sinensis* was first discovered in the bile ducts of a Chinese carpenter in Calcutta, India, in 1875. In 1994, archaeologists found a large number of *C. sinensis* eggs in the bowel content of a corpse buried at the middle stage of the Warring States Period (475–221 BC) in Hubei, China ([Wu et al., 1996](#)), indicating that this parasite has been present in this province for more than 2300 years. In a nationwide sampling survey on the epidemiological status of parasitic diseases in China, a total of 356629 persons were investigated, and 2065 were found to be infected with *C. sinensis*, with an overall infection rate of 0.58% (Office of the National Survey on the Important Parasitic Diseases, 2005). In a recent survey in Chinese endemic areas, a total of 217829 persons were investigated, and 5230 were

found to be infected with *C. sinensis*, resulting in an infection rate of 2.4%. From this, an estimate of the number of infected persons in China was calculated to be 12.5 million ([Fang et al., 2008](#)).

*C. sinensis* is currently the most prevalent human parasitic helminth in the Republic of Korea, as detected by faecal examination. There has been no decrease in the average national infection rate of *C. sinensis* for almost 30 years; the detection rate was 4.6% in 1971, 1.8% in 1976, 2.6% in 1981, 2.7% in 1986, 2.2% in 1992, 1.4% in 1997 and 2.9% in 2004, and about 1.3 million people in the Republic of Korea are estimated to be infected ([Korea Association of Health Promotion, 2004](#); [Rim, 2005](#)). In endemic areas of the Republic of Korea, along the main rivers, prevalence values up to 40% have been reported ([Rim, 1986, 2005](#)).

Due to a lack of available data from their national survey, there is no accurate number for infected people in Viet Nam. A study of 1155 villagers in northern Viet Nam reported a prevalence of *C. sinensis* infection of 26% ([Dang et al., 2008](#)).

A prevalence of *C. sinensis* infection is suspected in the south-eastern part of the Russian Federation, in the Amur River basin where, based on scarce reports, it was estimated at >20% in some villages (e.g. Nanay district) ([Semenova et al., 1995](#); [Dyk et al., 1997](#)).

#### 1.2.2 Transmission and risk factors for infection

The definitive host is infected by the liver fluke primarily through the ingestion of raw (dried, pickled or salted) or undercooked infected fish, which contain metacercariae – this is the infective stage in the life cycle of liver flukes ([Sithithaworn & Haswell-Elkins, 2003](#)). Many surveys show that people in Thailand ([Kaewpitoon et al., 2008](#)), Viet Nam ([Dang et al., 2008](#)), China ([Fang et al., 2008](#); [Lun et al., 2005](#)), Laos ([Hohmann et al., 2001](#)),

and the Republic of Korea ([Lim et al., 2006](#)) have these eating habits.

In southern China and among the Cantonese population in the Hong Kong Special Administrative Region, raw fish is traditionally eaten after being dipped in rice porridge. Alternatively, large fish are sliced and eaten with ginger and garlic known as “*yushen*.” This mode of transmission tends to increase with age. In contrast, many children in hilly areas of Guangdong and eastern China such as Jiangsu, Shandong, and Anhui provinces, often catch fish during play, and roast them incompletely before consumption. This mode of transmission tends to decline with age ([Fang et al., 2008](#)).

The population of the Republic of Korea eat raw fish soaked in vinegar, red-pepper mash or hot bean paste with rice wine at social gatherings. The fact that men do so more frequently than women has been given as a reason for the higher prevalence of infection among men; however, in heavily endemic areas, often no significant differences are seen between the genders. When fish is abundant, raw fish is eaten very regularly as opposed to being saved for special occasions ([Choi, 1984](#); [Rim, 1986](#)). Vietnamese people eat raw fish in salads ([Kiêu et al., 1990](#)).

In Thailand and the lowland region of Laos, three types of uncooked fish preparations are noted ([Sadun, 1955](#); [Sithithaworn & Haswell-Elkins, 2003](#)):

- *koi pla*, eaten soon after preparation;
- *pla som*, moderately fermented, and stored for a few days to weeks; and,
- *pla ra* and *jaewbhong*, extensively fermented, highly salted fish, stored for at least 2–3 months.

*Koi pla* is probably the most infective dish, followed by fish preserved for <7 days, then *pla ra* and *jaewbhong*, in which viable metacercariae are rare ([Sithithaworn & Haswell-Elkins, 2003](#)). Several factors can directly or indirectly lead to the transmission of the liver flukes to humans (for reviews, see [Sithithaworn &](#)

[Haswell-Elkins, 2003](#) for *O. viverrini*; [Lun et al., 2005](#) for *Clonorchis sinensis*): 1) poor educational level of local residents ([Jongsuksuntigul & Imsomboon, 2003](#)); 2) lack of sanitation: it is common in some endemic regions in China, particularly in the province of Guangdong and Guangxi, that “lavatories” are built adjacent to ponds, so that human excrement containing *C. sinensis* eggs enters the pond water ([Lun et al., 2005](#)). Also, in Laos, 95.5% of houses in some rural villages in Bolikhhamxay Province do not have a latrine, and more than half of the village people use animal and/or human faeces as fertilizer ([Hohmann et al., 2001](#)); 3) habit to eat raw or undercooked freshwater fish; 4) freshwater aquaculture is developing rapidly, but adequate testing of fish products is lacking ([Fang et al., 2007](#)); 5) dinner-set contamination from infected fish ([Fang et al., 2007](#)); and 6) the absence of systematic control activities to limit transmission in many endemic areas ([Fang et al., 2007](#)).

### 1.2.3 Persistency and latency

It has been reported that *C. sinensis* may survive up to 26 years in a human host, as has been shown in a Chinese immigrant living in Australia ([Attwood & Chou, 1978](#)). The life expectancy of *O. viverrini* is approximately 10 years ([Sithithaworn & Haswell-Elkins, 2003](#)).

## 2. Cancer in Humans

### 2.1 Cholangiocarcinoma

#### 2.1.1 *Opisthorchis viverrini*

The Working Group of the previous *IARC Monograph* on liver flukes ([IARC, 1994](#)) evaluated infection with *O. viverrini* based on a dozen of descriptive studies (case reports, cases series, and correlation studies), and three cross-sectional or case-control studies ([Kurathong](#)

**Table 2.1 Descriptive study of *Opisthorchis viverrini* and liver cholangiocarcinoma**

Reference	Area and period of study	Measure of exposure to Ov	Number of subjects	Egg positivity	Association	Comments
<a href="#">Sriamporn et al. (2004)</a> Thailand	20 districts in Khon Kaen province 1990–2001	Stool microscopy	18393	Adjusted proportion of Ov-infected subjects, by age and sex ( $\geq 35$ -yr-old)	Truncated incidence of CCA (age 35–69 yr)	The Pearson's correlation coefficient (r) for the overall districts was 0.009.
			1122	10.5	93.8 (Nam Phong)	Results reported for selected districts with more than 1000 tested for Ov
			1026	13.4	114.9 (Phon)	
			3884	21.5	288.6 (Mancha Khiri)	
			1003	25.7	135.7 (Muang)	
			4059	29.9	317.6 (Chonnabot)	

CCA, cholangiocarcinoma; Ov, *Opisthorchis viverrini*; yr, year or years.

[et al., 1985](#); [Parkin et al., 1991](#); [Haswell-Elkins et al., 1994](#)), which demonstrated a positive association between infection with *O. viverrini* and cholangiocarcinoma.

Currently, primary liver cancer is the leading cancer in Thailand in men (annual standardized ratio [ASR], 33.4/100000 population), and the third in women (ASR, 12.3/100000) ([Khuhaprema & Srivatanakul, 2007](#)), with cholangiocarcinoma being the predominant type. In addition, the highest incidence of liver cancer (ASR of up to 113.4/100000 in men) is found in the north-eastern regions where *O. viverrini* is endemic, and is 20 times higher than that in the south of Thailand where *O. viverrini* is rare ([Sripa & Pairojkul, 2008](#)). Furthermore, the proportion of histologically verified cases of cholangiocarcinoma in men diagnosed with liver cancer in the north-eastern regions has been reported to be as high as 85.5% compared to 10% in the south ([Khuhaprema & Srivatanakul, 2007](#)). A recent correlation study ([Sriamporn et al., 2004](#)) found a significant positive association between the incidence cases of cholangiocarcinoma from the cancer registry and *O. viverrini* infection in Khon Kaen, a province in north-east Thailand,

with the highest incidence of cholangiocarcinoma cancer in the world (see [Table 2.1](#)).

[Table 2.2](#) presents the results from all the available cross-sectional and case-control studies, all conducted in Thailand (descriptive studies are not presented). The odds ratios ranged from 1.3–27.1. The highest relative risk, reported by [Honjo et al. \(2005\)](#), was adjusted for sex, age, residence, alcohol consumption, and smoking. [Haswell-Elkins et al. \(1994\)](#) reported adjusted prevalence odds ratios (POR) of 1.7 in the light infection group, 3.2 in the moderate infection group, and 14.1 in the heavy infection group (based on 14 exposed cases stratified by intensity of infection).

## 2.1.2 *Clonorchis sinensis*

The Working Group of the previous *IARC Monograph* on liver flukes ([IARC, 1994](#)) evaluated infection with *C. sinensis* as probably carcinogenic to humans (Group 2A), based on nine case series and three case-control studies ([Gibson, 1971](#); [Kim, 1974](#); [Chung & Lee, 1976](#)). Since then, several studies have been published, and are summarized here.

**Table 2.2 Cross-sectional and case-control studies on *Opisthorchis viverrini* infection and cholangiocarcinoma**

Reference, study location and period	Characteristics of cases	Characteristics of controls	Detection method	Exposure categories	No. of exposed cases (%)	Relative risk (95%CI)	Adjusted potential confounders	Comments
<a href="#">Kurathong et al. (1985)</a> Thailand 1981–83	13 cases clinically diagnosed and confirmed by ultrasound biopsy	479 in- and out-patients without hepatobiliary tract diseases	Stool specimens Stool, bile duct aspirate or liver biopsy	Eggs in stool Eggs in any tissue or fluid	9/13 13/13	[0.94 (0.26–4.22)] <sup>a</sup> X <sup>2</sup> test p<0.05 [10.95 (1.10–108.48)]		
<a href="#">Parkin et al. (1991)</a> North-east Thailand 1987–88	103 consecutive patients from 3 hospitals	103 controls matched to cases by sex, age, residence, hospital, non-malignant diseases not related to tobacco or alcohol	Antibody titre by ELISA		NR	5.0 (2.3–11.0)	Consumption of 'sticky rice' and areca nut chewing	
<a href="#">Haswell-Elkins et al. (1994)</a> Thailand, 1990–91	15 cases of suspected CCA among 1807 patients screened by ultrasound scanning		Stool microscopy	Ov eggs+ 0 EPG ≤1500 EPG 1500–6000 >6000 EPG	1 3 4 7	1.0 (ref) 1.7 (0.2–16.3) 3.2 (0.4–30) 14.1 (1.7–119)	Age, sex, district	
<a href="#">Honjo et al. (2005)</a> Thailand, 2000	129 cases of CCA diagnosed by ultrasound, 9 with histology, serology and fetoprotein	129 population-based controls matched by age, sex, residence	Serology	Anti-Ov Ab+ ≤0.200	65	27.09 (6.30–116.57)	Smoking, alcohol, age, sex, residence	

<sup>a</sup> Continuity correction was applied to calculate OR

CCA, cholangiocarcinoma; ELISA, enzyme-linked immunosorbent assay; EPG, egg per gram; NR, not reported; Ov, *Opisthorchis viverrini*

**Table 2.3 Descriptive study of *Clonorchis sinensis* infection and cholangiocarcinoma**

Reference, study location and period	Area	Number of subjects	Measure of exposure to Cs	Egg positivity (%)	Association	Comments
<a href="#">Lim et al. (2006)</a> Korea 2000–04	Three areas by endemicity		Faecal egg		Incidence of cancer <sup>a</sup> per 100000 persons	In the survey, alcohol drinking and raw freshwater fish were significant risk factors for egg positivity (adjusted for age)
	Low (Chuncheon)	659		14 (2.1%)	0.3	
	Medium (Chungju)	568		44 (7.8%)	1.8	
	High (Haman)	1942		607 (31.3%)	5.5	

<sup>a</sup> drawn from cancer registry in 1999–2001 (ICD-10, C22.1)

Cs, *Clonorchis sinensis*

The incidence of primary liver cancer in the Republic of Korea is the highest in the world (ASR, 44.9 in men and 12.0 in women), with a proportion of microscopically verified cases of cholangiocarcinoma of 22.3% and 36.1% in men and women, respectively ([Curado et al., 2007](#)). According to the Korean Cancer Registry, the incidences of cholangiocarcinoma vary by geographic area, with up to 4-fold differences ([Shin et al., 2008](#)). The region with the highest incidence (7.2/100000 in men) was reported to be that with the highest prevalence of *C. sinensis* infection in a nationwide survey conducted 20 years ago ([Seo et al., 1981](#)).

A recent correlation study from the Republic of Korea showed a high correlation between the endemicity of *C. sinensis* infection with the incidence as well as mortality of cholangiocarcinoma ([Lim et al., 2006](#); [Table 2.3](#)).

Since the previous IARC Monograph, two case series from China have been published, both supporting a relationship between *C. sinensis* and cholangiocarcinoma ([Cheng et al., 2000](#); [Wang et al., 2003](#); [Table 2.4](#)). Furthermore, three case-control studies have been published from the Republic of Korea ([Table 2.5](#)). All three showed significant positive associations between *C. sinensis* infection and cholangiocarcinoma. The

study by [Choi et al. \(2006\)](#) reported an (unadjusted) odds ratio for any evidence of infection of 7.3 (95%CI: 3.9–13.3). [Shin et al. \(1996\)](#) reported an odds ratio of 2.7 (95%CI: 1.1–6.4), adjusted for alcohol consumption, smoking, hepatitis B and C, and [Lee et al. \(2008\)](#) found an odds ratio of 13.6 (95%CI: 6.1–30.3) after adjusting for hepatitis B, alcohol consumption, and liver cirrhosis.

In two of the studies ([Shin et al., 1996](#); [Choi et al., 2006](#)), higher odds ratios were reported for evidence of past *C. sinensis* infection (i.e. based on positive history, serology, skin test, radiology) compared to current infection (i.e. based on positive stool microscopy or pathology).

## 2.2 Hepatocellular carcinoma

### 2.2.1 *Opisthorchis viverrini*

A correlation analysis of the prevalence of *O. viverrini* infection and liver cancer incidence, conducted in five regions with different frequencies of cholangiocarcinoma and hepatocellular carcinoma (HCC), showed little geographic variation in the incidence of HCC, with a correlation of  $-0.37$  ( $P = 0.54$ ) for antibody titre  $\geq 1:40$ , and of  $0.02$  ( $P = 0.96$ ) for faecal egg count ([Srivatanakul et al., 1991a](#)).

**Table 2.4 Case series and case reports of cholangiocarcinoma associated with *Clonorchis sinensis***

Reference and study location	Case history	Clinical manifestations	Treatment	Pathological diagnosis
<a href="#">Liang (1995)</a> Guangdong Province, People's Hospital, China	27 CCA cases with Cs 24 CCA cases without Cs	The same CT findings were observed in the cases with or without Cs	Operation	Development of CCA
<a href="#">Kim et al. (1999)</a> Korea University Hospital, Seoul, Republic of Korea	69-yr-old man Eating raw freshwater fish, pulmonary tuberculosis	5-kg weight loss, moderate dilatation of left IHD and CBD, obstruction of proximal left HD, Cs eggs + by left HD cytology, CBD polyp	Hepaticojejunostomy, partial resection of left proximal HD Pzq, 75 mg/kg	Papillary hyperplasia
<a href="#">Cheng et al. (2000)</a> Lecong Hospital, China	35 CCA cases (28 positive for Cs)	Cs egg+, abdominal pain, weight loss	Operation (14 cases)	CT finding pathology proven
<a href="#">Kim et al. (2000)</a> Yonsei Medical Center, Seoul, Republic of Korea	64-year-old man	Abdominal pain, Cs worms were removed by percutaneous transbiliary drainage, CBD polyp	Pancreaticoduodenectomy	Composite small cell neuroendocrine carcinoma and adenocarcinoma
<a href="#">Wang et al. (2003)</a> Guangzhou, Zhujiang Hospital, China	29 CCA cases	Clonorchiasis 100%	Operation	Average 20 years of liver fluke infection
<a href="#">Shim et al. (2004)</a> Yonsei Medical Center, Seoul, Republic of Korea	69-year-old man Diabetes, cured tuberculosis	Abdominal pain, 8-cm-sized mass in right liver	Right hepatectomy, recurred metastasis	Mucinous adenocarcinoma

CBD, common bile duct; CCA, cholangiocarcinoma; Cs, *Clonorchis sinensis*; CT, computerized tomography; HD, hepatic duct; IHD, intrahepatic duct; Pzq, praziquantel

**Table 2.5 Cross-sectional and case-control studies on *Clonorchis sinensis* infection and cholangiocarcinoma**

Reference, study location and period	Characteristics of cases	Characteristics of controls	Detection method	Exposure categories	No. of exposed cases (%)	Relative risk (95%CI)	Adjusted potential confounders	Comments
Gibson (1971) Hong Kong SAR 1964–66	17 cases among 1484 autopsies, including 83 patients with HCC	1384 autopsies without CCA or HCC	Gross examination at autopsy		11/17	[3.1 (0.1–8.4)]		
Kim (1974) Low and high prevalence areas, Republic of Korea 1961–72	54 cases among 1843 records of autopsy and surgical specimens with liver diseases	1348 autopsies or surgery with non-cancerous liver lesions	Stool samples, liver tissue		NR	[6.5 (3.7–12)]		
Chung & Lee (1976) Pusan, Republic of Korea 1963–74	36 consecutive cases diagnosed in 2 hospitals	559 subjects admitted to hospital, with liver diseases	Stool specimen		NR	[6.0 (2.8–13)]		
Shin et al. (1996) Pusan Paik Hospital, Busan, Republic of Korea 1990–93	41 CCA cases	203 patients of other diseases (Control I), 203 healthy controls (Control II)	Stool microscopy	Cs eggs+ (current) Liver fluke history (past)	33.3 7.3	2.7 (1.1–6.4) 5.0 (1.2–21.3)	Age, sex, HBsAg, anti-HCV, drinking and smoking history, hepatitis history and SES	

**Table 2.5 (continued)**

Reference, study location and period	Characteristics of cases	Characteristics of controls	Detection method	Exposure categories	No. of exposed cases (%)	Relative risk (95%CI)	Adjusted potential confounders	Comments
<a href="#">Choi et al. (2006)</a> Republic of Korea 2003–04	185 CCA cases identified from 1 hospital in Seoul: 51 intrahepatic CCA, 53 hilar CCA, and 81 extrahepatic CCA  51 cases of intrahepatic CCA among the 185 cases above	185 patients with non-hepatobiliary diseases in the Department of Gastroenterology at same hospital	Stool microscopy, pathology, serology, radiology, history	Stool eggs + Pathology + Serology + Skin test + Radiology + History + Any evidence +	3 13 25 19 156 94 167	0.6 1.6 2.3 1.7 8.6 2.4 7.3 (3.9–13.3)		Age, sex, and area
<a href="#">Lee et al. (2008)</a> Seoul, Republic of Korea 2000–04	622 histologically confirmed intrahepatic CCA cases	2488 healthy controls admitted for routine examinations	Histology, stool, microscopy, serology, radiology, history	Stool eggs +	26	13.6 (6.1–30.3)	Age, sex, date of visit	

CCA, cholangiocarcinoma; HCC, hepatocellular carcinoma; NR, not reported; SAR, Special Administrative Region

**Table 2.6 Cross-sectional and case–control studies on infection with liver flukes and hepatocellular carcinoma**

Reference, study location and period	Characteristics of cases	Characteristics of controls	Detection method, fluke	Exposure categories	No. of exposed cases (%)	Relative risk (95%CI)	Adjusted potential confounders	Comments
<i>Opisthorchis viverrini</i>								
<a href="#">Kurathong et al. (1985)</a> Thailand 1981–83	Cases among 72 patients with hepatobiliary tract diseases: 12 clinically diagnosed 5 biopsy proven	479 in- and out-patients without hepatobiliary diseases	Stool specimen	Eggs in stool			Crude ratio	
					9/12	[1.21 (0.30–7.07)]		
<a href="#">Srivatanakul et al. (1991b)</a> North-east Thailand 1987–88	65 patients living and born in the area	65 patients with non-malignant diseases matched for sex, age, residence, hospital	ELISA for Ov antibody	Anti-OV titre≥1/40	4/5	[1.62 (0.16–80.28)]		
<i>Clonorchis sinensis</i>								
<a href="#">Gibson (1971)</a> Hong Kong SAR, China 1964–66	83 cases of HCC in a consecutive series of 1484 autopsies	1384 autopsies without HCC or CCA	Gross examination	Clonorchiasis	24	[0.73 (0.45–1.2)]	Age, sex	Expected proportion infected was 35%
<a href="#">Kim (1974)</a> Seoul & Pusan, Republic of Korea 1961–72	386 and 109 cases in low and high prevalence areas, respectively; histologically proven cases among records of autopsies and surgical specimens	1061 and 287 subjects with liver diseases from low and high prevalence areas, respectively	Examination of liver tissue or stool samples	Cs infection	423	[1.2 (0.80–1.7)]		
<a href="#">Chung &amp; Lee (1976)</a> Pusan, Republic of Korea 1963–74	206 cases in consecutive series of 368 cases of primary liver carcinoma	559 subjects admitted to hospitals without liver disease	Stool specimens	Eggs in stool	170	1.1 (0.65–1.7)	None (crude odds ratio)	Overlap with study by <a href="#">Kim (1974)</a> for cases from Pusan

CCA, cholangiocarcinoma; Cs, *Clonorchis sinensis*; HCC, hepatocellular carcinoma; NR, not reported; Ov, *Opisthorchis viverrini*

One cross-sectional study ([Kurathong et al., 1985](#)) and one case-control ([Srivatanakul et al., 1991b](#)) study were carried out in north-east Thailand to evaluate the association between *O. viverrini* infection and the risk for HCC ([Table 2.6](#)). Neither study showed a significant association.

### 2.2.2 *Clonorchis sinensis*

A few studies have evaluated the association between *C. sinensis* infection and the risk for HCC ([Table 2.6](#)). One study was conducted in the Hong Kong Special Administrative Region ([Gibson, 1971](#)) and found no association.

Three studies were conducted in the Republic of Korea; one ([Kim, 1974](#)) in two separate regions, of low and high prevalence of *C. sinensis* infection, respectively; the other two studies were conducted in Pusan, one of the areas with the highest prevalence of *C. sinensis* infection ([Chung & Lee, 1976](#); [Shin et al., 1996](#)). In the two earlier studies, no increased risks for HCC were observed [from crude odd ratios]. In the most recent study ([Shin et al., 1996](#)), neither *C. sinensis* eggs in stool samples (OR, 2.7; 95%CI: 0.9–7.7) nor a history of liver fluke infection (OR, 2.6; 95%CI: 0.6–11.3) were significantly associated with HCC in a conditional logistic regression analysis adjusted for socioeconomic status ([Table 2.6](#)).

### 2.3 Cofactors

The intake of raw freshwater fish is traditionally combined with alcohol consumption in the Republic of Korea. In this country, one study reported a significantly increased risk of *C. sinensis* infection with alcohol consumption ([Lim et al., 2006](#)).

[Shin et al. \(1996\)](#) reported odds ratios of 4.6 (95%CI: 1.4–15.2) for heavy alcohol consumption, 5.0 (95%CI: 1.2–21.3) for a history of liver fluke infection, and 2.7 (95%CI: 1.1–6.3) for *C.*

*sinensis* in stool samples, all adjusted for the other factors. [Lee et al. \(2008\)](#) reported odds ratios of 6.6 (95%CI: 4.8–9.2) for heavy alcohol consumption and 13.6 (95%CI: 6.1–30.3) for *C. sinensis* in stool samples. [Honjo et al. \(2005\)](#) found odds ratios of 4.31 (1.12–16.57) for regular alcohol drinking and 27.09 (95%CI: 6.3–116.6) for presence of *O. viverrini* by antibody detection. No specific interactions between alcohol drinking and liver fluke infection were estimated in any of these studies.

## 3. Cancer in Experimental Animals

The association between *O. viverrini* and *C. sinensis* infections and cancers was extensively studied in experimental animal models in the 1970s and 1980s. All of these studies were reviewed in the previous *IARC Monograph* ([IARC, 1994](#)). Only one additional study has been published since ([Wang et al., 1994](#)).

[Thamavit et al. \(1978\)](#) first reported that hamsters given *O. viverrini* and *N*-nitrosodimethylamine in drinking-water could develop cholangiocarcinoma. The gross morphology and histology of the experimentally induced cholangiocarcinomas are similar to those found in humans, and are considered a suitable model for the study of cholangiocarcinoma. Following this experiment, many studies on the administration of *N*-nitroso compounds (*N*-nitrosodimethylamine or *N*-nitrosodihydroxydi-*n*-propylamine) in combination with *O. viverrini* infection were performed, and all resulted in increased incidences of cholangiocarcinoma. Intraperitoneal administration induced cholangiocarcinoma but also hepatic neoplastic nodules, and a few HCCs. All of these studies clearly established the role of *O. viverrini* in promoting cholangiocarcinoma in hamsters ([Flavell & Lucas, 1982, 1983](#); [Thamavit et al., 1987a, b, 1988a, b, 1993, 1994](#)).

**Table 3.1 Studies in experimental animals exposed to liver flukes (*Opisthorchis viverrini* and *Clonorchis sinensis*)**

Species, strain (sex) Duration Reference	Dosing regimen, Animals/group at start	Incidence of tumours	Significance	Comments
<b><i>Opisthorchis viverrini</i></b>				
Hamster, Syrian golden (M) 23 wk <a href="#">Thamavit et al. (1978)</a>	Ov 100 MC, NDMA 0.0025% at Week 4 in drinking-water for 10 wk Group 1: Untreated control (n=18) Group 2: NDMA alone (n=21) Group 3: Ov (n=18) Group 4: Ov+ NDMA (n=21)	CCA: Group 1: 0/18 Group 2: 0/21 Group 3: 0/18 Group 4: 15/15	[p <0.001] <sup>a</sup>	This is the first experiment of NDMA + liver fluke-induced CCA in the hamster
Hamster, Syrian golden (M) 490 d <a href="#">Flavell &amp; Lucas (1982, 1983)</a>	Ov 50 MC, NDMA 1.6 mg single oral dose Group 1: Ov+NDMA (41 days after infection) (n=50) Group 2: NDMA+Ov (96 h later) (n=46) Group 3: NDMA (n=30) Group 4: Ov (n=50)	CCA: Group 1: 5/50 (10%) Group 2: 9/46 (20%) Group 3: 0/30 (0%) Group 4: 0/50 (0%)	[NS] <sup>a</sup> [p <0.01]	High mortality in Ov+NDMA groups. Tumours found in right lobe. No significant difference between 2 combination groups for tumour latency
Hamster, Syrian golden (M) 40 wk <a href="#">Thamavit et al. (1987a)</a>	Ov 12.5, 25, 50 or 100 MC NDMA 6 or 12.5 mg/L in drinking-water for 10 wk (2 wk later) Group 1: Untreated Groups 2: Ov 12, 25, 50 or 100 MC Groups 3: NDMA 3, 6 or 12 mg/L Groups 4: NDMA 6 or 12.5 mg/L + Ov 12, 25, 50 or 100 MC Total n=280	Group 1: No CCA Groups 2 and 3: No CCA in Groups 2 or 3 at doses of 3 or 6 mg/L Groups 3: CCA: 2/17 (12%) NDMA 12.5 mg/L Groups 4: CCA: 4/10, 7/10, 9/15, 13/19, 8/15, 10/17, 16/19, 14/15 in NDMA+Ov, respectively	p <0.01, all groups 4 (versus relevant group 3)	Cholangiofibrosis was also observed in Groups 3 and 4. Number of animals per group at start unspecified

**Table 3.1 (continued)**

Species, strain (sex) Duration Reference	Dosing regimen, Animals/group at start	Incidence of tumours	Significance	Comments
Hamster, Syrian (F) 32 wk <a href="#">Thamavit et al. (1987b)</a>	OV 60 MC, NDEA 10, 20 or 40 mg/L in drinking-water for 12 wk Group 1: Untreated control [n=20] Group 2: Ov only [n=20] Groups 3: Ov + NDEA (4 wk later) [n=20–30]  Groups 4 NDEA only [n=20–25] [Total n= 180]	Group 1: 0/20 Group 2: 0/20 Groups 3: hepatocellular nodules, 12/19 with 2.5 nodules/animal (OV+NDEA20), 23/25 with 7.1 nodules/animal (Ov+NDEA40). Groups 4: 3/19 with 0.2 nodules/animal (NDEA20), 9/21 with 0.9 nodules/animal (NDEA40)	[p<0.01] <sup>a</sup> , [p<0.01]	Hamsters in group 3 showed high incidence of cholangiofibrosis. One CCA observed in Group 3 (OV+NDEA20)
Hamster, Syrian (M) 22 wk <a href="#">Thamavit et al. (1988a)</a>	Ov 100 MC, NDHDPA 1000 mg/kg bw (two i.p. injections at 2 wk intervals) 2 wk later Group 1: Ov 100 MC + NDHDPA Group 2: DHPN Group 3: Ov 100 MC Group 4: Untreated control	Group 1: CCA, 6/19; liver neoplastic nodules, 9/19 Group 2: CCA, 0/20; liver neoplastic nodules, 0/20 Group 3: CCA, 0/14; liver neoplastic nodules, 0/14 Group 4: CCA, 0/14; liver neoplastic nodules, 0/14	p <0.05; p <0.01 (versus Group 2)	Initial number of animals not specified

**Table 3.1 (continued)**

Species, strain (sex) Duration Reference	Dosing regimen, Animals/group at start	Incidence of tumours	Significance	Comments
Hamster, Syrian (M) 30 wk <a href="#">Thamavit et al. (1988b)</a>	Ov 100 MC, 0.1% Sodium nitrite and 0.1% aminopyrine in the drinking-water for 8–12 wk Group 1: Untreated control Group 2 0.1% Sodium nitrite Group 3: 0.1% Aminopyrine Group 4: Sodium nitrite and Aminopyrine Group 5: Ov 100 MC Group 6: Ov 100 MC + sodium nitrite (4 wk later) Group 7: Ov 100 MC + aminopyrine (4 wk later) Group 8: Ov 100 MC + sodium nitrite and aminopyrine (4 wk later) Total n=150	Group 8 and 4: 8/18, 2/17 hepatocellular nodules and 14/18, 3/17 CCA, respectively; no tumours observed in group 1, 2, 3, 5, 6 and 7	P<0.05 (versus Group 4) and P<0.01 (versus Group 4)	Prior infection with Ov induced more inflammation and bile duct proliferation and is associated with a higher incidence of hepatocellular nodule, cholangiofibrosis and CCA
Hamster Syrian (M) 52 wk <a href="#">Moore et al. (1991)</a>	Ov 80 MC, NDHDPA 500 mg/bw (3 i.p. injections at 1 wk interval) 16 wk later Group 1: Ov 80 MC + NDHDPA (n=40) Group 2: NDHDPA (n=30) Group 3: Ov 80 MC (n=20) Group 4: Untreated control (n=10)	Group 1: CCA, 8/16 Group 2: CCA, 0/16 Group 3: CCA, no tumours Group 4: CCA, no tumours	[p=0.001] <sup>a</sup> (versus Group 2)	

**Table 3.1 (continued)**

Species, strain (sex) Duration Reference	Dosing regimen, Animals/group at start	Incidence of tumours	Significance	Comments
Hamster, Syrian (F) 38 wk <a href="#">Thamavit et al. (1993)</a>	NDHDPA 1000 mg/kg bw (i.p.) at 2 wk intervals, Ov 60 MC, PZ 250 mg/kg bw suspended in corn oil at Weeks 4, 12 or 20	CCA: Group 1: 4/22 (18%) Group 2: 6/22 (28%) Group 3: 10/16 (63%)  Group 1: NDHDPA +Ov+PZ(4) Group 2: NDHDPA +Ov+PZ(12) Group 3: NDHDPA +Ov+PZ(20) Group 4: NDHDPA +Ov Group 5: NDHDPA Group 6: Ov Group 7: Untreated  Total n= 205, 25–40 animals/group	P<0.05 (between Group 1 and Group 4); [p=0.024 between Group 4 and 5] <sup>a</sup>	It was found that whereas praziquantel administration at the later two time points was ineffective at reducing cholangiocellular lesions. Significant reduction only being evident in hamsters treated 4 wk after parasite infestation. The findings thus indicate that promotion of DHPN-initiated bile duct carcinogenesis by opisthorchiasis is both rapid and to a large degree irreversible
Hamster, Syrian (M) 45 wk <a href="#">Thamavit et al. (1994)</a>	Ov 80 MC, NDMA 20 mg/kg bw i.p. injection  Group 1: NDMA + Ov (19 d later) (n=50) Group 2: NDMA (n=25) Group 3: Ov (n=15) Group 4: Untreated control (n=15)	Group 1: 19/43, CCA; 15/43, mucinous cystadenomas; 2/43, HCC.  No such tumours in Group 2 (0/20), 3 (0/15) and 4 (0/15).	[p <0.001] <sup>a</sup> , [p <0.005], [NS]	

**Table 3.1 (continued)**

Species, strain (sex) Duration Reference	Dosing regimen, Animals/group at start	Incidence of tumours	Significance	Comments
<b><i>Clonorchis sinensis</i></b>				
Rat, Fischer F334 (M) 40 wk <a href="#">Jang et al. (1990)</a>	Cs 60 MC, NDMA 25 mg/L in the drinking-water for 8 wk Group 1 Cs + NDMA (4 wk later) ( <i>n</i> =20) Group 2: Cs + NDMA at the same time ( <i>n</i> =20) Group 3: NDMA + Cs 1 wk later ( <i>n</i> =20) Group 4: NDMA ( <i>n</i> =19) Group 5: Cs ( <i>n</i> =10) Group 6: Untreated control ( <i>n</i> =12) [Total <i>n</i> =101]	0/101		No malignant tumours seen in the rat model. Animals infected before NDMA administration had significantly ( <i>p</i> < 0.05) increased numbers of glutathione S-transferase P-positive liver foci. No such effect was seen when animals were infected during or after exposure to NDMA
Hamster, Syrian golden (F) 54 wk <a href="#">Iida (1985)</a>	2-AAF 0.03% in the diet for 40 wk, Cs 40 MC Group 1: 2-AAF + Cs ( <i>n</i> =60) Group 2: 2-AAF ( <i>n</i> =50)	CCA: Group 1: 11/14 animals that lived beyond Week 25 Group 2: 6/17 animals that lived beyond Week 25	<i>p</i> <0.05	In group 1, of 11 animals with liver tumours, 5 had metastases. No metastases were observed in Group 2
Hamster, Syrian golden (NR) 11 wk <a href="#">Lee et al. (1993)</a>	NDMA 15 mg/L in the drinking-water for 8 wk, Cs 10 MC. Group 1: NDMA + Cs 10 MC (7 d later) ( <i>n</i> =12) Group 2: NDMA ( <i>n</i> =12) Group 3: Cs ( <i>n</i> =12) Group 4: Untreated control ( <i>n</i> =12) Total <i>n</i> =48	Group 1: 6/8 CCA and 8/8 cholangiofibromas Group 2: 2/12 cholangiofibromas Group 3: 0/12 Group 4: 0/12	[ <i>p</i> <0.001] <sup>a</sup> , CCA and cholangiofibromas	In the hamsters that received either DMN or <i>C. sinensis</i> alone, the livers showed only hyperplastic changes of the bile duct epithelial cells

**Table 3.1 (continued)**

Species, strain (sex) Duration Reference	Dosing regimen, Animals/group at start	Incidence of tumours	Significance	Comments
Hamster, Syrian golden (NR) 13 wk <a href="#">Lee et al. (1994)</a>	NDMA 15 mg/L in the drinking-water for 4 wk, Cs 15 MC, Praziquantel 200 mg/kg bw daily for 3 d Group 1: NDMA+ Cs (1 wk later) + praziquantel (5 wk later) Group 2: Cs (5 wk) + Praziquantel + NDMA (3 d later after treatment with praziquantel) Group 3: Cs + NDMA at the same time Group 4: NDMA alone Group 5: Cs alone Group 6: Untreated control Total n= 90, 15 animals/group	CCA: Group 1: 3/15 Group 2: 0/15 Group 3: 11/15 Group 4: 0/15 Group 5: 0/15 Group 6: 0/15	[p<0.001] <sup>a</sup> , versus all groups	Synergistic effect of <i>Clonorchis</i> infection and NDMA promoted the development of CCA
Hamster, Syrian golden (unspecified) 21 wk <a href="#">Wang et al. (1994)</a>	Cs 20 MC, NDMA 25 mg/L in the drinking water for 17 wk (30 d later)	HCC; CCA Group A: Cs + NDMA Group B: NDMA Group C: Cs Group D: Untreated	Group A: 4/11; 1/11 Group B: 3/15; 0/15 Group C: 0/12; 0/12 Group D: 0/15; 0/15	[NS] <sup>a</sup> The authors concluded that <i>C. sinensis</i> "may" promote the formation of HCC though the comparison between Group A and B is not significant. Only one CCA in Group A.

<sup>a</sup> Fisher Exact test

2-AAF, 2-Acetylaminofluorene; bw, body weight; CCA, cholangiocarcinoma; Cs, *Clonorchis sinensis*; d, day or days; DHPN, 2,2'-dihydroxy-di-n-propylnitrosamine; DMN, dimethylnitrosamine; HCC, hepatocellular carcinoma; i.p., intraperitoneal; MC, Metacercariae; NDHDPA, N-Nitrosodihydroxidi-n-propylamine; NDMA, N-Nitrosodimethylamine; NR, not reported; NS, not significant; Ov, *Opisthorchis viverrini*; PZ, Praziquantel; SAR, Special Administrative Region; wk, week or weeks

Similar experiments were also performed following *C. sinensis* infection in combination with 2-acetylaminofluorene or *N*-nitroso compounds (*N*-nitrosodimethylamine or *N*-nitrosodihydroxydi-*n*-propylamine) in hamsters (Iida, 1985; Lee et al., 1993, 1994; Wang et al., 1994), and rats (Jang et al., 1990). Three of these (Iida, 1985; Lee et al., 1993, 1994) supported the role of *C. sinensis* in promoting cholangiocarcinoma in hamsters.

See [Table 3.1](#).

## 4. Other Relevant Data

### 4.1 Pathological changes in vivo

The main histopathological features of liver fluke infection both in man and the rodent models are inflammation, epithelial desquamation, epithelial and adenomatous hyperplasia, goblet cell metaplasia, periductal fibrosis, and granuloma formation. Liver fluke infection in humans may also result in cholangiocarcinoma, but not in rodents unless they are also given a chemical carcinogen (IARC, 1994; Sripa, 2003; Rim, 2005; Sripa et al., 2007; see also Section 3).

Liver fluke antigens stimulate both inflammatory and hyperplastic changes in the bile ducts. The liver fluke excretes or secretes metabolic products from the tegument and excretory openings into the bile *in vivo* or culture medium *in vitro*, some of which are highly immunogenic (Wongratanacheewin et al., 1988; Sripa & Kaewkes, 2000; Choi et al., 2003). The metabolic products themselves, apart from inducing host immune responses, may be toxic to or interact with the biliary epithelium (Sripa, 2003). Sripa & Kaewkes (2000) demonstrated that *O. viverrini* excretory–secretory (ES) antigens can be detected in both the parasite and biliary epithelium. The presence of *O. viverrini* ES antigens in the biliary epithelium in association with severe inflammation has also been seen in the small

bile ducts, which the flukes cannot inhabit (Sripa & Kaewkes, 2000). Hong et al. (1993) observed strong stimulation of the proliferation of bile duct epithelial cells located at the base of the mucosal layer in Sprague-Dawley rats infected by *C. sinensis*. This finding was directly related to hyperplasia of the bile duct epithelium that may have been due to direct and local stimulation by *C. sinensis*.

### 4.2 Carcinogenicity of liver fluke infections

#### 4.2.1 Cell proliferation *in vitro*

Adult *O. viverrini* worms were co-cultured with mouse NIH-3T3 fibroblasts. Even though worms and fibroblasts were separated by Transwell membrane, fibroblast proliferation was stimulated more than 4-fold. Moreover, *O. viverrini* ES products increased cell proliferation by stimulating the expression of phosphorylated retinoblastoma (pRB) and cyclin D1, the key proteins in driving cells through the G1/S transition point of the cell cycle. This led to the induction of cells going into the S-phase of the cell cycle (Thuwajit et al., 2004). In similar experiments with *C. sinensis*, ES products, and the human embryonic kidney epithelial cell line HEK293, the ES products induce HEK293 cell proliferation, associated with the upregulation of cyclin E and the transcription factor E2F1 (Kim et al., 2008a). Furthermore, *C. sinensis* ES products upregulate the phosphorylation of pRB and *N*-nitrosodimethylamine (NDMA) upregulates cyclin-dependent kinases, and both synergistically drive the cells to proliferate (Kim et al., 2008b). An anti-apoptotic effect of *C. sinensis* ES products in human cholangiocarcinoma cells has been reported (Kim et al., 2009).

Gene microarrays were used to explore transcriptional changes induced in NIH-3T3 murine fibroblasts co-cultured with *O. viverrini* ES products. mRNAs encoding certain

growth-promoting proteins such as transforming growth factor (TGF), PKC, EPS 8 and TGF- $\beta$  1I4, that are downstream of epidermal growth factor (EGF) or TGF- $\beta$ -mediated signalling, were found to be overexpressed ([Thuwajit et al., 2006](#)). Moreover, human cholangiocarcinoma cell line (KKU-100) underwent excessive proliferation upon stimulation with *O. viverrini* worms ([Sripa, 2003](#)). The promotion of proliferation *in vitro* is consistent with the histopathological findings of hyperplasia of biliary epithelial cells in opisthorchiasis and clonorchiasis ([Bhamarapravati et al., 1978; Sripa & Kaewkes, 2000; Rim, 2005](#)).

#### *4.2.2 Oval cell proliferation and differentiation *in vivo**

Oval cells are typically seen in response to certain liver injuries, and more than likely represent progenitor cells with the potential to differentiate along biliary or hepatocytic lineages, including into hepatic neoplasms ([Sell & Leffert, 2008](#)). [Lee et al. \(1997\)](#) reported the appearance of increased numbers of periductal oval cells in the portal and/or periportal areas of hamster liver infected with *C. sinensis* and administered NDMA.

#### *4.2.3 DNA damage and adduct formation *in vivo**

Diffuse nitrosative and oxidative DNA damage (8-nitroguanine and 8-oxo-7,8-dihydro-2'-deoxyguanosine [8-oxodG]) has been reported in the biliary epithelium of hamsters infected with *O. viverrini* ([Pinlaor et al., 2003](#)). These DNA lesions still persisted for at least 180 days post-infection. Moreover, repeated infections with liver flukes result in enhanced biliary DNA damage ([Pinlaor et al., 2004a, b](#)). This may be explained by the fact that repeated infection increased inducible nitric oxide synthase (iNOS) expression in the bile duct epithelium. The DNA damage in infected biliary cells is probably a result

of the inflammatory response caused by *O. viverrini* because 8-nitroguanine and 8-oxodG disappear after praziquantel treatment ([Pinlaor et al., 2006](#)). However, in promoting parasite antigen dispersal, treatment with praziquantel may transiently increase inflammation, in association with increased NF- $\kappa$ B and iNOS expression in the bile duct epithelium and inflammatory cells, and elevated levels of plasma nitrate, of end-products of nitric oxide, and of malondialdehyde in the treated hamsters ([Pinlaor et al., 2008](#)).

Individuals infected with *O. viverrini* also show evidence of oxidative DNA damage. Urinary 8-oxodG levels were significantly higher in *O. viverrini*-infected patients ( $4.45 \pm 0.25 \mu\text{g/g}$  creatinine) than in healthy subjects ( $3.03 \pm 0.24 \mu\text{g/g}$  creatinine;  $P < 0.01$ ). This level decreases significantly 2 months after praziquantel treatment, and is comparable with levels in healthy subjects 1 year after treatment. Urinary 8-oxodG levels were significantly correlated with leukocyte 8-oxodG levels ([Thanan et al., 2008](#)).

The excretion of lipid peroxidation-derived etheno DNA adducts – 1,N<sup>6</sup>-etheno-2'-deoxyadenosine (edA) and 3,N<sup>4</sup>-etheno-2'-deoxycytidine (edC) – was measured in urine samples collected from healthy volunteers and *O. viverrini*-infected Thai subjects. Mean excreted edA and edC levels were 3–4 times higher in the urine of *O. viverrini*-infected patients and correlated with an increased level of urinary malondialdehyde, urinary nitrate/nitrite, and plasma alkaline phosphatase. After fluke elimination by treatment with praziquantel, the level of the two etheno adducts, malondialdehyde, nitrate/nitrite, and alkaline phosphatase was decreased. The promutagenic DNA etheno adducts that are thought to derive from bile duct epithelial cells may increase the risk of developing cholangiocarcinoma in *O. viverrini*-infected patients. This study highlights the biomarker potential of urinary edA and edC levels as non-invasive risk markers for developing

opisthorchiasis-related cholangiocarcinoma ([Dechakhamphu et al., 2008](#)).

[The Working Group noted that all the studies described above relate to *O. viverrini*; studies regarding DNA damage in response to *C. sinensis* infection were not available to the Working Group.]

## 4.3 Gene mutation, methylation, and altered expression in cholangiocarcinoma

### 4.3.1 *O. viverrini*-endemic areas

Differences in *Ki-RAS* mutational status have been described when comparing cholangiocarcinoma from Japanese patients (where fluke infections are very rare) with those from cholangiocarcinoma arising in patients living in areas of Thailand endemic for *O. viverrini* (the incidence of *Ki-RAS* mutation was higher in Thai patients) ([Kiba et al., 1993](#)). Hypermethylation of the promoter of the DNA mismatch repair enzyme hMLH1 has also been shown in another group of Thai patients ([Limpaliboon et al., 2005](#)). However, these studies did not specifically document liver fluke infection status in the two groups of patients.

Gene microarray transcriptional profiling of cholangiocarcinoma from Japanese versus Thai patients (again without certain knowledge of liver fluke status) led [Jinawath et al. \(2006\)](#) to propose a signature of *O. viverrini*-associated cholangiocarcinoma with an elevated expression of genes involved in xenobiotic metabolism (*UGT2B11*, *UGT1A10*, *CHST4*, *SULT1C1*) in cases from Thailand, but a lower expression of genes related to growth-factor signalling (*TGFBI*, *PGF*, *IGFBP1*, *IGFBP3*).

### 4.3.2 Studies in experimental animals

Few mutations of the *Ki-RAS* gene were observed in *O. viverrini*-NDMA-induced cholangiocarcinomas in hamsters ([Tangkawattana et al., 2008](#)), but *TP53* overexpression was reported in nearly all *O. viverrini*-induced hamster cholangiocarcinomas ([Tesana et al., 2000](#)).

[Loilome et al. \(2006\)](#) investigated the molecular mechanism of *O. viverrini*-NDMA-induced cholangiocarcinogenesis in hamsters by using fluorescence differential display-PCR, and found 23 upregulated and one downregulated transcripts among 149 differentially amplified bands. Among the upregulated genes in the liver was the signal transduction protein kinase A regulatory subunit Iα (*Prkar1α*), which was significantly higher in cholangiocarcinoma and its precursor lesion when compared with normal liver and normal gallbladder epithelia ( $P < 0.05$ ). *Prkar1* expression tended to increase along with the progression of biliary transformation from hyperplasia and precancerous lesions to carcinoma.

## 4.4 Host immune system and genetic susceptibility

[Tesana et al. \(2000\)](#) explored the role of immunization in hamsters administered a subcarcinogenic dose of NDMA with *O. viverrini* infection. Pre-immunization with a crude somatic fluke antigen accelerated carcinogenesis at 30 weeks (71%) compared with the non-immunized group (20%), suggesting that an increased immune response to liver fluke antigens may increase the susceptibility of developing cholangiocarcinoma.

The relationship between immune responses to infection with *O. viverrini* and the synthesis of the carcinogen NDMA, nitric oxide (NO) and nitrosation of amines in humans has been described. The intake of exogenous nitrate and nitrite was minimized and assessments were carried out before and 4 months after elimination

of the infection with praziquantel treatment. No variation was observed in the amount of NDMA excreted in the urine between the control, moderate and heavy liver-fluke-infected groups ( $n = 40\text{--}50$  subjects per group). However, during active infection, a strong negative association was observed between *in vitro* lymphoproliferative responses to some liver fluke antigens and NDMA excretion. This association was reduced after praziquantel treatment. Multivariate statistical models revealed a highly significant relationship between NDMA levels and urinary nitrate, stimulation indices for two T-cell responses to two parasite antigens (molecular weight, 37 kDa and 110 kDa), and gallbladder dimensions. NDMA levels after treatment were best described by the ratio between parasite-specific IgG2 and IgE, background levels of T-cell proliferation, a urinary marker of nitrosation (*N*-nitrosothioproline), and a normal level of alcohol consumption. Thus, individual background immunological activity, parasite-specific responses and/or parasite products and NO synthesis may all be determinants of endogenous generation of nitrosamines in *O. viverrini*-infected humans ([Satarug et al., 1998](#)).

In the only study of host genetic polymorphisms, a population-based case-control study in Thailand failed to show any association between glutathione S-transferase polymorphisms and cholangiocarcinoma risk ([Honjo et al., 2005](#)).

## 4.5 Synthesis

Although liver fluke ES products may stimulate cell proliferation and anti-apoptosis directly, liver-fluke-induced cholangiocarcinoma is more likely the result of chronic inflammation ([Holzinger et al., 1999](#); [Sirica, 2005](#); [Kawanishi & Hiraku, 2006](#)), involving the activation of oxidative stress pathways. Studies on *O. viverrini* provide most of the mechanistic data.

## 5. Evaluation

There is *sufficient evidence* in humans for the carcinogenicity of chronic infection with *Opisthorchis viverrini*. Chronic infection with *Opisthorchis viverrini* causes cholangiocarcinoma.

There is *sufficient evidence* in humans for the carcinogenicity of chronic infection with *Clonorchis sinensis*. Chronic infection with *Clonorchis sinensis* causes cholangiocarcinoma.

There is *limited evidence* in experimental animals for the carcinogenicity of infection with *Opisthorchis viverrini*.

There is *limited evidence* in experimental animals for the carcinogenicity of infection with *Clonorchis sinensis*.

Chronic infection with *Opisthorchis viverrini* is *carcinogenic to humans (Group 1)*.

Chronic infection with *Clonorchis sinensis* is *carcinogenic to humans (Group 1)*.

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# **SCHISTOSOMA HAEMATOBIUM**

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*Schistosoma haematobium* was considered by a previous IARC Working Group in 1994 ([IARC, 1994](#)). Since that time, new data have become available, these have been incorporated into the *Monograph*, and taken into consideration in the present evaluation.

## **1. Exposure Data**

### **1.1 Taxonomy, structure, and biology**

#### *1.1.1 Taxonomy*

Schistosomes are parasitic blood-dwelling fluke worms belonging to the genus *Schistosoma*; family, Schistosomatidae; order, Digenea; class, Trematoda; phylum, Platyhelminths; and kingdom, Animalia. The genus *Schistosoma* contains six species that are of major pathological importance to man, *Schistosoma haematobium* (*S. haematobium*), *S. mansoni*, *S. japonicum*, *S. mekongi*, *S. intercalatum*, and *S. guineensis* ([Webster et al., 2006](#)). The species differ in their final location in the human host, the species of the intermediate (snail) host they use in their life cycle, the pathology they induce, and the number, size and shape of the eggs they produce. This *Monograph* is restricted to *S. haematobium*.

#### *1.1.2 Structure*

Unlike all other pathologically important trematodes, schistosomes are not hermaphroditic, but have separate sexes. The adult worms are 1–2 cm long with a cylindrical body that features two terminal suckers, a complex tegument, a blind digestive tract, and reproductive

organs. The male's body forms a groove or gynaecophoric channel, in which it holds the longer and thinner female. As permanently embraced couples, the schistosomes live within the perivesical (*S. haematobium*) or mesenteric (other species) venous plexus. Schistosomes feed on blood particles through anaerobic glycolysis ([Rumnajek, 1987](#)).

#### *1.1.3 Structure of the genome*

The genome of *S. mansoni* is currently being sequenced and is almost completed; an Expressed Sequence Tag (EST) sequencing project has also started for *S. japonicum*, and *S. haematobium* ([El-Sayed et al., 2004](#)).

#### *1.1.4 Life cycle and biology of the worm*

The life cycle of *S. haematobium* is illustrated in Fig. 1.1. The female *S. haematobium* worm produces hundreds of eggs per day throughout her life. The eggs (144x58 µm, with a characteristic terminal spine) penetrate through the bladder wall where they are excreted with urine. Each ovum contains a ciliated larva (miracidium), which secretes proteolytic enzymes that help the eggs migrate into the lumen of the bladder. About half of the eggs produced do not reach the vesical lumen, and are carried

away with the bloodstream, and/or trapped in the tissues. These retained eggs provoke a granulomatous inflammatory response, which is the main cause of pathology in the human host. The excreted eggs hatch if they come into contact with water, and release the miracidium. These remain viable for up to 48 hours and are able to locate a suitable freshwater snail host (i.e. *Bulinus spp.* for *S. haematobium*) using external stimuli such as light and snail-derived chemicals. In the snail, asexual multiplication takes place, and several generations of multiplying larvae (sporocysts) develop. Eventually, these sporocysts produce large numbers of infective larvae with a typical bifurcated tail (cercariae). These leave the snail at a rate of thousands per day after a period of weeks. Shedding of these cercariae can continue for months. The cercariae survive for up to 72 hours and use water turbulence and skin-derived chemicals to locate the human host. They attach to and penetrate the human skin within 3–5 minutes. They lose their tail, and the young parasites (schistosomulae) migrate with the bloodstream via the lungs to the liver, where they mature into adult worms in the portal vein and mate. The paired worms migrate against the bloodstream to the perivesicular veins, where in a total of 4–7 weeks after infection they start producing eggs throughout their adult life. The lifespan of an adult worm averages 3–5 years, but can be as long as 30 years ([Wilkins, 1987](#)). An infected person probably harbours an average of hundreds (range, 10s–1000s) of worms ([Gryseels & De Vlas, 1996](#)).

## 1.2 Epidemiology of infection

### 1.2.1 Prevalence, geographic distribution

Human schistosomiasis is endemic in large areas of the (sub)tropics. It has been estimated that over 700 million people in 74 countries are exposed to the risk of schistosomal infection, and almost 200 million were estimated to be infected

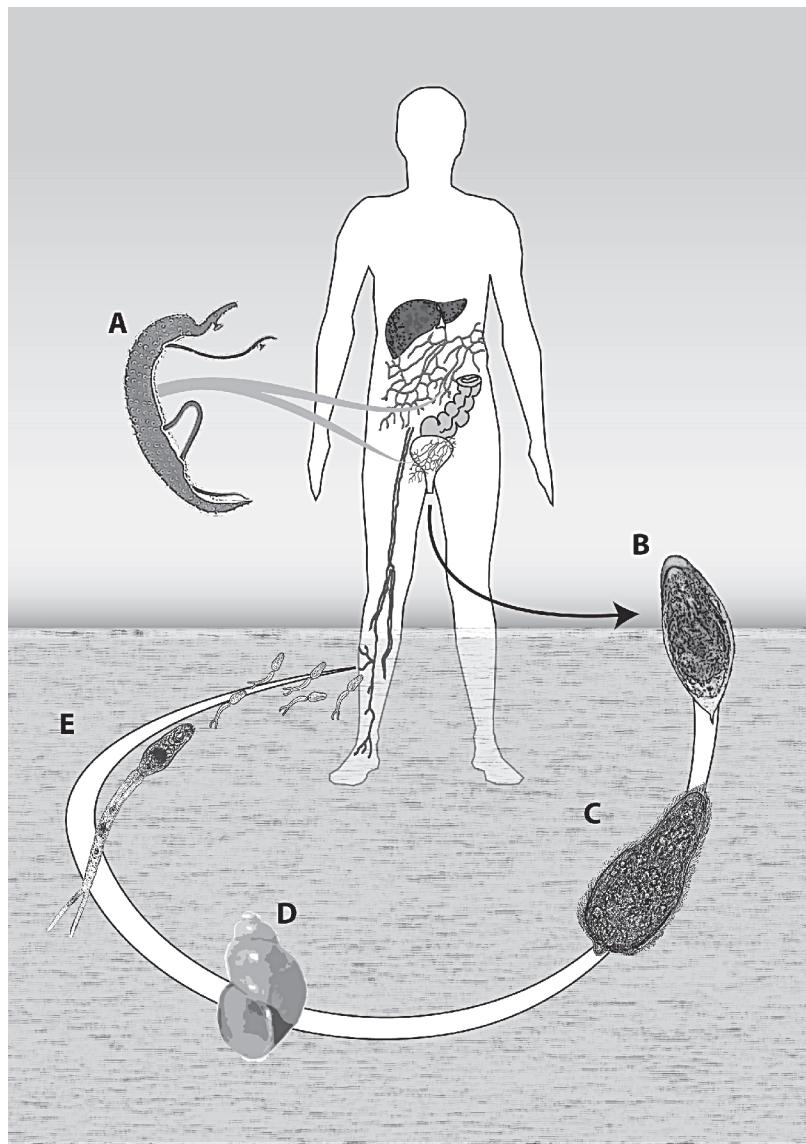
in 2003 ([Fenwick, 2006](#)), of which 85% in sub-Saharan Africa. About 95% of the cases are due to *S. mansoni* and *S. haematobium* infections. *S. haematobium* is endemic in 53 countries, in the Middle East and most of the African continent (Fig. 1.2; [Chitsulo et al., 2000](#)).

Schistosomiasis is largely an infection found in rural areas, but urban schistosomiasis is an increasing problem in many countries ([Mott et al., 1990](#)). Natural streams, ponds and lakes are typical sources of infection, but over the past few decades, man-made reservoirs and irrigation systems, as well as population growth and migration, have contributed to the spread of schistosomiasis ([Gryseels et al., 2006](#); [McManus & Loukas, 2008](#)).

Within countries, regions and villages, the distribution of schistosomiasis can be very focal, depending on variations in snail populations and human–water contact behaviour ([Gryseels & Nkulikyinka, 1988](#)). Also, the distribution of schistosomiasis can be highly uneven across individuals. The majority of the parasites are usually present in a small fraction of the infected individuals. Prevalence and intensities of infection generally show a typical convex-shaped curve with a peak at the ages of 5–15 years, and a decrease in adults. Sex-related patterns vary in relation to behavioural, professional, cultural, and religious factors ([Jordan & Webbe, 1993](#)).

### 1.2.2 Transmission, and risk factors for infection

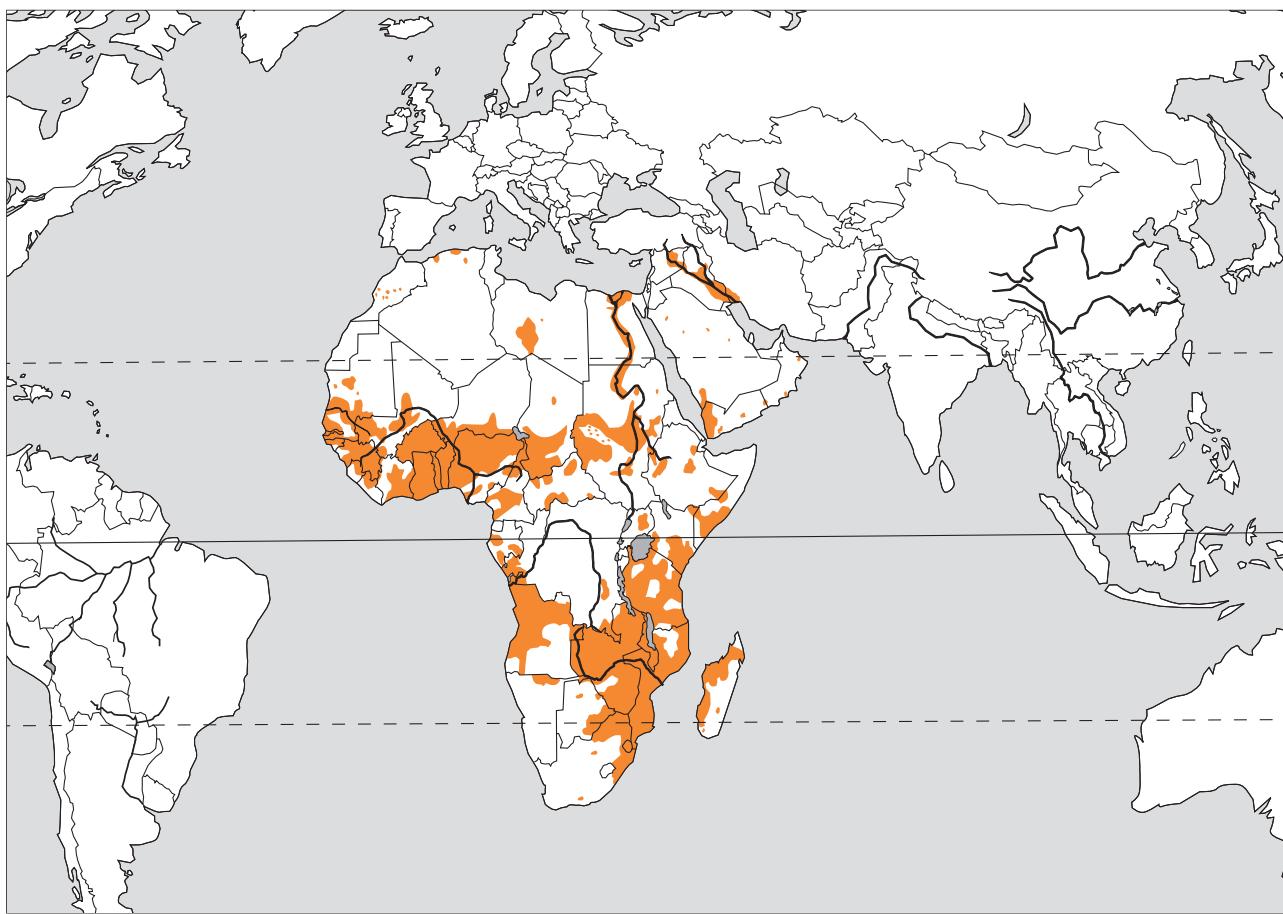
All *Schistosoma* infections follow direct contact with freshwater-harbouring cercariae (see life cycle). Three major factors are responsible for maintaining the transmission of the infection: 1) contamination of fresh water with excreta containing schistosome eggs, 2) the presence of the snail intermediate hosts, and 3) human contact with water-infested with cercariae ([Jordan & Webbe, 1993](#)).

**Figure 1.1 Life cycle of *Schistosoma haematobium***

A: paired adult worms; B: egg; C: miracidium; D: intermediate snail host; E: cercariae  
Courtesy of Pr. Bruno Gryseels, Institute for Tropical Medicine, Antwerp, Belgium.

Contact with contaminated freshwater is the major risk factor of infection ([Jordan & Webbe, 1993](#)). The main risk groups are school-age children, specific occupational groups (fishermen, irrigation workers, farmers), and women and other groups using infested water for domestic purposes ([WHO Expert Committee, 2002](#)). Many other host-related and environmental risk factors have been identified that may affect the

risk of acquiring schistosome infection, and/or influence the distribution, prevalence, intensity of infection, morbidity and mortality of schistosomiasis. Among these are genetic factors ([Quinnell, 2003](#)), behaviour, household clustering ([Bethony et al., 2001](#)), climate, immune response of the host, and concomitant infections (e.g. hepatitis) ([IARC, 1994](#)).

**Figure 1.2 Global distribution of *Schistosoma haematobium***

Main foci *Schistosoma haematobium*: found mainly in sub-Saharan Africa, Nile valley in Egypt and Sudan, the Maghreb, and the Arabian peninsula.

Courtesy of Pr. Bruno Gryseels, Institute for Tropical Medicine, Antwerp, Belgium.

### 1.2.3 Persistence, latency, and natural history of infection

#### (a) Persistence

Schistosome worms do not multiply in the host. The infection status is the result of an accumulation of consecutive infections, where individuals with the most intense infections usually have a higher risk of developing morbidity ([Gryseels et al., 2006](#)). In the absence of re-infection, the infection subsides when the schistosome worm dies, which is usually after 3–5 years. However, in endemic areas with continuous exposure, re-infection is the rule rather than the exception.

In highly endemic areas, children start to accumulate worms as soon as they are old enough to have contact with water and may, because of the chronic nature of the infection and continued susceptibility to re-infection, remain infected throughout their lives.

The possibility that adults might develop immunity to schistosome infections was initially suggested based on the shape of the age–intensity curve in endemic communities, which characteristically shows a rise in intensity during the first two decades of life, followed by a decline in adults to very low levels ([Butterworth, 1998](#)). Indeed, numerous studies have provided epidemiological

and clinical evidence that people living in endemic areas acquire some form of protective immunity after years of exposure. However, age-related innate resistance mechanisms may also play an important part in the epidemiology of schistosomiasis ([Butterworth, 1993](#); [Gryseels et al., 2006](#)).

#### (b) Latency and natural history

Not much is known about the latency between the onset of infection and the appearance of cancer, nor about the steps that might lead to cancer.

Infection with *S. haematobium* is not synonymous with clinical disease, and many infections are asymptomatic. Of those infected, a small proportion develops serious chronic disease, after varying durations of exposure and infection ([Homeida et al., 1988](#); [Vennerveld & Dunne, 2004](#)). [Mostafa et al. \(1999\)](#) noted that the incidence of bilharzial bladder cancer in various African countries peaks between the ages of 40–49 years, while infection with *S. haematobium* begins in childhood (as early as 6 months of age), and peaks usually in the second decade of life (between the ages of 5–15 years). This would imply a latency period of 20–30 years.

## 2. Cancer in Humans

### 2.1 Cancer of the urinary bladder

Earlier studies reported in the previous *IARC Monograph* ([IARC, 1994](#)) have supported an association between the occurrence of urinary bladder cancer and *S. haematobium* infection (for more recent reviews, see for example [Badawi et al., 1995](#); [Mostafa et al., 1999](#); [Mayer & Fried, 2007](#)). A substantial number of descriptive studies from Africa have shown that: the estimated incidence of urinary bladder cancer was higher in areas with a high prevalence of *S. haematobium* infection than in areas with a low prevalence; the

estimated incidence of urinary bladder cancer was related to the proportion of cancerous urinary bladder specimens containing *S. haematobium* eggs or egg remnants; the sex ratio of urinary bladder cancer cases varied widely and corresponded to the relative involvement of men and women in agricultural work (a risk factor for *S. haematobium* infection); and squamous cell cancers of the urinary bladder were proportionately more common in populations with a high prevalence of *S. haematobium* infection and a high proportion of urinary bladder cancers showing histological evidence of infection than in areas without these characteristics.

A large number of case series and case reports have repeatedly emphasized the prevalence of squamous cell urinary bladder tumours among patients with evidence of schistosomal infection. Clinically, the most notable and consistent feature described was the relatively young age of the cases that had evidence of a link to *S. haematobium* infection ([IARC, 1994](#)).

A more recent descriptive study by [Groeneveld et al. \(1996\)](#) on the incidence of different histological types of bladder cancer in various racial groups living within the same geographic area of Kwazulu-Natal, South Africa, reported similar results: squamous cell carcinoma occurred in 53% of the African patients (who have, according to the authors, a much higher risk of exposure and infestation to *S. haematobium* due to socioeconomic, cultural and educational factors), and in 2% of the Caucasian patients. Moreover, eggs of *S. haematobium* were seen in microscopic sections of the bladder tumour in 85% of the patients with squamous cell carcinoma, and in 10% of the patients with transitional cell carcinoma. [The Working Group noted that no mention is made of the percentages of *S. haematobium* ova in microscopic sections of African patients with bladder cancer of the squamous-cell-carcinoma type].

The mean age at presentation of African patients was at least 20 years younger than that of Caucasian patients.

Table 2.1 (available at <http://monographs.iarc.fr/ENG/Monographs/vol100B/100B-09-Table2.1.pdf>) summarizes the case-control studies on the association between *S. haematobium* infection and urinary bladder cancer. A total of 7/8 case-control studies showed significant, positive associations between the occurrence of urinary bladder cancer and infection with *S. haematobium*, with estimated risks ranging from 2–15. The most recent study, from Egypt ([Bedwani et al., 1998](#)), found that the odds ratio (OR) for urinary schistosomiasis was higher in subjects who were younger at first diagnosis (OR, 3.3 for <15 years), and with a long time since first diagnosis (OR, 3.0 for ≥35 years), suggesting a duration-risk relationship and a long-term effect of urinary schistosomiasis on bladder cancer ([Bedwani et al., 1998](#)).

At the time of writing, no cohort studies on urinary bladder cancer and *S. haematobium* infections have been reported.

In contrast to some of the earlier case-control studies, all studies after 1994 considered possible confounding by age, sex, and smoking.

In one study that considered tobacco smoking as a confounding factor ([Parkin et al., 1994](#)), no significant effect was observed due to tobacco smoking (OR, 1.1 for squamous cell bladder tumours).

In a study of schistosomiasis and the risk of bladder cancer in Egypt, [Bedwani et al. \(1998\)](#) assessed the interaction of history of urinary schistosomiasis with smoking. The interaction was significant ( $P<0.01$ ), with an odds ratio of 15.8 (95%CI: 5.1–48.4), and odds ratios for schistosomiasis only and for ever smoking only were 11.8 (95%CI: 2.8–50.1) and 13.8 (95%CI: 4.7–40.1), respectively.

## 2.2 Others

A number of studies have been conducted on the association of *S. haematobium* infection with other cancers, the results of which are summarized per cancer site below.

### 2.2.1 Cancers of the female genital tract

Other than urinary bladder cancer, cervical cancer and other malignancies of the female genitalia have been the most frequently reported cancers in association with *S. haematobium* infection, usually in the form of case reports ([IARC, 1994](#)). Recently, a number of additional cases of female genital malignancy in association with evidence of *S. haematobium* infection have been published (e.g. [North et al., 2003](#); [Chenault & Hoang, 2006](#)).

Two cross-sectional, one case-control and one pooled reanalysis studies have been published on the association between *S. haematobium* and cervical cancer ([Wright et al., 1982](#); [Moubayed et al., 1994](#); [Parkin et al., 1994](#); [Riffenburgh et al., 1997](#); Table 2.2, available at <http://monographs.iarc.fr/ENG/Monographs/vol100B/100B-09-Table2.2.pdf>). [Parkin et al. \(1994\)](#) consulted the same cancer registry from Zimbabwe as previously described for bladder cancer ([Vizcaino et al., 1994](#)). [Riffenburgh et al. \(1997\)](#) pooled the data from the two earlier published studies. None of these studies showed a positive association between the risk for cervical cancer and infection with *S. haematobium*. [The Working Group noted that possible confounding by age, smoking, or human papillomavirus (HPV) was not considered in any of the studies.]

### 2.2.2 Other cancers

Eight cases of prostatic schistosomiasis associated with adenocarcinoma of the prostate have been reported, three of which were associated with *S. haematobium*, the others with *S. mansoni* or the parasite species was not specified ([Cohen](#)

[et al., 1995](#); [Bacelar et al., 2007](#)). [Cohen et al. \(1995\)](#) reported on three patients in South Africa (27–29 years of age) with advanced prostate cancer associated with the presence of multiple eggs of *S. haematobium* (some viable and others calcified).

Other malignancies that have been reported in association with *S. haematobium* infection include: squamous cell cancer of the female genitals, ovarian cystadenocarcinoma, teratoma and Brenner tumours, uterine leiomyosarcoma, male breast cancer, hepatocellular carcinoma, lymphoma, bladder sarcoma, rectal carcinoid tumour, and renal cell carcinoma. The number of patients with each malignancy is usually small, which precludes any evaluation of the data ([IARC, 1994](#); [Mayer & Fried, 2007](#)).

### 2.3 Impact of *Schistosoma* eradication

Although several authors have suggested prevention or control of *S. haematobium* as a way to manage schistosomiasis-associated bladder cancer ([Badawi et al., 1995](#); [Groeneveld et al., 1996](#); [Mostafa et al., 1999](#); [Mayer & Fried, 2007](#)), no such intervention study has been published so far. Some studies have investigated the effect of praziquantel, the drug of choice for the treatment of urinary schistosomiasis, on lesions in the urinary and genital tract ([Richter, 2003](#); [Kjetland et al., 2006](#)).

In a recent study of schistosomiasis-associated bladder cancer conducted in Egypt ([Koraitim et al., 1995](#)), both the proportion of transitional cell carcinoma (31% in 1960s versus 42% in late 1980s) and mean age at diagnosis (47 in 1960s versus 53 in late 1980s) in *Schistosoma*-positive cases had increased over time. Lower levels of schistosome infection (based on the number of eggs in the bladder wall) were observed among patients with bladder cancer in the late 1980s when compared to patients in the 1960s, which is likely to result from national efforts to control schistosomiasis and the availability of new drugs

with minimal side-effects ([Koraitim et al., 1995](#)). Transitional cell carcinomas were slightly more common among patients with low infection rates (56%), and squamous cell carcinomas were slightly more common (58%) among patients with moderate-to-high levels of infection ([Michaud, 2007](#)).

## 3. Cancer in Experimental Animals

Earlier studies reported in the previous *IARC Monograph* on infection with schistosomes ([IARC, 1994](#)) have examined experimental *S. haematobium* infections in mice, rats, hamsters, opossums, and non-human primates.

In mice, hamsters, and opossums, hyperplasia of the urinary bladder was observed. Urinary bladder tumours were also reported in one opossum ([IARC, 1994](#)). In several studies in non-human primates, hyperplasia and a few lesions described as tumours of the urinary bladder and ureter were observed. Overall, non-invasive papillary and nodular transitional cell carcinomas of the urinary bladder were observed in one talapoin monkey (*Cercopithecus talapoin*), five capuchin monkeys (*Cebus apella*), and one gibbon (*Hylobates lar*). These types of carcinomas were morphologically similar to tumours observed in human urinary bladder ([IARC, 1994](#); [Mostafa et al., 1999](#)).

In studies where animals infected with *S. haematobium* were treated with known urinary bladder carcinogens, an increase in urinary bladder tumour incidence was observed in infected mice administered 2-acetylaminofluorene, and in infected baboons (*Papio sp.*) treated with *N*-butyl-*N*-(4-hydroxybutyl)nitrosamine. The previous *IARC Monograph* Working Group commented on the short duration, the lack of verification of infection and inadequate documentation of experimental details for the study in mice, and that some of the diagnostic terms in

the report were difficult to interpret in the study in baboons ([IARC, 1994](#); [Mostafa et al., 1999](#)).

Since then, a new study ([Vuong et al., 1996](#)) has described histopathological findings following experimental *S. haematobium* infection in ten rodents including three marsh rats (*Holochilus brasiliensis*), and seven jirds (four *Meriones shawi* and three *M. Unguiculatus*). Comparisons were made with findings in two infected mice and six infected hamsters. All animals were about 3 months of age, and were exposed to 60–400 cercariae per animal by transcutaneous exposure through water (in an individual bath) containing the cercariae, except one marsh rat that received 750 cercariae transcutaneously on the abdominal skin. Eight rodents died naturally 117–256 days after infection (three marsh rats, three jirds, one mouse and one hamster); the remaining ten were sacrificed 100–195 days post infection. One marsh rat developed an in-situ squamous cell carcinoma of the urinary bladder (175 days post infection). Another marsh rat developed precancerous dysplastic changes of the bladder mucosa (123 days post infection). Only one jird (*Meriones shawi*) developed squamous metaplasia associated with mild-to-moderate dysplasia of the urothelial lining (117 days post infection). One mouse developed a squamous cell carcinoma of the urinary bladder (185 days post infection). No neoplastic or preneoplastic lesions were observed in the four hamsters.

## 4. Other Relevant Data

Several reviews have been published on the mechanisms of carcinogenesis induced by *S. haematobium* ([Rosin et al., 1994a, b](#); [Badawi, 1996](#); [Mostafa et al., 1999](#); [Herrera & Ostrosky-Wegman, 2001](#); [Mayer & Fried, 2007](#)). Several studies indicate that the carcinogenicity of *S. haematobium* is a multifactorial and multistage process where several

mechanisms are involved. *S. haematobium* eggs induce a chronic inflammation and irritation in the urinary bladder that seems to be associated with an increased initiation of cancer at the site of inflammation ([Rosin et al., 1994b](#)). The inflammatory response around the eggs gives rise to genotoxic factors and products that may cause genomic instabilities of host cells, leading to modifications in the regulation of tumour-suppressor genes and oncogenes as well as stimulating a proliferative response of the host cells to repair tissue damage caused by the inflammation ([Rosin et al., 1994a](#); [Badawi, 1996](#)). Furthermore, schistosomiasis-induced inflammatory cells have been shown to participate in the metabolic activation of procarcinogens, such as aflatoxins, and in the formation of carcinogenic nitrosamines from nitric oxide ([Rosin et al., 1994a](#); [Mostafa et al., 1999](#)).

The following is a review of the studies published since the previous *IARC Monograph* ([IARC, 1994](#)). The majority of the studies were conducted on bladder tissue samples from cancer patients with and without schistosomiasis.

### 4.1 Experimental data

#### 4.1.1 Studies *in vitro*

##### (a) Mutagenicity

Neither worm nor egg extracts were shown to exhibit any *in vitro* mutagenicity by the *umu*-test or the V79/HGPRT assay with or without S9 mix ([Osada et al., 2005](#)).

##### (b) Effect on cell proliferation and on cell-cycle regulators

The effect of crude soluble egg antigen of *S. haematobium* on urothelial proliferation was tested on bovine endothelial cells (Endo), urothelial human transitional-cell carcinoma (J82), and human smooth muscle cell lines. Soluble egg antigen induced proliferation in a dose-dependent manner in both Endo and J82 cells

but not in smooth muscle cells. Furthermore, soluble egg antigen enhanced the expression of mRNA for the cell-cycle regulators, peripheral cell nuclear antigen and B-cell translocation protein (*BTG1*) in urothelial cells ([El-Awady et al., 2001](#)).

#### 4.1.2 Studies in experimental animals

##### (a) Mitogenicity and carcinogenesis

The effect of *S. haematobium* infection and low doses of the bladder carcinogen *N*-butyl-*N*-(4-hydroxybutyl)nitrosamine on the development of urothelial neoplasia was studied in a small group of baboons (*Papio sp.*). The study showed that proliferative and inflammatory changes occurred in infected animals not exposed to the carcinogen, whereas the addition of nitrosamine treatment induced neoplastic changes (carcinoma *in situ*). The carcinogen alone did not produce any changes ([Hicks, 1982](#)).

##### (b) Inhibition of glutathione-S-transferase (GST) activity in animals

Inhibition of GST activity may enhance the effect of many environmental carcinogens and toxic agents. The activity of GST, glutathione reductase (GR), and the levels of glutathione and free radicals were measured in different organs of male hamsters infected with *S. haematobium* 2, 4, 6, 8 and 10 weeks post infection. The total activity of GST was significantly increased in bladder tissue from infected animals when compared to uninfected controls 2, 4 and 6 weeks post infection ( $P < 0.001$ ,  $P < 0.025$  and  $P < 0.001$ , respectively). The GST activity was significantly decreased 8 and 10 weeks post infection ( $P < 0.001$ ). The expression of GST isoenzymes was reduced in the kidney and bladder tissues at later stages (8 and 10 weeks) of the infection, and reduced in spleen and liver tissues at all infection stages. Free radicals (measured as thiobarbituric acid reactive substances) increased to high levels in the bladder, and to a lesser extent in other

organs ([Sheweita et al., 2003](#)). [The Working Group noted that the number of animals in the *S. haematobium* and the control groups was unspecified.]

## 4.2 Studies in exposed humans

The majority of the data concerning genotoxicity linked to *S. haematobium* infection are derived from studies on bladder tissue samples from cancer patients with schistosomiasis or a history of exposure to schistosomiasis. A representative sample covering various mechanisms such as DNA adducts, gene methylation, gene mutations, chromosomal alteration, expression of oncogenes and presence of oxidative stress markers are summarized below.

### 4.2.1 Production of *N*-nitroso compounds

Bacteria from a superimposed bacterial infection may reduce dietary nitrate to nitrite, which may react with compounds in urine to produce *N*-nitroso compounds. In a study in Egypt, higher ( $P=0.01$ ) levels of *N*-nitroso compounds and *N*-nitrosodimethylamine (NDMA) were found in the urine of individuals infected with *S. haematobium* compared to uninfected controls ([Abdel Mohsen et al., 1999](#)). [The Working Group noted that many of the study participants were smokers, that the history of contracting schistosomiasis infection was not available, and that the study did not include a control group with bacterial infection only.]

### 4.2.2 Oxidative stress markers

Levels of 8-hydroxy-2'-deoxyguanosine were markedly increased in bladder squamous cell carcinomas ( $P=0.001$ ) and transitional cell carcinomas ( $P=0.045$ ) associated with schistosomiasis when compared to non-schistosomiasis-associated cancers. This was accompanied by strong overexpression of DNA-repair genes

8-oxyguanine-DNA-glycosylase ( $P=0.0047$ ) and apurinic/apirimidinic endonuclease ( $P=0.0121$ ); increased formation of DNA single strand breaks ( $P=0.0106$ ); and higher inducible nitric oxide synthase ( $P=0.022$ ), when comparing squamous cell carcinomas associated with schistosomiasis with non-schistosomiasis-associated cancers ([Salim et al., 2008](#)).

#### 4.2.3 DNA adducts

DNA alkylation damage indicated by increased levels of  $O^6$ -methyldeoxyguanosine has been demonstrated in tissue samples from schistosomiasis-associated bladder cancer patients.  $O^6$ -alkylguanine-DNA alkyltransferase (ATase) activity was significantly higher in normal bladders than in bladders with cancers ( $P<0.005$ ). A negative correlation between the levels of  $O^6$ -methyldeoxyguanosine previously measured in the same samples and ATase activity was demonstrated ( $r=-0.67$ ;  $P<0.001$ ) ([Badawi et al., 1992, 1994](#)).

Paired samples from bladder tumour tissue and tissue without macroscopic signs of tumour invasion were collected in Egyptian patients.  $N^7$ -methyldeoxyguanosine 3'-monophosphate ( $N^7$ -MedGp) was measured by  $^{32}P$  post-labelling. Levels of  $N^7$ -MedGp were highly variable both in tumour and normal tissue and the mean difference in adduct levels between tumour and normal DNA was statistically significant.  $N^7$ -MedGp levels were not associated with gender, age or presence of schistosomiasis ([Saad et al., 2006](#)). [The Working Group noted that the study had limited power with respect to examining the effect of schistosomiasis on the level of adducts because most of the patients were infected or had a history of infection.]

#### 4.2.4 Gene methylation index

Methylation-specific polymerase chain reaction showed that schistosomiasis-associated bladder cancer samples had more genes methylated than non-schistosomal bladder cancer samples in a study in Egyptian patients ([Gutiérrez et al., 2004](#)).

#### 4.2.5 Deletions and/or mutations in tumour-suppressor genes or oncogenes

Deletions and mutations in the  $p16^{INK4}$  gene were found to be more frequent in schistosomiasis-associated bladder tumours from Egypt than in other bladder tumours from the Netherlands ([Tamimi et al., 1996](#)).

Expression of p53, Rb, EGFR and c-erbB-2 proteins was detected by immunohistochemistry and screened by single-strand conformation polymorphism and mutations in the *ras* (H, N, K) hotspots (12, 13, 61) and *p53* (exons 4–9) genes. Among 21 invasive bladder squamous cell carcinoma cases from South Africa infected with *S. haematobium*, positive staining for p53, EGFR and c-erbB-2 was reported in 38%, 67% and 28% of the tumours, respectively. Only one poorly differentiated tumour showed an absence of nuclear Rb staining. Changes in the *H-ras* gene were detected in three squamous cell carcinoma cases; two of which had mutations in *H-ras* codon 13 (gly→arg). The *H-ras* change most commonly seen in transitional cell carcinoma (gly→val change in codon 12) was not seen in any of the squamous cell carcinomas examined. The detection of multiple mutations at the *p53* locus in the schistosomiasis-related cancers suggests the involvement of a specific carcinogenic agent, possibly nitrosamines ([Ramchurren et al., 1995](#)).

Mutations in the *p53* gene were assessed in tissue specimens from 92 Egyptian bladder cancer patients from an area hyperendemic for schistosomiasis. About 90% of the patients had a history of schistosomiasis and/or evidence of

schistosome eggs adjacent to the carcinoma. Thirty patients had mutations in exons 5–8 of the *p53* gene. Of 19 mutations in squamous cell carcinoma, 16 were base-pair substitutions, two were deletions, and one an insertion. All the mutations in transitional cell carcinoma were base-pair substitutions. Combining the 33 mutations from this study with six obtained from another study ([Habuchi et al., 1993](#)) of Egyptian schistosomiasis-associated squamous cell carcinoma, a mutational spectrum was compiled and compared with a non-schistosomal bladder cancer spectrum assembled from 118 mutations reported in the literature. The proportion of base-pair substitutions at CpG dinucleotides was significantly higher (18/34 versus 25/103,  $P=0.003$ ) in schistosomiasis-associated bladder cancer than in non-schistosomal bladder cancer ([Warren et al., 1995](#)).

[The Working Group reported that several studies compared tumours from schistosomiasis-infected patients to those from countries not endemic for schistosomiasis (e.g. [Shaw et al., 1999](#); [Swellam et al., 2004a](#)). These studies were not reviewed because of the difficulty of ascribing differences to schistosomiasis or to other factors that vary between these countries.]

#### 4.2.6 Expression of oncogenes

A significant correlation was recognized between bcl-2 overexpression and bladder squamous cell carcinoma with schistosomiasis. Bcl-2 expression was 74.8 U/mg protein in squamous cell carcinoma and 45.2 U/mg protein in transitional cell carcinoma. It was 82.41 U/mg protein in schistosomiasis cases compared to 35.8 U/mg protein in non-schistosomiasis cases ([Swellam et al., 2004b](#)).

#### 4.2.7 Biomarkers of bladder cancer

The detection of the biomarkers associated with bladder cancer BLCA-4 (a nuclear matrix protein involved in gene regulation and produced only in neoplastic bladder cells) and quantitative nuclear grading was performed in a population-based study from an area in Ghana endemic for *S. haematobium*. The results showed a close correlation between BLCA-4, quantitative nuclear grading and severe bladder damage such as bladder wall masses and polyps. The overall prevalence of BLCA-4 positivity was 40%. A total of 62/73 cytopathology Papanicolaou-stained smears were seen to have squamous metaplasia ([Shiff et al., 2006](#)).

#### 4.2.8 Effect of *S. haematobium* infection on detoxifying enzymes

*S. haematobium* infection has been found to markedly decrease the activity of the carcinogen-metabolizing enzymes GST and NDMA-N-demethylase in human bladder cancer tissue ([Sheweita et al., 2004](#)). [The Working Group noted that this may change the capacity of the bladder to detoxify many endogenous compounds, and may potentiate the effects of bladder carcinogens such as *N*-nitrosamines.]

### 4.3 Host susceptibility

Cytological abnormalities in urine sediment were investigated in a cross-sectional survey of a population (1014 individuals aged 1–91 years) living in an area in Kenya endemic for *S. haematobium*. No cancers were detected in the study population. The prevalence of inflammation (39%), hyperkeratosis (30%), metaplasia (33%) and frank atypia (0.4%) was much higher than what had been reported from non-endemic areas. *S. haematobium* infection was strongly associated with an increased risk of metaplasia or hyperkeratosis (relative risk > 2.8-fold,  $P<0.001$ ).

Among children, the incidence of metaplasia was linked with concurrent schistosome infection, whereas older individuals tended to have metaplasia incidences independent of the level of infection or inflammation. The incidence of moderate or severe metaplasia displayed two age-related peaks; one among the age group 10–14 years, where the intensity of infection is highest, and a second among individuals over 60 years of age ([Hodder et al., 2000](#)).

#### 4.4 Synthesis

It is well established that *S. haematobium* with egg deposition in the tissue leads to severe inflammation of the urinary bladder wall with accumulation of inflammatory cells resulting in increased oxidative stress. Overall, the studies summarized above suggest that the observed increased levels of oxidative stress in the schistosomiasis-associated bladder carcinomas correlate with genotoxicity and activation of repair genes, and point towards a relationship between oxidative stress induced by continuous and chronic inflammation due to schistosome infection and possibly nitric-oxide-mediated DNA genotoxicity. The excess of DNA alterations could result from nitric oxide produced by the inflammatory response provoked by *Schistosoma* eggs, and alkylation of DNA by *N*-nitroso compounds.

#### 5. Evaluation

There is *sufficient evidence* in humans for the carcinogenicity of chronic infection with *Schistosoma haematobium*. Chronic infection with *Schistosoma haematobium* causes cancer of the urinary bladder.

There is *limited evidence* in experimental animals for the carcinogenicity of infection with *Schistosoma haematobium*.

Chronic infection with *Schistosoma haematobium* is *carcinogenic to humans (Group 1)*.

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# ***HELICOBACTER PYLORI***

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*Helicobacter pylori* was considered by a previous IARC Working Group in 1994 ([IARC, 1994](#)). Since that time, new data have become available, these have been incorporated into the *Monograph*, and taken into consideration in the present evaluation.

## **1. Exposure Data**

*Helicobacter pylori* is a highly heterogenous bacterium with a large genomic diversity. In addition, humans may sometimes harbour multiple strains, and *H. pylori* can change genotypically and phenotypically during colonization in a single host ([Suerbaum & Josenhans, 2007](#)).

### **1.1 Taxonomy, structure, and biology**

#### *1.1.1 Taxonomy*

The presence of spiral-shaped bacteria on human gastric mucosa was first recognized nearly one hundred years ago ([Pel, 1913](#)). These bacteria were originally named *Campylobacter pylori* (*C. pylori*) ([Warren, 1983](#)).

In 1989, a new genus, *Helicobacter*, was proposed, and *C. pylori* was renamed *Helicobacter pylori* ([Goodwin et al., 1989](#)). Recently ([Garrity et al., 2005](#)), the genus *Helicobacter* has been included with the genus *Wolinella* in the family *Helicobacteraceae* which, with the family *Campylobacteraceae*, constitutes the *Epsilonproteobacteria*.

Over the past 20 years, 23 *Helicobacter* species have been validated, and two candidates

and several strains are awaiting official classification ([Table 1.1](#)).

According to the usual site of colonization, *Helicobacter* species can be divided into gastric and enteric or enterohepatic *Helicobacter* types.

Some gastric *Helicobacter* species from animals can infect humans: *H. bizzozeroni*, *H. salomonis*, *H. felis*, *H. candidates*, *H. suis*. Because they are extremely difficult to grow in cultures, the exact speciation is usually not done, and they are known as “*Gastrospirillum hominis*” or “*H. heilmannii*” ([De Groot et al., 2005](#)).

#### *1.1.2 Structure of the bacterium*

*H. pylori* is a spiral or slightly curved Gram-negative rod with 2–6 characteristic unipolar flagella. The bacterium has bluntly rounded ends and measures 2.5–4.0 µm in length and 0.5–1.0 µm in width. The cell wall is smooth and may be coated with a prominent glycocalyx with a thickness of up to 40 nm ([Goodwin et al., 1989](#)); it is covered with ring-like subunits with a diameter of 12–15 nm. Occasionally, the bacterium may contain bacteriophages. The flagella measure 2.5 µm in length and around 30 nm in thickness, and have a distinctive terminal bulb ([Goodwin & Worsley, 1993](#)). The bacterium

**Table 1.1 Validated species of the genus *Helicobacter***

Species
<i>Helicobacter pylori</i>
<i>Helicobacter acinonychis</i>
<i>Helicobacter aurati</i>
<i>Helicobacter bilis</i>
<i>Helicobacter bizzozeronii</i>
<i>Helicobacter canadensis</i>
<i>Helicobacter canis</i>
<i>Helicobacter cholecystus</i>
<i>Helicobacter cinaedi</i>
<i>Helicobacter felis</i>
<i>Helicobacter fennelliae</i>
<i>Helicobacter ganmani</i>
<i>Helicobacter hepaticus</i>
<i>Helicobacter mesocricetorum</i>
<i>Helicobacter muridarum</i>
<i>Helicobacter mustelae</i>
<i>Helicobacter nemestrinae</i>
<i>Helicobacter pametensis</i>
<i>Helicobacter pullorum</i>
<i>Helicobacter rodentium</i>
<i>Helicobacter salomonis</i>
<i>Helicobacter trogontum</i>
<i>Helicobacter typhonius</i>
<i>Candidatus Helicobacter bovis</i>
<i>Candidatus Helicobacter suis</i>

displays remarkable motility in viscous solutions, and the flagella play a central role in this motility ([Hazell et al., 1986; Suerbaum et al., 1993](#)).

In certain circumstances, *H. pylori* can evolve from this typical helical form to a coccoidal form. Some studies suggested that they are live organisms ([Sisto et al., 2000; Willén et al., 2000](#)), but others concluded that they are degenerating organisms ([Kusters et al., 1997](#)).

### 1.1.3 Structure of the genome

The genome of three *H. pylori* strains has now been fully sequenced: strain 26695 from a patient with gastritis, strain J99 from a duodenal ulcer patient, and strain HPAG1 from a patient with

chronic atrophic gastritis ([Tomb et al., 1997; Alm et al., 1999; Oh et al., 2006](#)).

Strains 26695, J99 and HPAG1 have a circular chromosome of 1667867 base pairs (bp), 1643831 bp and 1596366 bp, respectively. HPAG1 also has a single 9369 bp plasmid, pHPAG1.

The percentage of genome-coding sequences of strains 26695, J99 and HPAG1 is around 92%, and they contain 1552 ([Alm et al., 1999; Boneca et al., 2003](#)), 1495 ([Tomb et al., 1997; Alm et al., 1999](#)), and 1536 ([Oh et al., 2006](#)) predicted protein-coding genes, respectively. In these three small genomes, 1379 open reading frames (ORFs) are common to all three strains and about 10% of the genes are strain-specific ([Alm et al., 1999](#)); 117 and 89 genes present in strains 26695 and J99, respectively, are absent in the other strain ([Alm et al., 1999](#)); in contrast, 43 of the HPAG1 genes are either not detectable at all or incompletely represented in the 26695 and J99 genomes ([Oh et al., 2006](#)).

A comparison of the three genomic sequences revealed that the genetic organization was similar in all three strains. However, it confirmed the panmictic structure of *H. pylori*, which is the result of a high mutation rate (microdiversity, i.e. high polymorphism among orthologous genes), and free recombinations ([Falush et al., 2003](#)). A significant macrodiversity (presence or absence of the genes) was also observed ([Raymond et al., 2004](#)). A comparative genomic analysis of isolates from 15 Caucasians ([Salama et al., 2000](#)) allowed to extend the pool of strain-specific genes from 6–7% (as determined from the comparison of the first two sequenced genomes) to 18–22%. More recently, a large study was conducted on 56 *H. pylori* strains and four *H. acinonychis* strains, with whole genome microarrays. They concluded that the core genome present in all *H. pylori* isolates contains 1111 genes, with a weighted average of 27% of the genome variably present in different isolates ([Gressmann et al., 2005](#)).

Besides the *cag* pathogenicity island, which is known to be a variable region, half of the

strain-specific genes are clustered in a hypervariable region, known as the ‘plasticity zone’ ([Salama et al., 2000](#)). The group of genes containing the most variability are those that comprise genes of unknown function (44%), genes associated with DNA metabolism (most of them are restriction-modification systems 54%), outer-membrane proteins (22%), cellular processes/cagPAI (40%) and others (100%, including transposases) ([Gressmann et al., 2005](#)).

The genomic analyses suggest that *H. pylori* strains have essentially identical metabolic potential ([Table 1.2](#)).

#### 1.1.4 Host range

*H. pylori* is the *Helicobacter* species of humans. *H. pylori* isolation from several other animal species (monkey, pig, cat, dog) has been reported, but these reports were anecdotal, and these bacteria were most likely acquired from humans.

#### 1.1.5 Target cells and tissues

The target cell of *H. pylori* is the gastric mucus-secreting cells. A low acid output leads *H. pylori* to also infect the corpus ([Louw et al., 1993](#)). *H. pylori* lives mainly in the surface mucus layer and within the pits, and can adhere to mucus-secreting cells especially close to intercellular junctions ([Hazell et al., 1986](#)). It is not found on intestinal-type cells in the case of intestinal metaplasia. In contrast, it has the ability to colonize metaplastic gastric cells present in the duodenum and elsewhere, for example, in the oesophagus, in Meckel’s diverticulum, and in the rectum ([Hill & Rode, 1998](#)).

The main cell receptor for this adherence is the blood group antigen A, and the corresponding adhesin is named BabA. In a low proportion of the cells, *H. pylori* may be intracellular, a situation which contributes to its persistence ([Dubois & Borén, 2007](#)).

*H. pylori* can be present transiently in the mouth when regurgitated, and may also be found in the faeces, but it cannot survive with competing organisms ([Parsonnet et al., 1999](#)).

#### 1.1.6 Function of gene products

##### (a) Colonizing factors

Colonization by *H. pylori* involves an interaction between a large family of *Helicobacter* outer membrane proteins (Hop) and the gastric epithelial cells of the host. Several genes involved in determining the composition of the outer membrane are differentially regulated by a phase variation called slipped-strand repair. This phenomenon is possible due to the presence of repeated intragenic sequences, allowing replicative shifts and mismatches, leading to changes in the status of a gene (“on/off”) ([Salaün et al., 2004](#)). Such proteins are the blood group antigen binding adhesion (BabA), sialic acid binding adhesion (SabA), adherence-associated lipoprotein (AlpA and AlpB), and HopZ.

Lipopolysaccharides play an important role in the interaction between Gram-negative bacteria and their host. They are potential stimulators of the immune system ([Moran et al., 1996](#)). The *H. pylori* lipopolysaccharides, however, have remarkably low activity, and their synthesis may involve over 20 genes, scattered throughout the genome, unlike other bacteria in which they are grouped into a single cluster.

The expression of fucosyltransferase, an enzyme essential for the lipopolysaccharide biosynthesis pathway, is also subject to phase change, and is a key enzyme allowing *H. pylori* to mimic human Lewis antigens, which allows it to escape the host immune response ([Lozniewski et al., 2003](#)).

It has been suggested that this differential regulation and the strain-specific outer-membrane-related genes may play a role in the severity of *H. pylori*-related disease, and the

**Table 1.2 Classification of the genes of two *Helicobacter pylori* strains sequenced**

Annotation category	No. of genes in:	
	<i>H. pylori J99</i>	<i>H. pylori 26695</i>
<b>Functionally classified</b>	877	898
Aminoacid biosynthesis	44	44
Biosynthesis of cofactors etc.	60	59
Cell envelope	160	164
Cellular processes	96	113
DNA replication	23	23
DNA restriction-modification, etc.	66	68
Energy metabolism	104	104
Fatty acid and phospholipid metabolism	28	29
Purine and pyrimidine biosynthesis	34	34
Regulatory functions	32	32
Transcription	13	13
Translation	128	128
Transport and binding proteins	88	87
<b>Conserved with no known function</b>	275	290
<b><i>Helicobacter pylori</i> specific</b>	343	364
<b>Total</b>	<b>1495</b>	<b>1552</b>

From [Doig et al. \(1999\)](#)

ability of *H. pylori* to persist chronically in its host ([Mahdavi et al., 2002](#)).

### (i) BabA

The blood group antigen Lewis b was identified as a receptor for *H. pylori* in 1993 ([Borén et al., 1993](#)). This is the dominant antigen in the gastric mucosa of secretor-positive individuals. The adhesion-recognizing Lewis b was characterized as an *H. pylori* outer-membrane protein, namely BabA ([Ilver et al., 1998](#)). Another protein with almost identical amino terminal and identical carboxy terminal domains but divergent central domains, BabB, does not bind to Lewis b antigen ([Aspholm-Hurtig et al., 2004](#)).

A *babA* allele and a *babB* allele are both present in each of the three sequenced strains, but in different locations. In the strain from which *bab* genes were initially cloned, there were two *babA* genes but only one of them (*babA2*) has Lewis b binding activity. According to two studies, the *babA* gene is present in approximately 70% of

*H. pylori* strains, and the *babB* gene is present in almost them all ([Colbeck et al., 2006](#); [Hennig et al., 2006](#)).

Several mechanisms have been elucidated for the regulation of BabA expression including chimera formation ([Pride & Blaser, 2002](#)) between *babA* and *babB*, and phase variation of *babA* through slipped-strain mispairing ([Solnick et al., 2004](#)).

To date, BabA-Lewis b is the adhesin-receptor interaction in *H. pylori* that is best characterized, and probably one of the most important ([Yamaoka, 2008](#)).

### (ii) SabA

Another outer-membrane protein conferring adherence to host sialyl Lewis x was identified as sialic acid-binding adhesin (SabA) ([Mahdavi et al., 2002](#)). The sialyl-Lewis x expression is induced in the gastric epithelium during persistent *H. pylori* infection, suggesting that the bacterium can trigger the host tissue to modify

the mucosal glycosylation patterns for enhanced adherence. The event could occur via induction of 3GnT5, a GlcNAc transferase essential for the biosynthesis of Lewis antigens ([Marcos et al., 2008](#)). SabA could in this way contribute to the chronicity of *H. pylori* infection. SabA can also bind specifically to granulocytes and induce an oxidative burst ([Unemo et al., 2005](#)).

#### (iii) *AlpA* and *AlpB*

A genetic locus involved in *H. pylori* adherence to Kato cells was identified in 1999 ([Odenbreit, 2005](#)). It was named *alpAB* (adherence-associated lipoprotein A and B), and encodes two outer-membrane proteins. However, because no receptor has been identified for these proteins, their role as adhesins is unclear.

#### (iv) *HopZ*

*HopZ* has been described as an adhesin ([Peck et al., 1999](#)) but no receptor has yet been identified for this putative adhesin.

### (b) Pathogenicity factors

#### (i) *cag* Pathogenicity island

In many parts of the world, including Asia and most of Africa, almost all *H. pylori* strains contain an intact *cag* pathogenicity island, whereas about 30% of strains from Europe and North America lack the entire island, and are considered “*cag*-negative”. However, both *cag*-positive and *cag*-negative strains can exist together in the same stomach, and the *cag* pathogenicity island can be partially deleted with the loss of type 4 secretory function ([Suerbaum & Josenhans, 2007](#)), therefore, the simple designation of *cag* status as positive or negative may not be absolute. The CagA protein encoded by the *cagA* gene within the *cag* pathogenicity island is a highly immunogenic protein that elicits serum antibody responses allowing for the detection of *cag*-positive strains by enzyme-linked immunosorbent assay (ELISA)

or Western blot analysis in serum samples for epidemiological studies.

The *cag* pathogenicity island of *H. pylori* is a DNA fragment of approximately 40 kbp which exhibits the characteristics of pathogenicity islands in general: i) a G+C% different from the rest of the chromosome (35% vs 39%), ii) two direct repeat sequences at its ends, iii) several genes linked to virulence, iv) a secretion system, and v) an insertion sequence ([Censini et al., 1996](#)).

This pathogenicity island is always located between the same two genes: HP519, a gene of unknown function, and *murI*, the glutamate racemase gene. It is integrated at the 3' end of the glutamate racemase gene and flanked by two repeated 31 bp sequences probably derived from the duplication of the 3' end of this gene ([Akopyants et al., 1998](#)). Because this island appears to be acquired *in toto*, it may then be separated into two regions, namely *cagI* and *cagII*, by an insertion sequence (IS605) coding for two transposases (*tnpA* and *tnpB*) ([Censini et al., 1996](#)). There are 27 potential coding sequences in the *cag* pathogenicity island ([Censini et al., 1996](#)).

Not all *H. pylori* strains possess the *cag* pathogenicity island. In addition, it can be lost or gained via recombination ([Kersulyte et al., 1999](#)). In addition, partial deletions have been described. Among the *cag* pathogenicity island genes, six show sequence similarity with genes coding for a secretion system in other bacteria. This is a type IV secretion system, a multi-protein complex that allows the bacterium to inject specific molecules into an eukaryotic cell ([Krause et al., 2000](#)). Among such translocated molecules is the product of one of the *cag* pathogenicity island genes, CagA. This immunodominant protein of 120–145 kDa was the first *H. pylori* protein to be linked to more severe disease ([Crabtree et al., 1991](#)). Phosphorylated CagA was also detected inside epithelial cells infected with *H. pylori* ([Yamazaki et al., 2003](#); [Higashi et al., 2005](#)).

### (ii) VacA cytotoxin

A cytotoxic activity was first reported in broth culture filtrates of *H. pylori* incubated with mammalian cells *in vitro* ([Leunk et al., 1988](#)). The protein responsible for the observed effect of large intracellular vacuoles was designated vacuolating cytotoxin VacA ([Cover & Blaser, 1992](#)). It is a high-molecular weight multimeric pore-forming protein encoded by the chromosomal gene *vacA* ([Cover et al., 1994](#); [Schmitt & Haas, 1994](#); [Telford et al., 1994](#)).

As with *cagA*, no close homologues of *vacA* exist in other *Helicobacter* species nor in other bacteria or eukaryotic cells. Mature 88 kDa VacA toxin molecules are secreted as soluble proteins into the extracellular space, but can also remain localized on the surface of *H. pylori* ([Ilver et al., 2004](#)). The secreted toxin can assemble into oligomeric structures ([Cover et al., 1997](#); [Adrian et al., 2002](#)) for insertion into planar lipid bilayers to form anion-selective membrane channels allowing anions and urea to exit ([Iwamoto et al., 1999](#); [Tombola et al., 1999](#)). The microscopic analysis of VacA oligomeric complexes has shown their dissociation into monomeric components with low vacuolating potential that can be activated by pH changes.

The secreted mature 88 kDa toxin can be cleaved in two fragments: p33 and p55 ([Nguyen et al., 2001](#)). The p55 domain has a role in the binding of VacA to host cells, and the p33 domain, together with 100 amino acids of the p55 domain, is sufficient to induce intracellular vacuolation ([de Bernard et al., 1998](#)).

Unlike *cagA*, *vacA* is conserved among all *H. pylori* strains, but exhibits a high level of genetic diversity. Several *vacA* alleles can be distinguished on the basis of diversity in the 5' region, namely in the signal region, (s region) s1 and s2, and in the mid-region (m region) m1 and m2 ([Atherton et al., 1995](#)). Among the different combinations, the s1m1 type produces the most active vacuolating activity in different

cell lines, s1m2 produces detectable vacuolation in a limited range of cell types, s2m2 is inactive, and s2m1 does not appear to exist.

More recently, a third group of alleles has been described in the intermediary region (i) – i1 and i2 ([Rhead et al., 2007](#)).

A strong association between *vacA* s1 and *cag* pathogenicity island has been described ([Van Doorn et al., 1999](#)).

## 1.2 Epidemiology of infection

### 1.2.1 Prevalence, geographic distribution

The most common test used to determine the prevalence of infection in healthy populations is serology for *H. pylori* IgG by ELISA ([Vaira et al., 2002](#); [Mueller et al., 2006](#)). Serological testing for CagA antibody is more sensitive for individuals who are infected with CagA<sup>+</sup> *H. pylori*, but *H. pylori* IgG ELISA is most often used in screening. Other diagnostic tests for *H. pylori* are available but less often used in large epidemiological studies or to estimate population prevalence ([Mégraud & Lehours, 2007](#)).

*H. pylori* infection is common, with a global prevalence of over 50%, but with substantial country-to-country variations ([Parsonnet, 1998](#); [Suerbaum & Michetti, 2002](#)). Prevalence rates differ by age, race/ethnicity, and socioeconomic characteristics. As a rule, rates are higher in developing countries than in developed ones; however, in several eastern European countries the prevalence of infection is high.

The prevalence of infection is highest in the older age groups ([The EUROGAST Study Group, 1993](#)). The rate of *H. pylori* infection has been shown to have decreased in successive birth cohorts over the past several decades in developed countries ([Roosendaal et al., 1997](#)). [Banatvala et al. \(1993\)](#) screened a total of 631 serum samples collected from adults and children in 1969, 1979, and 1989, and the cohort effect on *H. pylori* positivity was estimated by Western blot based

on year of birth. The seropositivity declined by 26% (8–41%) per decade ( $P < 0.008$ ). [Eslick \(2003\)](#) reported on 451 pregnant women screened for *H. pylori* infection in Australia, and found that infection rates declined from 44% in the birth cohort of 1951–60, to 29% in the birth cohort of 1961–70, to 20% in the birth cohort of 1971–80, to 9% in the birth cohort of 1981–90.

[Rothenbacher et al. \(1998\)](#) used  $^{13}\text{C}$ -urea breath tests for population screening of approximately 1000 preschool children as part of a physician-administered school fitness test. This demonstrated its utility for determining current infection status in a relatively large population of healthy subjects.

Because of the substantial differences in the prevalence of infection over time by age group and race/ethnicity within a country, international comparisons of the overall prevalence of *H. pylori* where the populations were tested during different time periods, and different age composition or race/ethnicity or type of test performed are crude comparisons at best, and likely to be misleading (for a review, see [Everhart, 2000](#)).

The results of the EUROGAST Study Group ([The EUROGAST Study Group, 1993](#)) illustrate the importance of age-specific prevalence estimates. In Japan, the population prevalence was 61% in the 25–34 years age group, and 89% in the 55–64 years age group; in Poland the prevalence was 69% in the 25–34 years age group, and 89% in the 55–64 years age group; in Denmark the prevalence was 15% in the 25–34 years age group, and 30% in the 55–64 years age group; in the United States of America, the prevalence rate was 15% in the 25–34 years age group, and 34% in the 55–64 years age group.

Similar variations are seen within the USA between racial and ethnic groups. [Hyams et al. \(1995\)](#) conducted serological testing of 1000 military personnel aged 17–50 years. The overall prevalence of infection in the group was 25%, ranging from 18% in Caucasians to 45% in Hispanics and 46% in Blacks, with other races at 29%. More

recently [Everhart et al. \(2000\)](#) conducted a larger seroprevalence survey in the USA in which 7465 adults were tested for *H. pylori* by IgG ELISA. The overall seroprevalence was 32.5%. It was substantially higher among non-Hispanic blacks (52.7%) and Mexican-Americans (61.6%) than among non-Hispanic Caucasians (26.2%).

## 1.2.2 Transmission

### (a) Person-to-person route

Humans are the only known significant reservoir of *H. pylori* ([Oderda, 1999](#)). Person-to-person contact is believed to be the primary route of transmission in developed countries, and is also important in developing countries. Close personal contact, particularly within the family including mother/parents to child, sibling to sibling and spouse to spouse, has been consistently demonstrated as a risk factor for transmission of infection ([Dominici et al., 1999](#); [Escobar & Kawakami, 2004](#)).

[Brenner et al. \(2006\)](#) determined current *H. pylori* infection in 670 spousal pairs by  $^{13}\text{C}$ -Urea breath test and monoclonal antigen immunoassay for *H. pylori* in stool. The prevalence of infection was significantly greater in women with infected partners, compared to women whose partner was not infected (34.9% vs 14.5%).

Person-to-person transmission can occur in several ways. [Parsonnet et al. \(1999\)](#) conducted a controlled clinical experimental study to determine how humans shed *H. pylori* into the environment. A total of 16 asymptomatic individuals positive for *H. pylori* were administered a cathartic and an emetic and 1/10 participating *H. pylori*-negative individuals was given an emetic. Stool and vomitus samples were collected. All vomitus specimens from *H. pylori* positive individuals grew *H. pylori* (confirmed by polymerase chain reaction (PCR)). Air was sampled during vomiting and *H. pylori* were grown from 6 of 16 samples (37.5%). Small quantities of *H. pylori* were grown in three (18.8%) and nine (56.3%)

saliva samples obtained from subjects before and after emesis, respectively. Cultures from 7/14 (50%) positive subjects had at least one positive culture and 22/101 cathartic stools (21.8%) grew *H. pylori*. Samples from negative subjects did not grow the organism on culture.

Transmission of *H. pylori* was also examined by [Perry et al. \(2006\)](#) who tested 2752 household members for *H. pylori* in serum or stool at baseline and then again 3 months later. A total of 30 new infections occurred among 1752 persons uninfected at baseline. Exposure to a household member with gastroenteritis was associated with a relative risk of 4.8 (95%CI: 1.4–17.1) for definite or probable new infection. Risk of infection was greater for exposure to vomiting (odds ratio (OR), 6.3) than to diarrhoea (OR, 3.0).

Significantly higher than expected prevalence rates of *H. pylori* infection have been observed in institutionalized adults and children ([Malaty et al., 1996](#); [Böhmer et al., 1997](#)).

#### (b) Oral-oral route

*H. pylori* DNA has been detected in the saliva of *H. pylori*-positive subjects by PCR ([Namavar et al., 1995](#); [Madinier et al., 1997](#)). *H. pylori* organisms have also been successfully detected from the dental plaque of infected persons ([Nguyen et al., 1993](#)). In general, isolation has not been uniformly successful, however, perhaps as a result of the transient presence of *H. pylori* in the oral cavity or poor detection capability resulting from the co-occurrence of many other bacteria in the oral cavity.

#### (c) Faecal-oral route

*H. pylori* has been detected in faeces by culture and its DNA by PCR ([Kelly et al., 1994](#); [Namavar et al., 1995](#)), although other investigators have failed to replicate this ([van Zwet et al., 1994](#)). One study found detectable DNA in the faeces of 73% of known infected subjects ([Gramley et al., 1999](#)). These data, together with those from [Parsonnet](#)

[et al. \(1999\)](#), document the possible role of faecal shedding of *H. pylori* into the environment.

#### (d) Waterborne transmission

Studies in the People's Republic of China and in Latin America found that the source of water used for consumption, bathing or swimming could possibly be associated with *H. pylori* infection ([Goodman et al., 1996](#); [Zhang et al., 1996](#)). Contamination of drinking-water and sewage water has been demonstrated. [Hegarty et al. \(1999\)](#) found *H. pylori* in 60% of the samples of surface water, and 65% of the shallow ground water collected in several states in the USA. A Japanese study also reported *H. pylori* contamination of water from rivers and ponds ([Sasaki et al., 1999](#)).

#### (e) Iatrogenic transmission

Endoscopes used routinely in upper gastrointestinal procedures may be the source of iatrogenic infection as a result of improper disinfection between procedures ([Langenberg et al., 1990](#); [Tytgat, 1995](#)).

### 1.2.3 Risk factors for infection

The best established risk factor for *H. pylori* infection is low socioeconomic status, particularly during childhood when initial infection generally occurs ([Malaty & Graham, 1994](#)). Both education and income as components of socioeconomic status are inversely related to risk of infection ([Replogle et al., 1995](#)). Factors closely linked to socioeconomic status that appear to contribute to this inverse relation between poverty and risk of infection include hygienic conditions, household density/crowding, and the number of young children in the household ([Goodman et al., 1996](#); [Ford et al., 2007](#)).

Neither smoking nor alcohol were found to be associated with the prevalence of *H. pylori* seropositivity in the large EUROPAST study of 17 asymptomatic populations ([The EUROPAST](#)

[Study Group, 1993](#)). Because the literature is inconsistent, the most recent studies also report no significant association between *H. pylori* infection and tobacco use ([Brown, 2000](#)). Alcohol, particularly wine consumption, was found to have an inverse association with *H. pylori* infection in several studies ([Brenner et al., 1997, 1999a, b](#)). Other studies have also found modest reductions in risk that were not statistically significant ([Fontham et al., 1995; Peach et al., 1997](#)).

#### 1.2.4 Persistence, latency, and natural history of infection

Acquisition of *H. pylori* infection typically occurs in childhood ([Malaty & Graham, 1994; Goodman et al., 1996; Brown, 2000](#)). Once infection is established, it usually lasts for life, unless treated. At present there is no vaccine available, and the treatment of infection is generally a 2-week course of triple therapy consisting of an antisecretory agent, and two antibiotics.

*H. pylori* antibody titre has been shown to decline over the progression of premalignant lesions, and impacts the validity of serology, particularly in retrospective studies. [Kokkola et al. \(2003\)](#) followed 47 men with advanced *H. pylori*-positive atrophic corpus gastritis by endoscopy over a 6-year period, and by serum levels of pepsinogen I and antibodies to *H. pylori* over a 10-year period. None was treated for *H. pylori* infection during the study. The mean *H. pylori* antibody titres (IgG and IgA) declined during the course of follow-up, and 11 (23%) men converted to a seronegative status, and no significant changes were observed in the grade of atrophy or intestinal metaplasia in the antrum, or in the grade of intestinal metaplasia in the corpus. Using material from a population-based case-control study, [Ekström et al. \(2001\)](#) re-evaluated the association between *H. pylori* and gastric cancer by comparing ELISA assay against *H. pylori* IgG with immunoblot against CagA antibodies to detect evidence of past *H. pylori*

infection. Among cases, the seroprevalence of *H. pylori* was around 70% by ELISA and around 90% by immunoblot; among controls, the seroprevalence was similar by the two methods (55% positive). The odds ratios relating *H. pylori* exposure to gastric cancer substantially increased when CagA antibody positivity rather than *H. pylori* IgG ELISA was used to classify past exposure.

[Yoo et al. \(2007\)](#) examined the positivity of several currently available diagnostic tests for *H. pylori* when atrophic gastritis and/or intestinal metaplasia, and presumably more advanced lesions as well, are present. The CLO test (based on urease activity), has lower sensitivity in cases of both atrophy or intestinal metaplasia. Histological identification of *H. pylori* with Giemsa stain was markedly reduced as the degree of intestinal metaplasia increased ( $P < 0.01$ ), but was not affected in cases of atrophy only. The culture test was not affected except at the highest grade of atrophy or intestinal metaplasia, with 0% positivity.

## 2. Cancer in Humans

### 2.1 Cancer of the stomach

The previous IARC Monograph ([IARC, 1994](#)) reviewed results from four cohort and nine case-control studies that considered gastric carcinoma. Since its publication, results from several further cohort studies have been published. Some of these, together with the cohort studies presented in the earlier Monograph, were included in a pooled reanalysis ([Helicobacter and Cancer Collaborative Group, 2001](#)).

In analysing the relationship between gastric carcinoma and *H. pylori*, there is a specific bias in retrospective determination of *H. pylori* status in that precancerous subjects may undergo a loss of infection, thus producing an underestimate of prevalence in cases but not in controls ([Kokkola et al., 2003; Yoo et al., 2007](#)). For this

reason, relatively little weight is given to case-control studies in the assessment of the relationship between gastric carcinoma and *H. pylori*, although some are cited below as they provide specific evidence.

Since the previous *IARC Monograph*, it has also been reported that *H. pylori* appears to have a different relationship with gastric carcinoma arising in the region of the stomach distal to the cardia (non-cardia gastric carcinoma) compared with the cardia region located adjacent to the oesophageal sphincter. As a consequence, the following presentation of results, where possible, distinguishes non-cardia from cardia gastric carcinoma.

### 2.1.1 Non-cardia gastric carcinoma

Results are available from 17 prospective cohort studies with nested case-control designs and six further full cohort studies. Whereas the nested case-control comparisons all specify cases defined as non-cardia gastric carcinoma, for the full cohort studies, incident cancers are generally defined as gastric carcinoma without further subsite specification. [The Working Group noted that in the main, these can be assumed to be of the non-cardia.]

#### (a) Nested case-control analyses within cohort studies

These are summarized in Table 2.1 (available at <http://monographs.iarc.fr/ENG/Monographs/vol100B/100B-10-Table2.1.pdf>). The pooled reanalysis ([Helicobacter and Cancer Collaborative Group, 2001](#)) presented results based on 762 cases of non-cardia gastric carcinoma and 2250 control subjects derived from 12 independent prospective cohort studies in nine countries (China, Finland, Iceland, Japan, Norway, Sweden, Taiwan (China), the United Kingdom, and USA). The overall matched odds ratio for the risk of non-cardia gastric carcinoma was 2.97 (95%CI: 2.34–3.77), and odds ratios for

individual studies varied from 1.52–11.1. There were no substantive differences in the odds ratios between men and women or between gastric carcinoma with intestinal or diffuse histological type. Younger cases at diagnosis had a higher odds ratio than older cases (OR, 7.10; 95%CI: 2.93–17.2, for those aged < 50 years at diagnosis).

Six of the individual studies included in the pooled reanalysis accrued more cases, and updated results have since been published ([Nomura et al., 2002a](#); [Kamangar et al., 2006a, 2007](#); [Knekt et al., 2006](#); [Hansen et al., 2007](#); [Simán et al., 2007](#)). Using ELISA for *H. pylori* IgG to determine *H. pylori* infection status, odds ratios in these studies varied from 1.6–7.9, and all were statistically significant. One study ([Simán et al., 2007](#)) used immunoblot against *H. pylori* or CagA to determine infection status, and reported an increase in the odds ratios from 11.1 using ELISA ([Simán et al., 1997](#)) to 16.8 or 17.8 respectively, using immunoblot.

New results have been reported from four further prospective studies with nested case-control designs ([Shin et al., 2005](#); [Sasazuki et al., 2006](#); [Palli et al., 2007](#); [Mitchell et al., 2008](#)). Using ELISA for IgG to determine *H. pylori* infection status, odds ratios for non-cardia gastric carcinoma varied from 1.07–5.10, and two were statistically significant ([Sasazuki et al., 2006](#); [Palli et al., 2007](#)). One study ([Mitchell et al., 2008](#)) also used immunoblot to determine infection status, and reported an increase in the odds ratio from 2.3 using ELISA to 10.6 using immunoblot.

[The Working Group noted that some of the variations between study results may result from variation in the sensitivity and specificity of the original ELISA assays used ([Feldman et al., 1995](#)). The Working Group noted the substantial increase in estimated odds ratios in recent studies using immunoblot assays ([Simán et al., 2007](#); [Mitchell et al., 2008](#)).]

In the pooled reanalysis ([Helicobacter and Cancer Collaborative Group, 2001](#)), when the results were stratified by period of follow-up, the

odds ratio for non-cardia gastric carcinoma with cases diagnosed less than 10 years after recruitment was 2.39 (95%CI: 1.82–3.12), and for those with cases diagnosed 10 or more years after recruitment, 5.93 (95%CI: 3.41–10.3). A similar relationship in the magnitude of the odds ratio with period of follow-up was reported in two of the updated studies ([Nomura et al., 2002a](#); [Knekt et al., 2006](#)), but not in two others ([Kamangar et al., 2006a](#), [2007](#)). A study from Iceland, of which preliminary results were included in the pooled reanalysis, has been reanalysed taking into account quantitative changes in antibody titre between the time of initial blood sample and the diagnosis of gastric carcinoma ([Tulinius et al., 2001](#)). Repeat blood samples were available from 23/41 of the original gastric carcinoma cases, and 128 controls matched for sex, age, time, and number of repeat samples. The odds ratio for gastric carcinoma (predominantly non-cardia) was 1.16 (95%CI: 1.05–1.28) for those showing a decline in antibody titre compared with those with constant or rising levels.

#### (b) Cohort studies

These studies are summarized in Table 2.2 (available at <http://monographs.iarc.fr/ENG/Monographs/vol100B/100B-10-Table2.2.pdf>). Three studies, two in Japan ([Yamagata et al., 2000](#); [Yanaoka et al., 2008](#)) and one in China ([You et al., 2000](#)) followed adult subjects undergoing health checks or general screening examinations in relation to *H. pylori* infection status. All three showed a statistically significant increased relative risk of gastric carcinoma associated with infection (in [Yamagata et al., 2000](#) only in men), with relative risks varying from 1.8–3.48.

Three further studies, two in Japan ([Uemura et al., 2001](#); [Watabe et al., 2005](#)) and one in Taiwan, China ([Hsu et al., 2007](#)) followed adult patients undergoing endoscopy procedures for non-malignant conditions, and found statistically significant higher rates of gastric carcinoma in *H. pylori*-positive subjects, with relative

risks [estimated by the Working Group] of 3.6 in one study ([Watabe et al., 2005](#)), and infinity (no *H. pylori*-negative subjects) in the other two. None of these studies was adjusted for potential confounders.

#### (c) Case-control studies

In a retrospective case-control study of 234 non-cardia gastric carcinoma cases and 238 population controls in Sweden ([Ekström et al., 2001](#)), the reported odds ratio when using conventional IgG ELISA to assess *H. pylori* infection status was 2.2 (95%CI: 1.4–3.6). The odds ratio increased to 21.0 (95%CI: 8.3–53.4) after exclusion from the reference group of all subjects who were ELISA-negative and CagA-positive by immunoblot [The Working Group noted that the immunoblot used was reported to be specific for CagA-positive strains of *H. pylori*.]

In a retrospective case-control study of 57 non-cardia gastric carcinoma cases and 360 controls (with colorectal cancer) in Germany ([Brenner et al., 2004](#)), the reported odds ratio using an IgG ELISA to assess *H. pylori* infection status was 3.7 (95%CI: 1.7–7.9). The odds ratio increased to 18.3 (95%CI: 2.4–136.7) after exclusion from the analysis of defined groups of subjects who might have been susceptible to a misclassified serological result.

#### (d) Meta-analyses

Four meta-analyses of the association between gastric carcinoma and *H. pylori* infection have been published. [Huang et al. \(1998\)](#) identified 19 studies, both retrospective and prospective, including data from 2491 cases and 3959 controls. For non-cardia gastric carcinoma, the summary odds ratio was 3.08 (95%CI: 1.78–5.31) over all study designs, with evidence of significant heterogeneity. An inverse monotonic association was observed between age at diagnosis and the magnitude of the odds ratio, for non-cardia and cardia gastric carcinoma combined,

from 9.29 (95%CI: 3.43–34.04) at 20–29 years to 1.05 (95%CI: 0.73–1.52) at 70 years or older.

[Danesh \(1999\)](#) identified 34 retrospective and 10 nested prospective case-control studies, which included data from 3300 and 800 cases, respectively. Because of concerns about the validity of the controls, no summary estimate of the odds ratio was calculated for the retrospective studies. For the nested case-control studies, the summary odds ratio for gastric carcinoma was 2.5 (95%CI: 1.9–3.4), with no evidence of significant heterogeneity [The Working Group noted that no distinction was made between non-cardia and cardia gastric carcinoma.]

[Eslick et al. \(1999\)](#) identified 42 studies: eight cohort and 34 case-control designs [The Working Group noted that, unlike other meta-analyses, this included studies where the assessment of *H. pylori* status included non-serological methods.] The summary odds ratio was 2.04 (95%CI: 1.69–2.45). Cancer subsite (non-cardia vs cardia) was not a significant effect modifier. There was statistically significant heterogeneity between studies, but no evidence of publication bias.

A more recent meta-analysis focused on 16 seroprevalence studies of CagA and gastric cancer, which included a total of 2284 cases and 2770 controls from diverse geographic populations ([Huang et al., 2003](#)). Overall, ten studies provided results stratified by subsite of the tumour. Evidence of *H. pylori* infection was associated with a 2.71-fold risk of developing non-cardia gastric cancer. Because antibodies against CagA may persist longer than antibodies against other *H. pylori* components normally detectable by *H. pylori* status serology, the risk of cancer in patients who were CagA-positive but *H. pylori*-negative was also evaluated. Compared with controls who were both *H. pylori*-negative and CagA-negative, the summary odds ratio of gastric cancer (non-cardia and cardia combined) was 2.89.

#### (e) Impact of *H. pylori* CagA status

Many of the nested case-control studies identified above reported odds ratios for the risk of gastric carcinoma associated with infection with CagA-positive strains of *H. pylori* to see if disease was exclusively or predominantly associated with this genotype. In the meta-analysis by [Huang et al. \(2003\)](#), the analysis confined to *H. pylori*-positive cases and controls showed an additional risk of 2.01 (95%CI: 1.21–3.32) associated with CagA-positive strains.

Eight of the nested case-control studies have reported separately on results relating to CagA status ([Parsonnet et al., 1997](#); [Nomura et al., 2002a, 2005](#); [Gwack et al., 2006](#); [Kamangar et al., 2006a, 2007](#); [Sasazuki et al., 2006](#); [Palli et al., 2007](#); [Simán et al., 2007](#)). Five of these studies compared adjusted odds ratios for non-cardia gastric carcinoma in subjects with both CagA-positive and -negative infection status against a baseline of *H. pylori*-negative subjects. The reported odds ratios were, respectively, 5.8 vs 2.2 ([Parsonnet et al., 1997](#)), 8.93 vs 6.55 ([Kamangar et al., 2006a](#)), 6.5 vs 1.6 ([Palli et al., 2007](#)), 12.5 vs 9.5 ([Sasazuki et al., 2006](#)), and 1.58 vs 1.62 ([Kamangar et al., 2007](#)). Thus, in 4/5 studies, the odds ratio associated with CagA-positive infection was substantively greater than that for CagA-negative infection. One other study ([Gwack et al., 2006](#)), in an analysis restricted to *H. pylori*-infected individuals, reported a statistically significant increased odds ratio in relation to CagA-positive status of 3.74 (95%CI: 1.10–12.73) compared with CagA-negative status, even though the risk associated with *H. pylori* *per se* was not significant ([Shin et al., 2005](#)). In the other two nested case-control studies reporting on CagA-positive status ([Nomura et al., 2002a](#); [Simán et al., 2007](#)), odds ratios were decreased in comparison with those for *H. pylori* infection alone.

### (f) Impact of *H. pylori* eradication

Results are available from six randomized intervention studies in which the subsequent risk of gastric carcinoma or gastric precancerous lesions has been evaluated in *H. pylori*-infected adult subjects who were randomized to receive *H. pylori* eradication therapy or placebo/no treatment (see Table 2.3 available at <http://monographs.iarc.fr/ENG/Monographs/vol100B/100B-10-Table2.3.pdf>). Only one study ([Wong et al., 2004](#)) was specifically designed to analyse gastric carcinoma outcomes. A total of 1630 *H. pylori* positive subjects undergoing endoscopy were followed up for a mean of 7.5 years after being randomized to eradication therapy ( $n = 817$ ) or placebo ( $n = 813$ ). The therapy was successful in eradicating the infection in 84% of subjects in the intervention arm. There were 7 (0.86%) and 11 (1.35%) incident cases of gastric carcinoma diagnosed in the intervention and placebo arms respectively, a non-significant difference ( $P = 0.33$ ). A *post-hoc* subgroup analysis of subjects with no precancerous lesions at recruitment showed 0 (0.0%) and 6 (1.2%) incident cases in the intervention and placebo arms ( $P = 0.02$ ) [The Working Group viewed the design of this study to be underpowered to assess adequately the relationship between eradication and gastric carcinoma outcomes.]

Three studies ([Leung et al., 2004](#); [Mera et al., 2005](#); [You et al., 2006](#)) were designed to analyse changes in precancerous histological pathology as the primary end-point but also reported results for gastric carcinoma outcomes. These studies respectively randomized 587, 795 and 2258 *H. pylori*-positive subjects to receive eradication therapy or placebo. In the intervention and placebo arms of the three studies, during follow-up, there were four and six, three and two, and 19 and 27 incident cases of gastric carcinoma diagnosed, respectively. None of these associations was statistically significant. In terms of progression or regression of precancerous pathology

compared with baseline, all three studies showed a statistically significant benefit in the intervention arms as did a further intervention study ([Ley et al., 2004](#)) of 248 subjects which did not specifically report on gastric carcinoma as an outcome.

[Fukase et al. \(2008\)](#) reported a study of 544 *H. pylori*-positive patients who were all diagnosed with early gastric carcinoma, and underwent endoscopic mucosal resection and were followed up for a mean of three years after being randomized to eradication therapy ( $n = 272$ ) or standard care ( $n = 272$ ). There were 9 (3.3%) and 24 (8.8%) incident metachronous cases of gastric carcinoma diagnosed in the intervention and placebo arms respectively, a statistically significant difference (hazard ratio [HR], 0.35; 95%CI: 0.16–0.78). In an earlier non-randomized study ([Uemura et al., 1997](#)), 132 *H. pylori* positive patients, 44–85 years of age, diagnosed with early gastric carcinoma and treated with endoscopic mucosal resection, were followed up for two years after 65 patients received eradication therapy, and 67 did not. There were no gastric carcinomas in the intervention group and six (9%) in the control group. [The Working Group viewed these two latter studies as not applicable to populations outside Japan.]

### (g) Synthesis

Since the previous *Monograph*, a substantial number of prospective observational studies, both nested case-control and cohort, had provided results supportive of an association between *H. pylori* infection and non-cardia gastric carcinoma. The magnitude of the risk is increased when more sensitive assay procedures are used and there appears to be a stronger association with CagA-positive strains of *H. pylori*. Results from randomized studies have not had sufficient power to evaluate the effect of the impact of *H. pylori* eradication on gastric carcinoma risk.

### 2.1.2 Cardia gastric carcinoma

Results are available from ten prospective cohort studies with nested case-control designs. These are summarized in Table 2.4 (available at <http://monographs.iarc.fr/ENG/Monographs/vol100B/100B-10-Table2.4.pdf>).

#### (a) Nested case-control analyses within cohort studies

The pooled reanalysis ([Helicobacter and Cancer Collaborative Group, 2001](#)) presented results based on 274 cases of cardia gastric carcinoma and 827 control subjects derived from 12 independent prospective cohort studies in nine countries (the United Kingdom, Finland, Sweden, Norway, Iceland, USA, Taiwan (China), and Japan) analysed using a nested design. Cases were matched to controls on the basis of study, sex, age and date of blood sample collection but no further adjustment in the analysis was possible. In all studies, *H. pylori* infection status was determined by a conventional ELISA for IgG antibodies against *H. pylori*. The overall matched odds ratio for the risk of cardia gastric carcinoma was 0.99 (95%CI: 0.72–1.35). Odds ratios for individual studies varied from 0.40–1.77.

Five of the individual studies included in the pooled reanalysis accrued more cases, and updated results have since been published ([Kamangar et al., 2006a, 2007; Knekt et al., 2006; Hansen et al., 2007; Simán et al., 2007](#)). Odd ratios showed a statistically significant reduced risk (0.27 and 0.31) in two studies, no significant difference from unity in two further studies (0.82 and 1.5), and a significantly increased risk in one study (1.64).

New results were reported from four further prospective studies with nested case-control designs ([Shin et al., 2005; Sasazuki et al., 2006; Palli et al., 2007; Mitchell et al., 2008](#)). Using ELISA for IgG status to determine *H. pylori* infection status, odds ratios for cardia gastric

carcinoma varied from 0.8–3.7 but none was statistically significant.

In the pooled reanalysis ([Helicobacter and Cancer Collaborative Group, 2001](#)) when the results were stratified by period of follow-up, the odds ratio for gastric carcinoma with cases diagnosed less than 10 years after recruitment was 1.23 (95%CI: 0.86–1.75), and for those with cases diagnosed 10 or more years after recruitment, 0.46 (95%CI: 0.23–0.90). A similar relationship in the magnitude of the odds ratio with period of follow-up was reported in one of the updated studies ([Kamangar et al., 2006a](#)), but not in another ([Kamangar et al., 2007](#)).

In the pooled reanalysis ([Helicobacter and Cancer Collaborative Group, 2001](#)), no substantive difference in the odds ratios between men (0.98; 95%CI: 0.68–1.40) and women (1.03; 95%CI: 0.55–1.92) was observed. There was, however, a difference between gastric carcinoma with intestinal (0.42; 95%CI: 0.24–0.75) and diffuse (0.93; 95%CI: 0.21–4.10) histological types. [The Working Group noted that this difference was unexplained.]

#### (b) Meta-analysis

Only one meta-analysis has reported specifically on the risk of cardia gastric carcinoma ([Huang et al., 1998](#)). Based on the results from six studies with several study designs, the summary odds ratio was 0.93 (95%CI: 0.62–1.38), with no significant heterogeneity.

#### (c) Impact of *H. pylori* CagA status

A meta-analysis ([Huang et al., 2003](#)) including results from both retrospective and prospective studies identified 16 eligible studies, ten of which provided results stratified by cardia subsite location of the tumour. The odds ratio associated with *H. pylori* infection (determined by *H. pylori* seroprevalence) was 1.13 (95%CI: 0.75–1.70); a further analysis, confined to *H. pylori*-positive cases and controls, showed a risk associated with CagA positivity of 0.70 (95%CI: 0.44–1.10).

Three nested case-control studies have reported results in which the risk of cardia gastric carcinoma in relation to CagA-positive and -negative status can be compared. Respectively, these odds ratios were 0.43 and 0.21 ([Kamangar et al., 2006a](#)), 0.8 and 0.8 ([Palli et al., 2007](#)), and 1.75 and 1.35 ([Kamangar et al., 2007](#)). In the other nested case-control study reporting on CagA-positive status ([Simán et al., 2007](#)), an increased odds ratio of 2.3 in comparison with that for *H. pylori* infection alone (OR, 1.3) was reported.

[The Working Group noted that there are substantial difficulties in the reliability of classification of cardia gastric carcinoma. Some studies may be more inclusive of distal non-cardia gastric carcinoma and other studies may be more inclusive of lower oesophageal adenocarcinoma cases, which may lead to variability of outcome between studies.]

## 2.2 Gastric mucosa-associated lymphoid tissue (MALT) lymphoma

Results are available from one prospective cohort study with a nested case-control design and one retrospective case-control study. These are summarized in Table 2.5 available at <http://monographs.iarc.fr/ENG/Monographs/vol100B/100B-10-Table2.5.pdf> and Table 2.6 available at <http://monographs.iarc.fr/ENG/Monographs/vol100B/100B-10-Table2.6.pdf>.

### 2.2.1 Nested case-control analysis within a cohort study

[Parsonnet et al. \(1994\)](#) analysed a database of 33 cases of gastric non-Hodgkin lymphoma and 132 matched controls. *H. pylori* infection status was determined by ELISA for IgG antibodies and the odds ratio for risk of gastric non-Hodgkin lymphoma was 6.3 (95%CI: 2.0–19.9).

For low-grade MALT lymphoma, the odds ratio was 2.8 (95%CI: 0.2–28.5).

### 2.2.2 Case-control study

A study from Spain ([de Sanjosé et al., 2004](#)) compared ten cases of gastric lymphoma (four gastric MALT) with matched hospital controls. *H. pylori* infection status was determined by ELISA for IgG antibodies, the odds ratio for the risk of both gastric lymphoma and gastric MALT was infinity (all cases infected).

### 2.2.3 Impact of *H. pylori* eradication

A total of 16 uncontrolled studies ([Wotherspoon et al., 1993](#); [Stolte et al., 1994](#); [Bayerdörffer et al., 1995](#); [Neubauer et al., 1997](#); [Pinotti et al., 1997](#); [Savio et al., 2000](#); [Chen et al., 2001, 2005](#); [Fischbach et al., 2004](#); [Nakamura et al., 2005, 2008](#); [Wündisch et al., 2005](#); [Hong et al., 2006](#); [El-Zahabi et al., 2007](#); [Terai et al., 2008](#); [Stathis et al., 2009](#)) reported on the effect of *H. pylori* eradication therapy on B-cell MALT gastric lymphoma regression (see Table 2.7 available at <http://monographs.iarc.fr/ENG/Monographs/vol100B/100B-10-Table2.7.pdf>). In all studies, the eradication rates were very high (in general over 94%), and were accompanied by high rates of complete remission of the MALT lymphoma (62–100%). Where assessed, remission was more strongly associated with the successfully treated patients. Subsequent relapse rates, where reported, were in the order of 10% over the 1–3 year follow-up periods. [The Working Group recognized that these results made it unlikely that ethics committees would approve randomized intervention studies on the effect of *H. pylori* eradication on MALT regression.]

### 2.2.4 Synthesis

Despite the small number of observational studies of B-cell lymphoma in relation to *H. pylori* infection, evidence from the eradication studies

is critically important. Treatment of patients to eradicate *H. pylori* is strongly associated with remission of low-grade lymphomas. Therefore, infection with *H. pylori* causes low-grade B-cell MALT gastric lymphoma in humans.

## 2.3 Cancer of the oesophagus

### 2.3.1 Oesophageal adenocarcinoma

Results are available from two prospective cohort studies with nested case-control designs, 15 retrospective case-control studies, and three meta-analyses.

#### (a) Cohort studies

Two studies ([de Martel et al., 2005](#); [Simán et al., 2007](#)) analysed a database of 51 and 12 cases of oesophageal adenocarcinoma, respectively, and reported adjusted odds ratios of 0.37 (95%CI: 0.16–0.88) and 0.46 (95%CI: 0.07–2.6) respectively (see Table 2.8 available at [http://monographs.iarc.fr/ENG/Monographs/vol100B/100B-10-Table2.8.pdf](#)). Analysis for CagA-positive infection status did not substantially modify these results. Both studies adjusted for smoking but only the former adjusted for body mass index [The Working Group noted that this is a potential confounder for this type of cancer.]

#### (b) Case-control studies

Among the available studies, four were population-based ([El-Omar et al., 2003](#); [Wu et al., 2003](#); [Ye et al., 2004](#); [Anderson et al., 2008](#)). Two studies ([Ye et al., 2004](#); [Anderson et al., 2008](#)) reported significantly reduced odds ratios of 0.3 and 0.38, respectively. The other two studies ([El-Omar et al., 2003](#); [Wu et al., 2003](#)) reported odds ratios that were not significantly different from unity; however, the odds ratios associated with CagA positivity decreased to 0.82 and 0.33, respectively, with the latter being statistically significant.

Three studies ([Wu et al., 2003](#); [Ye et al., 2004](#); [Anderson et al., 2008](#)) adjusted for body mass index, and all of them adjusted for other potential confounders. One further hospital-based study ([Früh et al., 2008](#)) that used friends and spouses as controls reported an odds ratio of 0.71 (0.95%CI: 0.4–1.0) for *H. pylori* infection, with borderline statistical significance after adjustment for confounders including body mass index.

Nine other case-control studies were based on comparisons within clinical patient groups and all reported odds ratios that were either significantly reduced or not significantly different from unity (see Table 2.9 available at [http://monographs.iarc.fr/ENG/Monographs/vol100B/100B-10-Table2.9.pdf](#)).

#### (c) Meta-analyses

A meta-analysis ([Islami & Kamangar, 2008](#)) reviewed the results from 13 studies (2 prospective and 11 retrospective case-control studies) of oesophageal adenocarcinoma. A comparison of 840 cases with 2890 controls and an assessment of *H. pylori* infection status mainly by ELISA resulted in a summary odds ratio of 0.56 (95%CI: 0.46–0.68). There was no statistically significant heterogeneity between studies, and no evidence of publication bias. Sensitivity analyses to include only large studies or only population-based studies or similar methods for assessment of infection did not substantially modify the odds ratio. Five studies included comparisons of CagA-positive and -negative strain status against *H. pylori*-negative subjects, and for these, the summary odds ratios were 0.41 (95%CI: 0.28–0.62) and 1.08 (95%CI: 0.76–1.53), respectively.

Two further meta-analyses ([Rokkas et al., 2007](#); [Zhuo et al., 2008](#)), which included fewer studies, also reported a significant decrease in risk with both *H. pylori* and CagA positivity (see Table 2.10 available at [http://monographs.iarc.fr/ENG/Monographs/vol100B/100B-10-Table2.10.pdf](#)).

#### (d) Synthesis

The observational epidemiological studies are all consistent in showing a lack of association between *H. pylori* infection and an increased risk of oesophageal adenocarcinoma. Several of these studies as well as the meta-analyses show a statistically significant reduced risk of oesophageal cancer.

#### 2.3.2 Oesophageal squamous cell carcinoma

Results are available from two prospective cohort studies with nested case-control designs, five retrospective case-control studies, and three meta-analyses.

##### (a) Cohort studies

Two studies ([Kamangar et al., 2007](#); [Simán et al., 2007](#)) analysed a database of 300 and 37 cases of oesophageal squamous cell carcinoma respectively. The adjusted odds ratios for *H. pylori* infection were 1.17 (95%CI: 0.88–1.57) and 0.56 (95%CI: 0.24–1.3), respectively. CagA infection status did not substantially modify these results (see Table 2.11 available at [http://monographs.iarc.fr/ENG/Monographs/vol100B/100B-10-Table2.11.pdf](#)).

##### (b) Case-control studies

Four studies were population-based ([El-Omar et al., 2003](#); [Wang et al., 2003a](#); [Ye et al., 2004](#); [Wu et al., 2005](#)). One of these (El-Omar et al., 2003) included 53 cases and reported a statistically significant increased odds ratio of 2.11; another study ([Wu et al., 2005](#)) included 127 cases and reported a significantly reduced odds ratio of 0.51. The other two studies ([Wang et al., 2003a](#); [Ye et al., 2004](#)) included 63 and 85 cases, respectively, and reported odds ratios not significantly different from unity. The studies all adjusted for age and sex but differed in the extent of adjustment for other confounding factors. Only one study ([Wu et al., 2005](#)) adjusted for alcohol consumption. One hospital-based study ([Iijima](#)

[et al., 2007](#)) reported a non-significantly increased odds ratio of 1.40 (95%CI: 0.62–3.15 [calculated by the Working Group]) (see Table 2.12 available at [http://monographs.iarc.fr/ENG/Monographs/vol100B/100B-10-Table2.12.pdf](#)).

##### (c) Meta-analyses

One meta-analysis ([Islami & Kamangar, 2008](#)) reviewed results from nine studies (two prospective and seven retrospective case-control studies) of oesophageal squamous cell carcinoma. A comparison of 921 cases of oesophageal squamous cell carcinoma with 2743 controls and an assessment of *H. pylori* infection status mainly by ELISA resulted in a summary odds ratio of 1.10 (95%CI: 0.78–1.55). There was statistically significant heterogeneity between studies, but no evidence of publication bias. Sensitivity analyses to include only large studies or only population-based studies or similar methods for assessment of infection did not substantially modify the risk. Four studies included comparisons of CagA-positive and -negative strain status against *H. pylori*-negative subjects: the meta-relative risks were 1.01 (95%CI: 0.80–1.27) and 1.41 (95%CI: 1.00–1.97), respectively. Two further meta-analyses ([Rokkas et al., 2007](#); [Zhuo et al., 2008](#)), which included fewer studies, reported similar results (see Table 2.13 available at [http://monographs.iarc.fr/ENG/Monographs/vol100B/100B-10-Table2.13.pdf](#)).

##### (d) Synthesis

The Working Group concluded that there was little evidence of an association between *H. pylori* infection and the risk of oesophageal squamous cell carcinoma.

## 2.4 Other cancers

### 2.4.1 Cancer of the liver

#### (a) Hepatocellular carcinoma

Results are available from 17 retrospective case-control studies, and a meta-analysis.

Two studies, based on 46 and 11 cases and using ELISA to detect IgG antibodies to *H. pylori*, showed odds ratios [calculated by the Working Group] that were significantly increased in one study (OR, 3.02; 95%CI: 1.12–8.34) ([Leone et al., 2003](#)), but not in the other (OR, 2.3; 95%CI: 0.15–15.1) ([Dore et al., 2002](#)) (see Table 2.14 available at <http://monographs.iarc.fr/ENG/Monographs/vol100B/100B-10-Table2.14.pdf>). No adjustment was carried out for potential confounders. All the other studies used PCR assays of liver biopsy samples from cases and controls to detect the presence of *Helicobacter* species (see Table 2.15 available at <http://monographs.iarc.fr/ENG/Monographs/vol100B/100B-10-Table2.15.pdf>). The primers used for the PCR assays (16s rDNA primers) were for genes associated with the *Helicobacter* genus but were not specific for *H. pylori*. In some of the studies, DNA from a subsample of positive samples was sequenced, and found to be specific for *H. pylori*. A higher proportion of positive results in 13/15 studies were observed among cases when compared to controls. Cases numbers were small in all studies, the largest having 48 cases, and studies varied in the extent to which they adjusted for potential confounding factors.

[The Working Group noted the small size of these studies, the potential problems of specificity associated with PCR assays, the use of opportunistic control series selected from patient groups, and the lack of adjustment for potential confounders.]

A meta-analysis ([Xuan et al., 2008](#)) reviewed results from ten studies (nine case-control and one cross-sectional) (see Table 2.16 available at <http://monographs.iarc.fr/ENG/Monographs/vol100B/100B-10-Table2.16.pdf>). A comparison of 242 cases of hepatocellular carcinoma with 280 hospital controls and an assessment of *H. pylori* infection status by PCR on liver biopsy samples resulted in a summary odds ratio of 13.63 (95%CI: 7.90–23.49). [The Working Group were concerned about the comparability of studies within this

meta-analysis as a wide variety of control groups and methodologies were employed.]

#### (b) *Cholangiocarcinoma*

A review ([de Martel et al., 2009](#)) summarized results from eight studies in which PCR was used to detect the presence of *Helicobacter* species in bile or tissue biopsies from patients with biliary tract cancers and controls usually with benign biliary tract diseases (see Table 2.17 available at <http://monographs.iarc.fr/ENG/Monographs/vol100B/100B-10-Table2.17.pdf>). The primers used for the PCR assays were not specific for *H. pylori*. In 2/8 studies, none of the cases was reported as positive but the other six studies all showed a higher proportion of positive results in cases compared to controls. None of these studies included more than 20 cases.

[The Working Group noted the small size of these studies, the potential problems of specificity associated with PCR assays, the use of opportunistic control series selected from patient groups, and the lack of adjustment for potential confounders.]

#### 2.4.2 *Cancer of the colorectum*

Results are available from two prospective cohort studies with a nested case-control design, 12 retrospective case-control studies, and one meta-analysis.

##### (a) *Nested case-control analyses with cohort studies*

Two studies ([Thorburn et al., 1998](#); [Limburg et al., 2002](#)) were based on 233 and 118 cases of colorectal cancer associated with *H. pylori* infection, and reported non-significant adjusted odds ratios of 0.9 (95%CI: 0.5–1.5) and 1.05 (95%CI: 0.63–1.74), respectively, associated with *H. pylori* infection (see Table 2.18 available at <http://monographs.iarc.fr/ENG/Monographs/vol100B/100B-10-Table2.18.pdf>). CagA-positive infection status

did not influence the latter result, and was not tested for in the former study.

#### (b) Case-control studies

Out of 11 studies, three ([Hartwich et al., 2001](#); [Machida-Montani et al., 2007](#); [Zumkeller et al., 2007](#)) were population-based (see Table 2.19 available at <http://monographs.iarc.fr/ENG/Monographs/vol100B/100B-10-Table2.19.pdf>). Two of these ([Hartwich et al., 2001](#); [Zumkeller et al., 2007](#)), based on 80 and 384 cases, respectively, reported statistically significant odds ratios of 3.78 and 1.41, respectively, for colorectal cancer associated with *H. pylori* infection. The third study ([Machida-Montani et al., 2007](#)) included 121 cases, and reported no significant risk. CagA-positive status did not affect the observed risk. Three studies ([Moss et al., 1995](#); [Fireman et al., 2000](#); [Siddheshwar et al., 2001](#)) were based within colonoscopy clinics, and included 41, 51 and 189 cases, respectively, with adjusted odds ratios for colorectal cancer associated with *H. pylori* infection reported to be between 0.74–2.43, none of which statistically significant. One other colonoscopy clinic study ([Fujimori et al., 2005](#)) included 154 cases of adenocarcinomas, and reported a statistically significant odds ratio of 1.8.

Four studies were hospital-based ([Penman et al., 1994](#); [Meucci et al., 1997](#); [Shmueli et al., 2001](#); [D'Onghia et al., 2007](#)), and did not adjust for any potential confounders.

#### (c) Meta-analysis

A meta-analysis ([Zumkeller et al., 2006](#)) reviewed results from 11 studies (two prospective and nine retrospective case-control studies) (see Table 2.20 available at <http://monographs.iarc.fr/ENG/Monographs/vol100B/100B-10-Table2.20.pdf>). A total of 899 cases of colorectal cancer were compared with 1476 controls; *H. pylori* infection status was assessed by ELISA in ten studies and by urea breath test in one study. The resulting meta-relative risk was 1.4 (95%CI: 1.1–1.8). The

meta-relative risk for the 2 prospective studies was 1.0 (95%CI: 0.8–1.4).

#### 2.4.3 Cancer of the pancreas

[Nilsson et al. \(2002\)](#) analysed pancreatic biopsy specimens from patients undergoing surgery for possible pancreatic cancer to detect the presence of *Helicobacter* species and *H. pylori* by bacterial culture, PCR, and DNA sequencing. Five of six pancreatic ductal carcinomas and one malignant neuroendocrine cancer were positive for *Helicobacter* species by PCR with genus-specific primers; however, none of the five was positive for *H. pylori* by PCR with genus-specific primers. Two of the 16S rDNA PCR fragments were sequenced and compared with the GenBank database by a BLAST search ([www.ncbi.nlm.nih.gov](http://www.ncbi.nlm.nih.gov)). One of those was 98% similar to the 16S rDNA of *Helicobacter* species liver 3, clustering to a phylogenetic group that includes *H. pylori*. The other was 99% similar to *H. pullorum*, and clustered to a phylogenetic group that also contains *H. bilis*.

Results are available from three informative prospective cohort studies with nested case-control designs, and one retrospective case-control study.

Two of the three prospective cohort studies ([de Martel et al., 2008](#); [Lindkvist et al., 2008](#)) based on 104 and 87 cases, respectively, of pancreatic cancer associated with *H. pylori* infection reported non-significant odds ratios of 0.85 (95%CI: 0.49–1.48) and 1.25 (95%CI: 0.75–2.09), respectively. The third study ([Stolzenberg-Solomon et al., 2001](#)) reported an odds ratio of 1.87 (95%CI: 1.05–3.34) for *H. pylori* seropositivity. CagA-positive infection status did not influence any of these results. All studies adjusted for age, sex, and smoking (see Table 2.21 available at <http://monographs.iarc.fr/ENG/Monographs/vol100B/100B-10-Table2.21.pdf>).

The retrospective case-control study ([Raderer et al., 1998](#)) included 92 cases of pancreatic

cancer. The odds ratio for the risk of pancreatic cancer associated with *H. pylori* infection was 2.1 (95%CI: 1.1–4.1) (see Table 2.22 available at <http://monographs.iarc.fr/ENG/Monographs/vol100B/100B-10-Table2.22.pdf>).

#### 2.4.4 Cancer of the lung

Results are available from four retrospective case–control studies (see Table 2.23 available at <http://monographs.iarc.fr/ENG/Monographs/vol100B/100B-10-Table2.23.pdf>).

Two retrospective case–control studies ([Gocyk et al., 2000](#); [Ece et al., 2005](#)) included 50 and 43 cases of lung cancer, respectively, and showed statistically significant associations with *H. pylori* infection with odds ratios [estimated by the Working Group] of 5.06 and 13.33, respectively. The first study was not adjusted for smoking, and the second included only smokers. Two other studies ([Philippou et al., 2004](#); [Najafizadeh et al., 2007](#)) included 72 and 40 cases of lung cancer, respectively, and neither showed a statistically significant association with *H. pylori* infection. [The Working Group noted that none of these four studies was adequately adjusted for smoking.]

#### 2.4.5 Cancer of the head and neck

Results are available from four retrospective case–control studies (see Table 2.24 available at <http://monographs.iarc.fr/ENG/Monographs/vol100B/100B-10-Table2.24.pdf>). The studies varied slightly in their case definitions. One ([Grandis et al., 1997](#)) included 21 cases of squamous cell carcinomas of the head and neck and the odds ratio for *H. pylori* infection was 0.82 (95%CI: 0.24–2.76). A second study among smokers ([Aygenc et al., 2001](#)) included 26 cases of squamous cell laryngeal cancers and the odds ratio for *H. pylori* infection [estimated by the Working Group] was 3.97 (95%CI: 1.32–11.89). A third study ([Rubin et al., 2003](#)) included 55 cases

of squamous cell cancer of the upper aerodigestive tract (excluding oesophagus) and six cases of laryngeal severe dysplasia, and the odds ratio for *H. pylori* infection [estimated by the Working Group] for the risk of cancer/severe dysplasia was 1.86 (95%CI: 1.03–3.35). The final study ([Nurgalieva et al., 2005](#)) included 119 cases of squamous cell carcinoma of the laryngopharynx, and the adjusted odds ratio for *H. pylori* infection was 1.27 (95%CI: 0.70–2.29).

#### 2.4.6 Childhood leukaemia

One prospective cohort study ([Lehtinen et al., 2005](#)) with a nested case–control design compared 341 children with acute lymphocytic leukaemia and 61 with other leukaemias with 1212 matched controls (see Table 2.25 available at <http://monographs.iarc.fr/ENG/Monographs/vol100B/100B-10-Table2.25.pdf>). *H. pylori* infection status was determined on maternal serum samples (first trimester) by ELISA for IgG and IgM antibodies. The adjusted odds ratio for risk of all leukaemias combined was 1.0 (95%CI: 0.8–1.2) for IgG antibodies. Results for IgM antibodies or specifically for acute lymphoblastic leukaemia did not differ substantially.

### 2.5 Cofactors

Two studies have found an effect modification between smoking, *H. pylori* infection, and gastric carcinoma ([Zaridze et al., 2000](#); [Brenner et al., 2002](#)). [Zaridze et al. \(2000\)](#) reported on the relative risk of gastric cancer associated with smoking by *H. pylori* status. Among men, the odds ratio for ever smoking compared to never smoking among *H. pylori* negatives was 1.0 (95%CI: 0.5–2.1), and ever smoking compared to never smoking among *H. pylori* positives was 2.3 (95%CI: 1.1–4.7), with a  $P = 0.07$  for the effect modification between smoking and *H. pylori*.

[Brenner et al. \(2002\)](#) evaluated the individual and joint association of smoking and *H. pylori*

infection as well as CagA-positive *H. pylori* infection. The adjusted relative risk of gastric cancer was 2.6 (95%CI: 1.2–5.7) for CagA-positive *H. pylori* infection in non-smokers compared to uninfected non-smokers, and CagA-positive *H. pylori*-infected smokers had a relative risk of 7.2 (95%CI: 2.2–23.6). When analyses were restricted to non-cardia gastric cancer, the corresponding estimates of relative risk were 6.1 (95%CI: 2.3–16.5) for CagA-positive non-smokers and 16.6 (95%CI: 4.3–67.2) for CagA-positive smokers. Not all studies found this effect. [Machida-Montani et al. \(2004\)](#) found gastric cancer risk associated with smoking and dietary factors to be independent of risk associated with *H.pylori* infection.

Plasma levels of vitamin C are inversely associated with gastric cancer risk for both cardia and non-cardia, diffuse, and intestinal subsites. Vitamin C plasma levels showed no effect modification with *H. pylori* infection ([Jenab et al., 2006](#)). [Ekström et al. \(2000\)](#), however, found an effect modification on non-cardia gastric carcinoma risk by dietary intake of ascorbic acid,  $\beta$ -carotene, and  $\alpha$ -tocopherol by *H. pylori* infection status. There was little or no association of these antioxidants in *H. pylori*-negative subjects, but 30–70% reductions in the relative risk of non-cardia gastric carcinoma in *H. pylori*-positive subjects were observed with dietary intake increments of 50 mg/day of ascorbic acid, 3.0 mg/day  $\beta$ -carotene or 8.0 mg/day  $\alpha$ -tocopherol.

A significant effect modification between *H. pylori* infection and salted, smoked foods and processed meat has been observed ([Phukan et al., 2006](#); [Shikata et al., 2006](#); [Epplein et al., 2008](#)). Two studies from East Asia have correlated dietary salt intake with an increased risk of non-cardia gastric cancer over and above that attributable to *H. pylori* alone ([Lee et al., 2003](#); [Shikata et al., 2006](#)).

In two recent studies, diets rich in fresh vegetables [and therefore high in antioxidants] intake were shown to be specially beneficial in reducing non-cardia gastric cancer risk among

those who are *H. pylori* infected ([Ekström et al., 2000](#); [Epplein et al., 2008](#)).

[Ekström et al. \(1999\)](#) found that risk of gastric cancer associated with *H. pylori* infection was independent of risk associated with specific occupational exposures.

### 3. Cancer in Experimental Animals

#### 3.1 Mongolian gerbil

The first report of gastric cancer induced by *H. pylori* in an animal model was published by [Watanabe et al. \(1998\)](#). The authors infected Mongolian gerbils (*Meriones unguiculatus*) with *H. pylori* strain TN2GF4, and observed the gastric lesions after 62 weeks. Gastric adenocarcinomas were detected in 10/27 infected animals vs 0/30 controls.

The Mongolian gerbil model infected with *H. pylori* has been cited in seven other publications ([Honda et al., 1998](#); [Hirayama et al., 1999](#); [Zheng et al., 2004](#); [Elfvin et al., 2005](#); [Franco et al., 2005, 2008](#); [Romero-Gallo et al., 2008](#)), and all except one ([Elfvin et al., 2005](#)) reported gastric adenocarcinoma developments. They developed late in the animals' lives (62–90 weeks) except in three studies ([Franco et al., 2005, 2008](#); [Romero-Gallo et al., 2008](#)) where the development was extremely rapid (8–12 weeks).

It is noteworthy that in other experiments using chemical carcinogens in Mongolian gerbils and where *H. pylori*-infected animals were used as controls, none of the controls developed gastric cancer ([Sugiyama et al., 1998](#); [Tokieda et al., 1999](#); [Shimizu et al., 1999](#); [Nozaki et al., 2002](#); [Kato et al., 2006](#)) with a follow-up of 40–53 weeks, or in the study of [Cao et al. \(2007\)](#) despite a follow-up of 70 weeks.

These results indicate that the Mongolian gerbil is not the most reliable model for the development of gastric adenocarcinomas even after a long follow-up. The reason could be linked to the animal strain used. Mongolian gerbils were first bred in Japan, then exported to the USA,

and later to Europe. The genetic background of the animals may have evolved differently among the different colonies. The *H. pylori* strain used may also be the cause of these discrepant results. Two strains were essentially used (ATCC 43 504 and TN2GF4) but when [Franco et al. \(2005\)](#) used a Mongolian gerbil adapted strain (7.13) derived from the parent strain B128, they observed gastric adenocarcinomas in 59% of the animals. The high susceptibility of this strain was confirmed in two further studies by [Franco et al. \(2008\)](#) and [Romero-Gallo et al. \(2008\)](#). Another variable is the criteria used for grading the observed pathology. This point was raised by [Elfvin et al. \(2005\)](#) who published a negative result. Furthermore, in the Mongolian gerbil model, no metastasis was reported nor did any gerbil die of gastric carcinoma.

The limitation of this model led to test the impact of *H. pylori* infection in Mongolian gerbils receiving well known chemical carcinogens, e.g. *N*-methyl-*N*-Nitrosourea or *N*-methyl-*N'*-nitro-*N*-nitrosoguanidine at different concentrations. Eight studies used such a design and in all of them, there was a synergistic effect of *H. pylori* infection on the incidence of gastric carcinomas compared to the incidence observed after treatment with the carcinogen alone.

In some studies gerbils were infected with *H. pylori* and then treated also with a chemical carcinogen to test the action of pharmacological agents such as a cyclooxygenase 2 inhibitor (etodolac) ([Magari et al., 2005](#)), and an antioxidative and anti-inflammatory compound (canolol) ([Cao et al., 2008](#)), both of which have a protective effect. The impact of a salty diet was also tested in two studies ([Nozaki et al., 2002](#); [Kato et al., 2006](#)), and a synergy between *H. pylori* infection and a high-salt diet was observed. [Romero-Gallo et al. \(2008\)](#) and [Nozaki et al. \(2003\)](#) showed the benefit of an early eradication using clarithromycin-based triple therapy, which decreased the gastric cancer incidence.

See [Table 3.1](#).

## 3.2 Mouse

### 3.2.1 Inbred mouse

The progression of the gastric lesions after *H. pylori* infection has also been observed in inbred mice, C57BL/6 and BALB/c. Despite a long follow-up of 80 and 100 weeks by [Kim et al. \(2003\)](#) and [Wang et al. \(2003b\)](#), respectively, no gastric adenocarcinoma occurred, only gastric lymphoma in the latter. When C57BL/6 mice crossed with 12996/SvEv mice were infected by *H. pylori* and submitted to a high-salt diet, high-grade dysplasia occurred but not adenocarcinoma ([Rogers et al., 2005](#)).

In a study where C57BL/6 mice infected with *H. felis* received an eradication treatment after different time periods, [Cai et al. \(2005\)](#) observed adenocarcinomas in all untreated infected animals after 24 months, no adenocarcinomas if the treatment was given after 2 or 6 months of infection, and a decrease in the incidence of adenocarcinomas if the treatment was delayed to the 12<sup>th</sup> month.

### 3.2.2 Transgenic mouse

The first model (of five transgenic models) used transgenic mice deficient for TGF-β infected with *H. pylori*. Gastric adenocarcinoma or dysplasia developed in 85.7% of the mice after 36 weeks ([Hahm et al., 2002](#)).

The second model involves transgenic mice overexpressing gastrin (INS-GAS mice). A first study showed that infection of INS-GAS male mice with *H. pylori* strain SS1 could induce gastric adenocarcinoma in 4/6 male animals within 30 weeks ([Fox et al., 2003a](#)). This finding was repeated in another study (3/7 gastric adenocarcinoma within 28 weeks), and the protective effect of estradiol was shown ([Ohtani et al., 2007](#)). In a study designed to determine the impact of *H. pylori* eradication therapy ([Lee et al., 2008](#)), gastric carcinogenesis was inhibited. In another study where an H2-receptor antagonist

**Table 3.1 Studies of gastric cancer in Mongolian gerbils infected by *H. pylori* by gavage with or without modifying agents**

Duration Reference	Dosing regimen, strain Gender Animals/group at start	Incidence of tumours	Significance	Comments
62 wk <a href="#">Watanabe et al. (1998)</a>	$10^7$ CFU <i>H. pylori</i> , TN2GF4 (M) 55, 30 (controls)	Adenocarcinoma: 10/27 cases 0/30 controls	[ <i>P</i> =0.0002]	Well differentiated intestinal type cancer of the pyloric region
18 mo <a href="#">Honda et al. (1998)</a>	$10^9$ CFU <i>H. pylori</i> , ATCC 43 504 (M) 15, 15 (controls)	Adenocarcinoma: 2/5 cases 0/5 controls	[NS]	
23 mo (average) <a href="#">Hirayama et al. (1999)</a>	<i>H. pylori</i> , ATCC 43 504 (M) 56 (total), 3 (controls)	Adenocarcinoma; Carcinoids 12–18 mo: 1/16; 4/16 18–24 mo: 0/14; 3/14 >24 mo: 0/24; 11/26 Controls 0/3; 0/3	[NS]	Carcinoids were found in the fundus region of the stomach
84 wk <a href="#">Zheng et al. (2004)</a>	<i>H. pylori</i> , ATCC 43 504 or <i>H. pylori</i> 161 (M, F) 1: <i>H. pylori</i> ATCC 43 504: <i>n</i> =18 2: <i>H. pylori</i> 161: <i>n</i> =18 3: Controls: <i>n</i> =10	Adenocarcinoma: 1: 1/6 (16.6%) 2: 2/11 (18.2%) 3: 0/10	[NS], Group 1 and 2 vs 3	
18 mo <a href="#">Elfvin et al. (2005)</a>	1: <i>H. pylori</i> , TN2GF4: <i>n</i> =23 2: <i>H. pylori</i> , SS1: <i>n</i> =20 3: Controls: <i>n</i> =18 (M) Interim sacrifices at 3, 6 and 12 mo Group size: 3–10 animals	No adenocarcinoma observed in all 12 groups		[Age of the animals at start is described as 'sixty-seven-week-old', which could be a typo.] Discussion on the interpretation of the pathological findings to conclude adenocarcinoma: the authors conclude that, so far, adenocarcinomas have not yet been shown convincingly to develop in infected gerbils
4 wk, 8 wk or 16 wk <a href="#">Franco et al. (2005)</a>	(M) Experiment 1 A. <i>H. pylori</i> B128: <i>n</i> =16 B. <i>H. pylori</i> 7.13: <i>n</i> =16  Experiment 2 C. <i>H. pylori</i> 7.13: <i>n</i> =116  D. Controls: <i>n</i> =42	Adenocarcinoma: A: 0/16 (8 wk) B: 4 wk: 2/8 (25%), 8 wk: 6/8 (75%) C: 4 wk: 17% [incidence NS], 8 wk: 59% [incidence NS], 16 wk: 59% [incidence NS] D: 0/42	[ <i>P</i> <0.0001]; Group B (8 wk) vs D	<i>H. pylori</i> strains 7.13 is gerbil adapted, derived from parent strain B128 (human gastric ulcer strain). Experiment 2 is very likely to be positive at Weeks 8 and 16

**Table 3.1 (continued)**

Duration Reference	Dosing regimen, strain Gender Animals/group at start	Incidence of tumours	Significance	Comments
14–18 wk <a href="#">Romero-Gallo et al. (2008)</a>	5 × 10 <sup>9</sup> CFU <i>H. pylori</i> strain 7.13 1. Eradication therapy at 4 wk post infection with lansoprazole, amoxicillin and clarithromycin, orally, for 2 wk (daily). Sacrifice 8 wk after end of treatment 2. Controls infected for 14 wk 3. Eradication therapy at 8 wk post infection with lansoprazole, amoxicillin and clarithromycin, orally, for 2 wk (daily). Sacrifice 8 wk after end of treatment 4. Controls infected for 18 wk	Adenocarcinoma: 0/8 6/20 (33%) 1/14 (7%) 5/10 (50%)	P=0.01 Group 1 vs 2  P=0.027 Group 3 vs 4	Eradication treatment decreased the carcinoma incidence
12–52 wk <a href="#">Franco et al. (2008)</a>	(M) 1. <i>H. pylori</i> 7.13 (12–16 wk, n=18; 24–30 wk, n=19; 40–52 wk, n=9) 2. <i>H. pylori</i> 7.13 cagA- (12–16 wk, n=18; 24–30 wk, n=20; 40–52 wk, n=17) 3. <i>H. pylori</i> 7.13 vacA- (12–16 wk, n=13; 24–30 wk, n=9; 40–52 wk, n=17) 4. Controls (12–16 wk, n=19; 24–30 wk, n=5; 40–52 wk, n=13)	Adenocarcinoma: 53% [9/17], 50% [9/18], 55% (5/9) 0% (0/18), 0% (0/20), 0% (0/17) 16% (2/13), 37% [3/8], 47.5% (8/17) 0% (0/19), 0% (0/5), 0% (0/13)	[p=0.0003], [NS], [p=0.005] [NS], [NS], [NS], [NS], [NS], [p<0.005]	<i>cagA</i> , cytotoxin-associated gene <i>vacA</i> , vacuolating cytotoxin gene
MNNG (in the drinking-water)				
52 wk <a href="#">Tokieda et al. (1999)</a>	10 <sup>9</sup> CFU <i>H. pylori</i> , ATCC 43 504 (M)	Adenocarcinoma: 1: 0/20 2: 0/14 3: 3/17 (17.6%) 4: 4/6 (66.7%)		Group 3: poorly differentiated GC Group 4: well differentiated GC

**Table 3.1 (continued)**

Duration Reference	Dosing regimen, strain Gender Animals/group at start	Incidence of tumours	Significance	Comments
50 wk <a href="#">Shimizu et al. (1999)</a>	$10^8$ CFU <i>H. pylori</i> , ATCC 43 504 (M)	Adenocarcinoma: 1: MNNG 300 ppm (10 wk) followed by <i>H. pylori</i> 2: MNNG 300 ppm (10 wk) alone 3: MNNG 60 ppm (10 wk) followed by <i>H. pylori</i> 4: MNNG 60 ppm (10 wk) alone 5: <i>H. pylori</i> followed by MNNG 100 ppm (30 wk) 6: MNNG 100 ppm (30 wk) alone 7: <i>H. pylori</i> followed by MNNG 20 ppm (30 wk) 8: MNNG 20 ppm (30 wk) alone 9: <i>H. pylori</i> alone 10: Controls	1: 12/27 (44%) 2: 1/19 (5.3%) 3: 6/27 (24%) 4: 0/20 5: 4/27 (14.8%) 6: 3/18 (16.7%) 7: 15/25 (60%) 8: 1/19 (5%) 9: 0/20 10: 0/20	<i>P</i> <0.01, Group 1 vs 2 <i>P</i> <0.05, Group 3 vs 4 <i>P</i> <0.001, Group 7 vs 8
<b>MNU (in the drinking water)</b>				
40 wk <a href="#">Sugiyama et al. (1998)</a>	$10^9$ CFU <i>H. pylori</i> , ATCC 43 504 (M) Experiment 1 (18–20/group) A. <i>H. pylori</i> followed by MNU 10 ppm (20 wk) B. MNU 10 ppm (20 wk) alone C. <i>H. pylori</i> followed by MNU 3 ppm (20 wk) D. MNU 3 ppm (20 wk) alone Experiment 2 E. MNU 30 ppm (6 wk) followed by <i>H. pylori</i> F. MNU 30 ppm (6 wk) alone G. MNU 10 ppm (10 wk) followed by <i>H. pylori</i> H. MNU 10 ppm (10 wk) alone I. Control: <i>H. pylori</i>	Adenocarcinoma: A: 7/19 (36.8%) B: 0/18 C: 1/20 (5%) D: 0/18 E: 6/18 (33.3%) F: 0/18 G: 1/19 (5.3%) H: 0/20 I: 0/17	<i>P</i> <0.01, Group A vs B <i>P</i> <0.05, Group E vs F <i>P</i> <0.005, Group 1 vs 3 NS; Group 2 vs 4	

**Table 3.1 (continued)**

Duration Reference	Dosing regimen, strain Gender Animals/group at start	Incidence of tumours	Significance	Comments
50 wk <a href="#">Nozaki et al. (2002)</a>	10 <sup>8</sup> CFU <i>H. pylori</i> , ATCC 43 504 (M) - Groups 1,2,3 & 4 MNU (20 ppm) orally alternate wk for a total of 5 wk - Groups 5,6,7 & 8 Controls <i>H. pylori</i> infection at Week 11 in Groups 1 & 2, 5 & 6 and vehicle in groups 3 & 4, 7 & 8. High salt diet at Week 12 in Groups 1 & 3, 5 & 7 and control diet in Groups 2 & 4, 6 & 8	Carcinoma: 1: 9/28 (32.1%) 2: 2/17 (11.8%) 3: 0/27 4: 0/20 5: 0/11 6: 0/6 7: 0/4 8: 0/4		Synergy between <i>H. pylori</i> infection & high salt diet to promote GC
75 wk <a href="#">Nozaki et al. (2002)</a>	10 <sup>8</sup> CFU <i>H. pylori</i> , ATCC 43 504 (M) - Groups A, B, C, D, E MNU (30 ppm) orally alternate wks for a total of 5 wk - Groups A, B, C, D, F <i>H. pylori</i> infection at Week 10 - Groups A, B, C Eradication therapy at Week 5, 25 and 45 post infection with lansoprazole, amoxicillin and clarithromycin, orally - Group G not infected	Carcinoma: A: 1/15 B: 3/11 C: 13/34 D: 9/16 E: 1/16 F: 0/8 G: 0/9	P<0.05 vs C, P<0.005 vs D P<0.05 vs E P<0.005 vs E and G	<i>H. pylori</i> eradication can reduce cancer incidence when given relatively early
53 wk <a href="#">Magari et al. (2005)</a>	3x10 <sup>8</sup> CFU <i>H. pylori</i> , ATCC 43 504; MNU: 10 ppm for 24 wk (M) A. <i>H. pylori</i> + MNU B. <i>H. pylori</i> + MNU + etodolac diet (5 mg/kg/d) C. <i>H. pylori</i> + MNU + etodolac diet (10 mg/kg/d) D. <i>H. pylori</i> + MNU + etodolac diet (30 mg/kg/d) E. Control (not infected)	Adenocarcinoma: A: 4/27 (14.8%) B: 8/34 (23.5%) C: 3/34 (8.8%) D: 0/39 E: 0/16	p<0.05, group A and B vs D	Etodolac is a selective cyclooxygenase 2 inhibitor preventing GC induction

**Table 3.1 (continued)**

Duration Reference	Dosing regimen, strain Gender Animals/group at start	Incidence of tumours	Significance	Comments
50 wk <a href="#">Kato et al. (2006)</a>	<i>H. pylori</i> , ATCC 43 504 (M) 20 groups G1–10: MNU (30 ppm) G11–20: no MNU G1–5 & G11–15: + <i>H. pylori</i> at Week 10 + increasing NaCl concentrations (0%, 2.5%, 5%, 10% in food or a gavage with diet with a saturated salt solution)	Adenocarcinoma: G1: 6/40 (15%) G2: 8/24 (33%) G3: 9/25 (36%) G4: 19/30 (63%) G5: 5/21 (24%) G8: 1/25 (4%) G6, G7, G9, G10–20: 0% (n=7–27)	<i>P</i> for trend <0.01 (Groups G1→G4) <i>p</i> <0.01 (Group G4 vs G1)	
70 wk <a href="#">Cao et al. (2007)</a>	10 <sup>8</sup> CFU <i>H. pylori</i> , ATCC 43 504 (M) 8 groups A to H A & E: inoculated at Week 0 B & F: inoculated at Week 12 C & G: inoculated at Week 18 D & H: Controls A, B, C & D received oral MNU (10 ppm) at Week 20 during 20 wk	Adenocarcinoma: A: 13/20 (65%) B: 2/10 (20%) C: 3/13 (23%) D: 0/16 E, F, G, H: 0/6	<i>P</i> <0.01; Group A vs D	
18 wk <a href="#">Cao et al. (2008)</a>	10 <sup>8</sup> CFU <i>H. pylori</i> strain ATCC 43 504; (M); MNU: 10 ppm; BHT: used at 0.5 ppm in the diet; Canolol: 0.1% in the diet - Experiment 1: A: <i>H. pylori</i> + Canolol + BHT B: <i>H. pylori</i> + BHT C: <i>H. pylori</i> D: Canolol + BHT E: BHT F: Control - Experiment 2: G: <i>H. pylori</i> + MNU + Canolol + BHT H: <i>H. pylori</i> + MNU + BHT I: <i>H. pylori</i> + MNU + Control diet J: Control + Canolol + BHT	Adenocarcinoma: A to F: 0/58 G: 6/40 (15%) H: 13/33 (39.4%) I: 15/36 (41.7%) J: 0/5	<i>P</i> =0.031 (Group G vs H) <i>P</i> =0.011 (Group G vs I)	Canolol, an antioxidative and anti inflammatory compound, prevents GC induction. BHT is an antioxidant additive No MNU-only treated group

ATCC, American type culture collection; BHT, butylated hydroxytoluene; CFU, colony forming units; d, day or days; F, female; GC, gastric cancer; *H. pylori*, *Helicobacter pylori*; M, male; MNNG, N-methyl-N'-nitro-N-nitrosoguanidine; MNU, N-methyl-N-nitrosourea; mo, month or months; NS, not significant; vs, versus; wk, week or weeks

(loxtidine) and a CCK-2-receptor antagonist were given for 6 months ([Takaishi et al., 2005](#)), gastric carcinogenesis was also inhibited in *H. felis*-infected animals. [The Working Group noted that no tumour incidences were provided.] Again, in the [Lee et al. \(2008\)](#) study, an early eradication (8 weeks) almost prevented gastric cancer development.

The third model used transgenic p27 deficient mice infected with *H. pylori* strain SS1. Infection and p27-deficiency were synergistic in gastric cancer development ([Kuzushita et al., 2005](#)).

The fourth model used Trefoil factor family 2 (TFF2)-deficient mice, with a B6129Sv strain background, infected with *H. pylori* strain SS1. Gastric adenocarcinomas were observed 19 months after infection ([Fox et al., 2007](#)).

The fifth model used transgenic mice over-expressing IL-1 $\beta$ . The two lines of mice built infected with *H. felis* developed gastric adenocarcinomas but at a low incidence ([Tu et al., 2008](#)).

### 3.2.3 Mouse models of gastric MALT lymphoma

Mouse models have been used as models of gastric MALT lymphomas. Gastric lymphomas histologically similar to the low-grade lymphoma observed in humans have been observed in mice infected for about 2 years with *Helicobacter* species, including *H. pylori* and *H. felis*. [Enno et al. \(1995\)](#) infected BALB/c mice with *H. felis*, and observed lymphoepithelial lesions (gastric lymphomas) in 25% of the mice after 22–26 months. Such lesions were observed in both BALB/c and C57BL/6 mice infected with *H. pylori* in the study by [Wang et al. \(2003b\)](#) (see above), and in BALB/c mice infected with *H. felis* ([Sutton et al., 2004](#)). However, the best model corresponds to neonatal thymectomized mice because all animals infected by *H. pylori* developed these lesions within 12 months ([Fukui et al., 2004](#)).

See [Table 3.2](#).

## 4. Other Relevant Data

It had been well established much before the discovery of *Helicobacter pylori* in 1983 that gastric cancers usually arose in a chronically inflamed stomach. Since the recognition of the role of *H. pylori* infection as the dominant cause of chronic gastritis, affecting approximately half of the world's population, considerable evidence has accumulated that the nature of the chronic inflammatory process driven by *H. pylori* is of critical importance in gastric carcinogenesis.

*H. pylori*-related gastric carcinogenesis is a slow process, typically developing over 4–6 decades, and accompanied by specific histological changes ([Correa et al., 1975](#)). The 'intestinal' subtype of gastric adenocarcinoma develops through a preneoplastic sequence from chronic superficial gastritis through atrophic gastritis, intestinal metaplasia, and dysplasia. The 'diffuse' subtype is also usually preceded by many years of chronic *H. pylori*-associated gastritis, although the molecular pathways and histological changes involved in progression to cancer are less fully characterized ([Peek & Blaser, 2002](#)).

Investigating the mechanisms responsible for gastric carcinogenesis through studies *in vivo* necessitates investigating simultaneously the effects of the bacterium together with the associated intense neutrophilic and mononuclear inflammatory response, which always accompanies *H. pylori* infection. In contrast, examining direct effects of the bacterium on gastric epithelial cells is only possible in co-culture experiments *in vitro* that are subject to numerous artefacts including the almost universal use of cancer-derived gastric epithelial lines, and the lack of the normal stroma, substrate and cell-cell interactions that exist *in vivo*.

**Table 3.2 Studies of gastric cancer in mice infected by *H. pylori* by gavage**

Strain Duration Reference	Dosing regimen, strain Gender Animals/group at start	Incidence of tumours	Significance	Comments
<b>Inbred mice</b>				
C57BL/6 80 wk <a href="#">Kim et al. (2003)</a>	10 <sup>6</sup> CFU <i>H. pylori</i> , SS1 Gender NR <i>H. pylori</i> : n=25 Controls: n=35	No gastric tumours observed		
C57BL/6 & BALB/c 23 mo <a href="#">Wang et al. (2003b)</a>	<i>H. pylori</i> , 119p <i>H. pylori</i> , G50 <i>H. pylori</i> , SS1 Gender NR  C57BL/6 <i>H. pylori</i> : n=9 (total) Controls: n=4	Gastric lymphoma  C57BL/6 <i>H. pylori</i> 119p: 3/4 <i>H. pylori</i> G50: 3/3 <i>H. pylori</i> SS1: 0/2 controls: 0/4		
	BALB/c <i>H. pylori</i> : n=9 (total) Controls: n=4	BALB/c <i>H. pylori</i> 119p : 2/4 <i>H. pylori</i> SS1 : 0/5 controls : 0/4		
C57BL/6 24 mo <a href="#">Cai et al. (2005)</a>	10 <sup>7</sup> CFU <i>H. felis</i> (M) <i>H. felis</i> eradication therapy* after 2 , 6 & 12 mo * made of tetracycline, metronidazole & bismuth subsalicylate orally for 14 d Controls: no eradication Number of animals per group NR	Adenocarcinoma when eradication of infection after 2 mo: 0%; 6 mo: 0%; 12 mo: 30% Adenocarcinoma when no eradication: 100%		
C57BL/6 × 12 996/SvEv 15 mo <a href="#">Rogers et al. (2005)</a>	10 <sup>8</sup> CFU <i>H. pylori</i> , SS1 (M, F) (gender distribution NR) 4 groups: 1. 0.25% salt diet 2. 7.5% salt diet 3. 0.25% salt diet + <i>H. pylori</i> 4. 7.5% salt diet + <i>H. pylori</i> Total n=62	Increased incidence of high-grade dysplasia (indefinite dysplasia or atypical hyperplasia) in Groups 3 & 4. No adenocarcinoma		High-salt diet switch Th1 to a Th2 immune response. Th1: <i>H. pylori</i> -specific IgG2c; Th2: <i>H. pylori</i> - specific IgG1.

**Table 3.2 (continued)**

Strain Duration Reference	Dosing regimen, strain Gender Animals/group at start	Incidence of tumours	Significance	Comments
<b>TGF-β deficient mice</b>				
pS2-dnRII derived from FVB/N strain (wild-type) 36 wk <a href="#">Hahn et al. (2002)</a>	10 <sup>9</sup> CFU <i>H. pylori</i> , 43 504 (M, F) 1. TGF-β-deficient mice (infected): n=48 2. wild-type (infected): n=48 3. TGF-β-deficient mice (non infected): n=38	Dysplasia or carcinoma: 6/7 (85.7%) 0/6 No lesion		
<b>INS-GAS mice</b>				
7 mo <a href="#">Fox et al. (2003a)</a>	10 <sup>8</sup> CFU <i>H. pylori</i> , SS1 (M/F) n=20 (total)	Carcinoma: 4/6 males; 0/6 females; 0/8 male and female controls		Only males developed gastric adenocarcinomas
28 wk <a href="#">Ohtani et al. (2007)</a>	10 <sup>8</sup> CFU <i>H. pylori</i> strain SS1 (M, F) 1. Males: n=29 2. Ovariectomized females: n=35 3. Intact females: n=29 4. Estradiol-treated* ovariectomized females: n=16 Part of each group was infected or not. Sacrifice occurred at Weeks 16 & 28 post-infection *Subcutaneous placement of a time-release estradiol pellet 16 wk post-infection	Adenocarcinoma: A. 3/7 (42%) infected males B. 1/10 (10%) infected ovariectomized females C. 0/7 infected intact females D. 0/8 E <sub>2</sub> treated ovariectomized females (infected) F. 0% non infected males and females		Protective effect of estradiol
28 wk <a href="#">Lee et al. (2008)</a>	10 <sup>9</sup> CFU <i>H. pylori</i> , SS1 (M) 1. Infected and untreated 2. Eradication therapy* at 8 wk post-infection 3. Eradication therapy* at 12 wk post-infection 4. Eradication therapy* at 22 wk post-infection 5. Non infected * with omeprazole, metronidazole and clarithromycin in 0.2 mL orally, for 7 d	Adenocarcinoma: 1. 10/10 (100%) 2. 1/11 (9%) 3. 8/9 (89%) 4. 6/12 (50%) 5. 0/7	[P<0.0001], Group 1 vs 5 [P<0.05], Group 1 vs 2 [P<0.05], Group 1 vs 4	Protective effect of eradication to the greatest extent when given early

**Table 3.2 (continued)**

Strain Duration Reference	Dosing regimen, strain Gender Animals/group at start	Incidence of tumours	Significance	Comments
<b>p27-deficient mice</b>				
17 mo <a href="#">Kuzushita et al. (2005)</a>	10 <sup>9</sup> CFU <i>H. pylori</i> , SS1 (M, F)	Gastric carcinoma or dysplasia		
C57BL/6 (p27-deficient) C57BL/6 (wild-type)	Sacrifice at 15, 30, 45, 60 and 75 wk post-inoculation			
	1. wild-type + <i>H. pylori</i>	0/40, carcinoma		
	2. p27 <sup>-/-</sup> + <i>H. pylori</i>	1/10 (30 wk), 1/6 (45 wk), 5/6 (60 wk), 2/6 (75 wk)	P<0.05 (60 wk vs Group 1), P<0.05 (75 wk vs Group 4)	Incidence for carcinoma (vs dysplasia) in Group 2 can not be ascertained from data provided
	3. Wild-type (controls)	0/9, carcinoma		
	4. p27 <sup>-/-</sup> (controls)	0/10, carcinoma		
	25–50 animals/group			
<b>TFF2-deficient mice</b>				
19 mo <a href="#">Fox et al. (2007)</a>	10 <sup>8</sup> CFU <i>H. pylori</i> , SS1 (M, F)	Gastric intraepithelial neoplasia		
TFF2 <sup>-/-</sup> C57BL/6 X Sv129 C57BL/6 X Sv129 (wild-type)	1. TFF2-deficient (infected): 20 2. TFF2-deficient (not infected): 10 3. Wild-type (infected): 20 4. Wild-type (not infected): 10	2/10 (20%) 0% 1/20 (10%) 0%		
<b>IL-1<math>\beta</math> transgenic mice</b>				
12 mo <a href="#">Tu et al. (2008)</a>	10 <sup>8</sup> CFU <i>H. felis</i> , ATCC 49179 (M)	Carcinomas:		
	1. Infected mice overexpressing human IL-1 $\beta$ (line 19) 2. Infected mice overexpressing human IL-1 $\beta$ (line 42) 3. Infected control mice 4. Uninfected line 42	1/12 (8.4%) 1/10 (10%) 0% 0%		

**Table 3.2 (continued)**

Strain Duration Reference	Dosing regimen, strain Gender Animals/group at start	Incidence of tumours	Significance	Comments
<b>MALT lymphoma mice models</b>				
26 mo <a href="#">Enno et al. (1995)</a>	$10^9$ CFU <i>H. felis</i> , ATCC 49179 (F) BALB/c	Lymphoepithelial lesions (gastric lymphoma)		Lesions are B-cell lymphoid infiltrates
	1. Infected			
	a. Sacrifice 0–19 mo: 167	1a: 0/167		
	b. Sacrifice 22–26 mo: 80	1b: 20/80 (25%) [ $P<0.0001$ ]		
	2. Controls (uninfected)			
	a. Sacrifice 0–19 mo: 95	2a: 0/95		
	b. Sacrifice 22–26 mo: 48	2b: 0/48		
22 mo <a href="#">Sutton et al. (2004)</a>	$10^8$ CFU <i>H. felis</i> , CS1 (F) BALB/c	MALT lymphoma	[ $P<0.0001$ ], Group B vs A [ $P<0.0001$ ], Group C positive vs A [NS], Group C negative vs A	
	A. Untreated (control): $n=17$	1/17		
	B. Infected: $n=15$	13/15		
	C. Immunised + infected.			
	- <i>H. felis</i> positive: $n=4$	4/4		
	- <i>H. felis</i> negative: $n=11$	5/15		
12 mo <a href="#">Fukui et al. (2004)</a>	$10^8$ CFU <i>H. pylori</i> , TN2GF4 (M, F) BALB/c	Lymphoepithelial lesions (gastric lymphoma)		
	Sacrifice at 2, 4, 6 and 12 mo			
	1. Non-thymectomized mice uninfected (Control): 40	0/40		
	2. Non-thymectomized mice infected: 40	0/40		
	3. Neonatal thymectomized (3 d) mice (nTx) uninfected: 40	0/40		
	4. Neonatal thymectomized (3 d) mice (nTx) infected: 40	2 mo: 6/10 4 mo: 8/10 6 mo: 9/10 12 mo: 10/10		

ATCC, American Type Culture Collection; CFU, colony forming units; d, day or days; F, female; GC, gastric cancer; *H. pylori*, *Helicobacter pylori*; IL-1 $\beta$ , interleukin-1 $\beta$ ; M, male; MALT, mucosa-associated lymphoid tissue; mo, month or months; TFF2, trefoilfactor family 2; vs, versus; wk, week or weeks

## 4.1 Data supporting the carcinogenicity of *H. pylori*

### 4.1.1 Genotoxicity linked to *H. pylori* infection

The intense gastric inflammatory infiltrate (gastritis) that accompanies gastric colonization by *H. pylori* in humans (or by related *Helicobacter* species in animal models) can generate potentially genotoxic reactive oxygen and nitrogen species from the inflammatory cells themselves, and from adjacent gastric epithelial cells ([Macarthur et al., 2004](#); [Ding et al., 2007](#)).

*H. pylori* is not directly genotoxic *in vitro*, and evidence for genotoxicity related to *H. pylori* and/or the associated inflammatory response *in vivo* is relatively limited. An increased frequency of micronuclei in the peripheral blood lymphocytes of *H. pylori*-infected patients ([Suárez et al., 2007](#)), more DNA strand breaks as shown by the comet assay ([Ladeira et al., 2004](#)), increased gastric mucosal DNA adduct (8-hydroxy-2'-deoxyguanosine) formation ([Farinati et al., 1998](#)), and an increased mutation frequency in the gastric mucosa of *H. pylori*- or *felis*-infected transgenic mutation reporter mice ([Touati et al., 2003](#); [Jenks et al., 2003](#)) have all been reported to occur relatively early during *Helicobacter* infection, before gastric cancer development.

Increased expression of activation-induced cytidine deaminase (AID), a DNA- and RNA-editing enzyme, was reported in *H. pylori*-infected human gastric biopsies, and was noted to decrease following *H. pylori* eradication. When human gastric adenocarcinoma epithelial cells were infected *in vitro* with *H. pylori*, the resulting AID overexpression occurred in parallel with the accumulation of mutations in *p53*, but much less frequently in *β-catenin* and *c-myc* genes; this supports a relatively specific role for AID in the generation of *p53* mutation by *H. pylori* ([Matsumoto et al., 2007](#)).

### 4.1.2 Changes in gene expression

Several reports have demonstrated that *H. pylori* alters the expression of specific oncogenes and tumour-suppressor genes implicated in gastric carcinogenesis. For example *H. pylori* infection promotes the nuclear translocation of *β-catenin*, thereby activating downstream *β-catenin*-responsive genes including cyclin D ([Franco et al., 2005](#)), upregulates the *p53* homologue *p73* in gastric cells to promote apoptosis ([Wei et al., 2008](#)), and decreases expression of the cell-cycle inhibitory protein *p27* ([Eguchi et al., 2003](#)) that is known to be lost in aggressive gastric cancers.

Hypermethylation of several genes has been found in *H. pylori*-associated chronic gastritis, including *E-cadherin* and *p14*, that are of potential importance mechanistically in gastric carcinogenesis. Methylation of the *E-cadherin* gene has been reported to reverse with the eradication of *H. pylori*. *In vitro*, *H. pylori* and the reactive oxygen species or nitric oxide released during the chronic inflammatory process may be responsible for gene methylation ([Hmadcha et al., 1999](#); [Tamura, 2004](#); [Chan et al., 2006](#); [Maekita et al., 2006](#); [Nardone et al., 2007](#); [Tahara et al., 2007](#)).

### 4.1.3 Altered cell turnover

It is well established that in human and experimental animal infections, *H. pylori* is associated with increased numbers of both apoptotic and proliferating gastric epithelial cells ([Shirin et al., 2001](#)). Increased cell turnover has long been linked to a risk of carcinogenesis, based on the increased chance of mutations arising under conditions of accelerated DNA replication ([Preston-Martin et al., 1990](#)). In contrast to the stimulation of apoptosis in association with *H. pylori* infection, some have reported that *H. pylori* may have anti-apoptotic effects ([Peek et al., 1997](#); [Mimuro et al., 2007](#)), which could directly promote aberrant tissue growth and perhaps gastric neoplasia.

The development of an acquired resistance to *H. pylori*-induced gastric epithelial apoptosis has been associated with decreased expression of p27 *in vitro* ([Shirin et al., 2000](#)), and downregulation of Fas-mediated signalling pathways *in vivo* ([Houghton et al., 2000](#)). However, there is no evidence that the balance between programmed cell death and cell proliferation is any different in subjects who are at higher risk for cancer compared with the *H. pylori*-infected population in general ([Moss et al., 1999](#)). [The Working Group noted that most studies addressing postulated mechanisms of carcinogenicity *in vivo* have compared only *H. pylori*-infected patients with uninfected patients (or the same patients before and after *H. pylori* eradication), but have not compared the minority of subjects who develop gastric cancer with the majority who do not.]

#### 4.1.4 Changes in gastric acid secretion

While some persons infected by *H. pylori* develop duodenal ulcers related to depletion of somatostatin secretion and increased gastrin and gastric acid secretion ([Calam, 1995](#)), in some others, there is a marked decrease of acid secretion from the loss of or damage to the acid-secreting parietal cells. This state of hypochlorhydria typically occurs in patients progressing towards the ‘intestinal’ type of gastric cancer, where the loss of acid secretion can promote intragastric colonization of non-*Helicobacter* bacteria, and the formation of luminal N-nitrosamines with some genotoxic potential ([Sanduleanu et al., 2001](#)).

#### 4.1.5 Inflammation and bone-marrow derived stem cells

An especially intense inflammatory response to *H. pylori* is thought to induce greater gastric epithelial cell damage, faster cell turnover, and the eventual emergence of gastric epithelial cells carrying cancer-prone mutations ([Moss & Blaser, 2005](#)). An alternative view of the detrimental

effects of the infiltrating inflammatory cells in the gastric mucosa is that of [Houghton et al. \(2004\)](#), who provided evidence in *H. felis*-infected mice that the bone-marrow-derived haematopoietic stem cells recruited to the gastric mucosa by *H. felis* can repopulate this mucosa, and progress through metaplasia and dysplasia to intraepithelial cancer. Whether this occurs during human gastric carcinogenesis remains untested thus far.

### 4.2 Host immune system and genetic susceptibility

Some of the genetic factors underpinning the pathophysiology of the loss of normal gastric secretory function and severe inflammation in gastric carcinogenesis have been uncovered in recent years. Based upon evidence that interleukin-1β (IL-1β) is both a potent gastric acid secretion inhibitor and a pro-inflammatory cytokine, [El-Omar et al. \(2000, 2001\)](#) documented the association of pro-inflammatory single nucleotide polymorphisms (SNPs) in both the *IL-1β* gene and the *IL-1RN* gene that encode the endogenous IL-1 receptor antagonist in association with hypochlorhydria and gastric atrophy in first-degree relatives of gastric cancer patients in a Scottish population. They also showed that pro-inflammatory *IL-1β* and *IL-1RN* SNPs were associated with a 2–3-fold increase in gastric cancer risk in a case-control study from Poland. [Figueiredo et al. \(2002\)](#) evaluated simultaneously *H. pylori vacA* and *cagA* genotypes together with *IL-1 β* and the *IL-1RN* genotypes of a large group of Portuguese shipyard workers with chronic gastritis and gastric cancer patients. They obtained risk ratios for gastric cancer among *H. pylori*-infected patients that differed markedly, based upon a combination of these four genes.

Moderate associations between SNPs in other genes encoding cytokines or genes involved in the initiation and maintenance of inflammation

have also been reported that include genes that encode tumour necrosis factor  $\alpha$  (TNF- $\alpha$ ), IL-10, IL-8, and toll-like receptor 4 ([Amieva & El-Omar, 2008](#)). Furthermore, increased non-cardia gastric cancer risk in *H. pylori*-infected subjects has also been related to polymorphisms in IL-1 $\beta$ , IL-1RN, TNF- $\alpha$  and IL-10 in subjects from a multicentre case-control study in the US population ([El-Omar et al., 2003](#)) but not, in that same study, to cardia gastric cancer or oesophageal adenocarcinoma risk. Thus, these SNPs appear to be relatively specific to increased distal gastric cancer risk following *H. pylori* infection.

There remains considerable debate regarding the reproducibility of many of these findings, reflecting perhaps differences in the populations studied, and the relationship of the cases to the controls in individual studies ([Perri et al., 2005](#); [Starzyńska et al., 2006](#)). Most studies are relatively small and evaluate only a few SNPs simultaneously, without adjustment for other potential confounding variables such as *H. pylori* genotype or other SNPs. Recent meta-analyses suggest that the influence of *IL-1 $\beta$*  and *IL-1RN* SNPs on gastric cancer risk is relatively weak, and/or confined to Caucasians ([Camargo et al., 2006](#); [Kamangar et al., 2006b](#)), whereas the relationship between non-cardia gastric cancer and two specific *TNF- $\alpha$*  SNPs (especially the -308AA SNP) is more consistent, though again mainly in Western populations ([Gorouhi et al., 2008](#); [Zhang et al., 2008](#)). [The Working Group noted that larger and more extensive studies are necessary to determine the importance of individual SNPs in gastric cancer risk in different populations, and in understanding how they may modulate the risk of cancer following *H. pylori* infection.]

## 4.3 Factors associated with gastric carcinogenesis

### 4.3.1 *H. pylori* virulence factors

#### (a) *cag* Pathogenicity island

As reviewed in Section 2, although CagA-positive strains confer an increased risk of gastric carcinogenesis, CagA-positive infections are also associated with the development of duodenal ulcer disease, which is itself inversely related to gastric cancer risk ([Hansson et al., 1996](#); [Nomura et al., 2002b](#)). This suggests that factors other than just the presence or absence of CagA may be important in gastric carcinogenesis. In regions such as south-eastern Asia, where almost all strains are CagA-positive, structural variations in tyrosine phosphorylation sites in the C-terminal domain of the CagA protein have been identified. These variations within regions encoded by the five amino acids sequence Glu-Pro-Ile-Tyr-Ala (EPIYA) serve as sites of CagA phosphorylation by gastric epithelial Src kinases. Four types of EPIYA motifs have been described (A through D), but the number of repeats of the EPIYA-C sequence in particular correlates with increased CagA phosphorylation, and a more marked phenotypic effect on infected gastric epithelial cells *in vitro*. EPIYA sequences differ markedly between east Asian strains (where gastric cancer is most prevalent), and western European/North American strains ([Yamaoka et al., 1998](#); [Basso et al., 2008](#)). In a multivariate analysis of 203 Italian *H. pylori*-infected patients, 53 of whom had gastric cancer, the number of repeats of the EPIYA-C motif was associated with the prevalence of both gastric cancer and its histological precursor, intestinal metaplasia ([Basso et al., 2008](#)). Similar associations of EPIYA motifs with gastric cancer have been described in smaller studies from east Asia ([Yamaoka et al., 1998](#)).

(b) Evidence of carcinogenic effect of *cag* genes in animal models

CagA-positive strains of *H. pylori* are associated with more vigorous gastric inflammatory responses. Several genes within the *cag* pathogenicity island, though not *cagA*, may facilitate colonization ([Marchetti & Rappuoli, 2002](#)). Two studies have highlighted the importance of an intact *cag* pathogenicity island in the induction of gastric cancer in rodent species. In the gerbil model infected by *H. pylori* strain 7.13, an isogenic mutant lacking the *cagA* gene failed to produce gastric cancer whereas cancers were seen in over 50% of the gerbils infected by wild-type or *vacA*-negative strains. There was also much less inflammation with the *cagA* knockout strain ([Franco et al., 2008](#)). Deletion of *cagE*, which is known to be important in the functionality of the type 4 secretory system, delayed, but did not prevent, gastric cancer in the INS-GAS mouse model ([Fox et al., 2003b](#)).

The only direct evidence of oncogenicity of the CagA protein *in vivo* is in transgenic mice overexpressing a virulent form of the *cagA* gene ([Ohnishi et al., 2008](#)). About 10% of such mice developed hyperplastic polyps and about 1% gastric or small intestinal cancer after 72 weeks, whether the CagA protein was targeted specifically to parietal cells in the stomach or expressed ubiquitously in all cells. Unexpectedly, transgenic CagA expression did not induce gastric inflammation, and leukaemias were also observed in some of the mice. The latter is consistent with the known effect of CagA on activating SHP-2 tyrosine phosphatase, an event that is also involved in leukaemogenesis.

In contrast to the paucity of data regarding the carcinogenic effects of *cag*-encoded proteins in animal models, there is a very extensive literature describing the cellular and molecular consequences of CagA translocation by the type 4 secretory system that is encoded by multiple genes within the *cag* pathogenicity island

(reviewed in [Buret et al., 2005](#); [Backert & Selbach, 2008](#); [Wen & Moss, 2009](#)). Following adherence of *H. pylori* to gastric epithelial cells, the CagL protein on the *cag* pilus interacts with the  $\alpha_5\beta_1$  host cell integrin to initiate CagA translocation. Translocated CagA is then phosphorylated by host cell Src kinases at EPIYA tyrosine phosphorylation sites to promote downstream signalling, resulting in reorganization of the actin cytoskeleton, and enhanced cellular motility (thus giving rise to the so-called “humming bird” phenotype in cultured cells). Activation of the transcription factor NF- $\kappa$ B by CagA leads to mitogenic signalling through mitogen-activated protein (MAP) kinase pathways, and also to pro-inflammatory gene activation. Some other cellular events activated by CagA translocation are not dependent on CagA phosphorylation. These events include the disruption of tight and adherent junctions between adjacent gastric epithelial cells through interactions with occludin, zonulin-1, junctional adhesion molecules, claudins, E-cadherin, and  $\beta$ -catenin.  $\beta$ -Catenin translocation to the nucleus promotes further mitogenic gene expression. One of the hallmark epithelial consequences of *cag*-positive infections is secretion of the pro-inflammatory chemokine IL-8, but it is currently unclear whether this is dependent on CagA translocation or by some other *cag*-island dependent stimulus. The muramylpeptide component of *H. pylori*'s cell wall, peptidoglycan, is also translocated to gastric epithelial cells by type 4 secretion, where it can interact with the intracellular NOD1 protein, a component of the innate immune response to pathogen-associated molecular patterns of bacteria. NOD1-binding also stimulates NF- $\kappa$ B-dependent signalling.

(c) *VacA*

In many studies in Western populations, peptic ulcer disease has been strongly associated with the *vacA* genotype (particularly s1m1), while in some of these studies the s1m1 genotype has been linked to gastric cancer ([Miehlke et al.,](#)

[2000](#)). East Asian strains are almost universally s1m1, and are not associated with any particular clinical outcome. The recent reports of *vacA* i region polymorphism where the i1 type was associated with gastric cancer in Iranian ([Rhead et al., 2007](#)) and Italian ([Basso et al., 2008](#)) populations are worthy of further corroboration by other groups. However, as for *cagA*, the *vacA* i region polymorphism may not correlate with gastric cancer risk in east Asia, where very few strains are of the i2 type ([Ogiwara et al., 2008](#)).

(d) *Evidence of carcinogenic effect of vacA in animal models*

VacA deletion does not alter the inflammatory or carcinogenic effects of *H. pylori* in the gerbil model ([Franco et al., 2008](#)). Multiple biological effects of VacA have been demonstrated *in vitro*, including the induction of apoptosis in epithelial cells, and the inhibition of T-cell activation and proliferation that may allow for *H. pylori* persistence, but the relevance of these observations to gastric carcinogenesis remains obscure ([Takeshima et al., 2009](#); [Matsumoto et al., 2011](#)).

(e) *BabA and SabA*

Some studies have demonstrated an association between the *babA2* genotype and gastric cancer ([Yu et al., 2002](#)) though this association may be confounded by the frequent co-association of *babA2* with *vacA* s1m1 and *cagA* positivity. Although the induction of *SabA* expression in *H. pylori* strains has been associated with gastric epithelial cell adhesion ([Marcos et al., 2008](#)), no good evidence points to a more direct role of *SabA* in carcinogenesis.

#### 4.3.2 Cofactors and co-infections

Effects of dietary salt on inflammation and proliferation have been observed in some animal studies, but the data from these is not consistent ([Rogers et al., 2005](#)).

*H. pylori* induces a predominantly Th1 immune response which is more typical of intracellular bacteria rather than extracellular organisms that produce typically a Th2 response. Because gastric cancer is thought to result from a Th1 pro-inflammatory immune response, it has been postulated that concurrent parasitic infection (which is more likely to induce a Th2 immune response) may modify the gastric inflammation and carcinogenic effects of *H. pylori*. Such a hypothesis is supported by *H. felis*-induced gastric atrophy in mice with concurrent intestinal nematode infection ([Fox et al., 2000](#)). Although gastric cancer was not evaluated in this model, this hypothesis may perhaps explain the low rate of *H. pylori*-associated cancer despite high *H. pylori* infection rate in areas of the world where parasitic infection is common ([Whary et al., 2005](#)).

## 4.4 Mechanisms of lymphomagenesis

### 4.4.1 Host immune system and genetic susceptibility in humans

Several different host polymorphisms associated with gastric lymphoma have been reported. These include the 49 G/G polymorphism in *CTLA4*, coding for a receptor on CD4-positive T cells, which inhibits T-cell functioning on ligand binding. This polymorphism was associated with a 6-fold higher risk of developing MALT lymphoma after *H. pylori* infection in a study from Taiwan, China ([Cheng et al., 2006](#)). Association of the R702W mutation in the *NOD2/CARD15* gene was demonstrated in a study in German/Austrian patients with a 2.4-fold risk of gastric lymphoma development ([Rosenstiel et al., 2006](#)). Susceptible polymorphisms for gastric lymphoma have also been reported at certain human leukocyte antigen (HLA) loci in a small Japanese cohort ([Kawahara et al., 2005](#)), and within the *TNF-α* gene (moderate effects at distinct loci, which were different for low- and

high-grade lymphoma) ([Hellwig et al., 2005a](#)), and in the toll-like receptor TLR4 in German/Austrian patients ([Hellwig et al., 2005b](#)). Each of these findings requires independent corroboration in other populations, and the understanding of additional factors that may predispose certain individuals or populations to gastric lymphoma following *H. pylori* exposure remains poor.

#### 4.4.2 *H. pylori* virulence factors

Unlike the large literature regarding the importance of certain *H. pylori* factors in association with gastric cancer, the data for gastric lymphoma are inconsistent, and generally not supportive of any specific association.

#### 4.4.3 Molecular mechanisms

The normal human gastric stomach contains few or no inflammatory cells. On infection by *H. pylori* or other *Helicobacter* species, such as *H. heilmanni*, an active infiltration of acute and chronic inflammatory cells (including B-cell lymphoid follicles) results. This so-called MALT lymphoma is the precursor of a low-grade lymphoma of B cells, gastric MALT lymphoma, originating in B cells of the marginal zone of the secondary follicles that are generated in the inflammatory response to *H. pylori* ([Du & Isaccson, 2002](#)). [Hussell et al. \(1993\)](#) demonstrated that in the early stages of MALT, lymphoma B-cell proliferation was dependent on both *H. pylori* antigens, and tumour-infiltrating T cells.

Most cases of MALT lymphoma are clonal, and will respond to *H. pylori* eradication. However, approximately 20% of MALT lymphomas are not responsive to eradication therapy, and it is thought that this is because they acquire further mutations, in particular the *API2-MALT1* fusion gene that results from a translocation of [t(11;18) (q21;q21)]. Three other relatively uncommon translocations [t(1;14)(p22;q32), t(14;18)(q32;q21), and t(3;14)(p13;q32)] are also associated with

gastric MALT lymphoma. Three of the four above translocations lead to the activation of NF-κB, a transcription factor important in inhibiting programmed cell death ([Sagaert et al., 2007](#)). Other mutations in gastric MALT lymphoma include *TP53*, *c-MYC* (approximately 20% of cases each), and epigenetic events such as gene promoter hypermethylation of *p16* ([Isaacson, 1999](#); [Huang et al., 2004](#)).

[The Working Group noted that how *H. pylori* and/or the associated inflammatory response promote downstream mutations that result in subsequent *H. pylori*-independent growth and high-grade disease that may be refractory to removing the offending antigen has not been defined. Whether high-grade lymphomas that are refractory to *H. pylori* eradication arise through the same pathways that lead *H. pylori* to promote low-grade lymphomagenesis is also unclear and worthy of further investigation. Little is known of possible environmental cofactors in the predisposition to *H. pylori*-induced gastric lymphomagenesis.]

### 4.5 Synthesis

Multiple lines of evidence point to a central role for the chronic gastric inflammatory response and resulting oxidative stress in *H. pylori*-associated gastric carcinogenesis. This leads to altered cellular turnover accompanied by changes in gene expression, methylation, and mutation. The nature and extent of the inflammatory response, and the subsequent effects of the inflammatory environment on gastric epithelial cells are associated with three inter-related factors: 1) host-determined modulation of inflammatory responses, 2) specific *H. pylori* virulence factors, including CagA, and 3) altered gastric secretory function.

## 5. Evaluation

There is *sufficient evidence* in humans for the carcinogenicity of chronic infection with *Helicobacter pylori*. Chronic infection with *Helicobacter pylori* causes non-cardia gastric carcinoma and low-grade B-cell MALT gastric lymphoma.

For oesophageal adenocarcinoma, there is *evidence suggesting lack of carcinogenicity* of chronic infection with *Helicobacter pylori* in humans.

There is *sufficient evidence* in experimental animals for the carcinogenicity of infection with *Helicobacter pylori*.

Chronic infection with *Helicobacter pylori* is *carcinogenic to humans (Group 1)*.

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## LIST OF ABBREVIATIONS

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4-NQO	4-nitroquinoline-N-oxide
5' SAGE	5' serial analysis of gene expression
8-oxodG	8-oxo-7,8-dihydro-2'-deoxyguanosine
AFB1	aflatoxin B1
AIDS	acquired immune deficiency syndrome
AIN1	anal intraepithelial neoplasia
<i>AlpA, AlpB, and HopZ</i>	adherence-associated lipoprotein
ALT	serum alanine aminotransferase
anti-HBc	antibody to hepatitis B core antigen
anti-HBs	antibody to hepatitis B surface antigen
anti-HCV	antibodies to HCV
ASD	Adult and Adolescent Spectrum of HIV Disease
ASR	annual standardized ratio
ATase	O6-alkylguanine-DNA alkyltransferase
ATL	adult T-cell leukaemia
ATLL	adult T-cell leukaemia/lymphoma
ATPase	adenosine triphosphatase
BabA	blood group antigen binding adhesion
BLCA-4	a nuclear matrix protein involved in gene regulation and produced only in neoplastic bladder cells
BLV	bovine leukaemia virus
BMI	body mass index
bp	base pairs
BRK	baby-rat kidney
BTG1	B-cell translocation protein
C	Core protein
cAMP	cyclic adenosine monophosphate
CCA	cholangiocarcinoma
ccc DNA	covalently closed circular
CDKIs	cyclin-dependent kinase-inhibitors
CDKs	cyclin-dependent kinases
CF/S	complement-fixing soluble antigens
CI	confidence interval
CLL/SLL	chronic lymphocytic leukaemia/small lymphocytic lymphoma
CMV	Cytomegalovirus
CRE	cAMP-responsive element
CREB/ATF	CRE-binding protein/activating transcription factor
Cryo-EM	Cryo-Electron Microscopy

C. pylori	Campylobacter pylori
C. sinensis	Clonorchis sinensis
CTAR	C-terminal activation region
CTL	cytotoxic T lymphocyte
CTLs	cytotoxic T cells
DDB1	UV-damaged DNA binding protein 1
DLBCL	diffuse large B-cell lymphoma
DS	dyad symmetry
E6-associated protein	E6AP
<i>E region</i>	early region
EAAs	early antigens
EBER	EBV-encoded RNA
EBNA	EBV nuclear antigens
EBV	Epstein-Barr virus
ECM	extracellular matrix
εdA	1,N6-ethenodeoxyadenosine
εdC	3,N4-etheno-2'-deoxycytidine
EGF	epidermal growth factor
EGFR	epidermal growth-factor receptor
EIAAs	enzyme immunoassays
ELISAs	enzyme-linked immunosorbent assays
EMT	epithelial-to-mesenchymal transdifferentiation
EPIYA	Glu-Pro-Ile-Tyr-Ala
ES	excretory–secretory
EST	Expressed Sequence Tag
EV	epidermodysplasia verruciformis
FLIP	FLICE (Caspase-8)-inhibitory protein
FR	family of repeats
gamma-HV68	gammaherpesvirus 68
GLUT1	glucose transporter 1
GR	glutathione reductase
GRE	glucocorticoid responsive element
GST	glutathione-S-transferase
HAART	Highly Active Anti-Retroviral Therapy
HAV	hepatitis A virus
HBs	hepatitis B surface proteins
HBV	hepatitis B virus
HBeAg	hepatitis B 'e' antigen
HbsAg	hepatitis B surface antigen
HBx	X protein
HBZ	HTLV-1 bZIP factor
HC2	hybrid capture 2
HCC	hepatocellular carcinoma
HCV	hepatitis C virus
HDL	High Density Lipoprotein
HF1-α	hypoxia-induced factor-1α
HGF	hepatocyte growth factor
HHV-4	human herpesvirus 4
HHV-8	human herpesvirus 8
HIV	Human immunodeficiency virus

HIV-1	Human immunodeficiency virus type 1
HIV-NHL	HIV-associated non-Hodgkin lymphoma
HNE	trans-4-hydroxy-2-nonenal
HNSCC	human head and neck squamous cell carcinomas
Hop	Helicobacter outer membrane proteins
HOPS	Project and the HIV Outpatient Study
<i>H. pylori</i>	Helicobacter pylori
HPNE	4-hydroperoxy-2-nonenal
HPV	Human papillomavirus
HRC	Hodgkin and Reed-Sternberg cells
HSCs	hepatic stellate cells
HTLV-1	human T-lymphotropic virus type 1
HVMF-1	herpesvirus Macaca fascicularis 1
ICC	intrahepatic cholangiocarcinoma
ICO	Catalan Institute of Oncology
IGF	insulin-growth factor
IGF-1	insulin-like growth factor 1
IGF-2	insulin-like growth factor-2
IgR	immunoglobulin-receptor
iNOS	inducible nitric oxide synthase
IRAK2	IL1R-associated kinase 2
IRS	insulin receptor substrates,
ISDR	interferon sensitivity determining region
ITAM	Immunoglobulin Transactivation Motif
ITS2	internally transcribed spacer region
K14	human keratin 14
KSHV	Kaposi-sarcoma-associated herpesvirus
L region	late region
LANA	latent nuclear antigen
LCR	long control region
LCV	lymphocryptovirus
LDLr	low-density lipoprotein receptor
LHBs	large hepatitis B surface proteins
LMP2	low molecular weight protein 2
LMPs	latent membrane proteins
LTR	long terminal repeat
M1dG	malondialdehyde-deoxyguanine
MALT	Gastric mucosa-associated lymphoid tissue
MALT	mucosa-associated lymphoid tissue
MALTs	muco-associated lymphoid tissue lymphomas
MAPK	Mitogen Activated Protein Kinase
MDA	malondialdehyde
MGUS	monoclonal gammopathy of undetermined significance
MHBs	medium hepatitis B surface proteins
MHC	major histocompatibility complex
MHC I	complex such as class I heavy chain
MHV 68	murine herpesvirus 68-
miRNA	micro-RNAs
mtCOI	mitochondrial cytochrome c oxidase subunit I
MTOC	microtubule-organizing centre

MTP	microsomal triglyceride transfer protein
N7-MedGp	N7-methyldeoxyguanosine 3' monophosphate
NAFLD	non-alcoholic fatty liver disease
NANBH	non-A, non-B hepatitis
NCRs	non-coding regions
NDMA	N-nitrosodimethylamine
NFKB	nuclear factor- $\kappa$ -B
NHANES III	National Health and Nutrition Examination survey
NRE	negative regulatory element
NTRK2	neurotropic tyrosin receptor kinase 2
NSI	non-syncytia-inducing
nuclear PML bodies	protein-specific nuclear bodies containing the promyelocytic leukaemia protein.
OR	odds ratio
ORCs	origin recognition complexes
ORFs	open-reading frames
OriP	origin of replication
O. viverrini	<i>Opisthorchis viverrini</i>
P	Polymerase
p42 MAPK1	p42 mitogen-activated protein kinase 1
PAHs	polycyclic aromatic hydrocarbons
PBM	PDZ-binding motif
PBMC	peripheral blood mononuclear cells
PCNA	proliferating cell nuclear antigen
PCR	polymerase chain reaction
PEG-IFN	pegylated interferon
PIN	Preneoplastic intraepithelial lesions
PKC	protein kinase C
PLA-2	phospholipase A2
PML	nuclear promyelocytic leukaemia bodies
POR	prevalence odds ratios
pRB	phosphorylated retinoblastoma
Prkar1 $\alpha$	protein kinase A regulatory subunit I $\alpha$
PTEN	Phosphatase and Tensin homology deleted on chromosome 10
PTLV	primate T-lymphotropic viruses
QNG	quantitative nuclear grading
RBV	ribavirin
rc DNA	relaxed-circular
RIG-I	retinoic-acid-inducible gene I
RR	relative risk
RT	reverse transcriptase
SabA	sialic acid binding adhesion
SEER	Surveillance, Epidemiology and End Results
SHBs	small hepatitis B surface proteins
<i>S. haematobium</i>	<i>Schistosoma haematobium</i>
SI	syncytia-inducing
SIGN	Safe Injection Global Network
sIL2R	interleukin-2 receptor- $\alpha$
SIR	standardized incidence ratio
SIR	standardized incidence ratio
SIV	simian immunodeficiency virus

SIVsm	SIV of sooty mangabees ( <i>Cercocebus atys</i> )
SMAHRT	SEER-Medicare Assessment of Hepatopoietic Malignancy Risk Traits
SMRs	standardized mortality ratios
SNPs	single nucleotide polymorphisms
SR-BI	scavenger receptor B1
STDs	sexually transmitted diseases
STLV	simian T-cell leukaemia virus
TAP1	transporter associated with antigen processing subunit 1
TGF- $\alpha$	transforming growth factor- $\alpha$
TNFR	TNF-receptor
TNF- $\alpha$	tumour necrosis factor- $\alpha$
TORCs	transducers of regulated CREB activity
TPA	tetradecanoyl phorbol ester acetate
TR	terminal-repeat
TRE	Tax-responsive element
TSP/HAM	tropical spastic paraparesis/HTLV-1-associated myelopathy
UVB	ultraviolet light
UNAIDS	United Nations programme on HIV/AIDS
UV	ultraviolet
VaIN	Vaginal intraepithelial neoplasia
VCA	viral capsid antigens
VEGF	vascular endothelial growth factor
VIN	vulvar intraepithelial neoplasia
vLDL	very low density lipoproteins
VLPs	virus-like particles
WHV	woodchuck hepatitis virus
XAP-1	HBx-associated protein
XLP	X-linked lymphoproliferative syndrome



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*Chemicals, Industrial Processes and Industries Associated with Cancer in Humans (IARC Monographs, Volumes 1 to 29)*

1982; 292 pages (out-of-print)

## **Supplement No. 5**

*Cross Index of Synonyms and Trade Names in Volumes 1 to 36 of the IARC Monographs*

1985; 259 pages (out-of-print)

## **Supplement No. 6**

*Genetic and Related Effects: An Updating of Selected IARC Monographs from Volumes 1 to 42*

1987; 729 pages (out-of-print)

## **Supplement No. 7**

*Overall Evaluations of Carcinogenicity: An Updating of IARC Monographs Volumes 1–42*

1987; 440 pages (out-of-print)

## **Supplement No. 8**

*Cross Index of Synonyms and Trade Names in Volumes 1 to 46 of the IARC Monographs*

1990; 346 pages (out-of-print)





Volume 100 of the *IARC Monographs, A Review of Human Carcinogens*, covers all agents previously classified by IARC as *carcinogenic to humans (Group 1)* and was developed by six separate Working Groups: Pharmaceuticals; Biological Agents; Arsenic, Metals, Fibres, and Dusts; Radiation; Personal Habits and Indoor Combustions; Chemical Agents and Related Occupations.

This Volume 100B covers Biological Agents, specifically Epstein-Barr Virus, Hepatitis B Virus, Hepatitis C Virus, Kaposi Sarcoma Herpesvirus, Human Immunodeficiency Virus-1, Human Papillomaviruses, Human T-cell Lymphotropic Virus Type 1, *Opisthorchis Viverrini* and *Clonorchis Sinensis*, *Schistosoma Haematobium*, and *Helicobacter Pylori*.

Because the scope of Volume 100 is so broad, its *Monographs* are focused on key information. Each *Monograph* presents a description of a carcinogenic agent and how people are exposed, critical overviews of the epidemiological studies and animal cancer bioassays, and a concise review of the agent's toxicokinetics, plausible mechanisms of carcinogenesis, and potentially susceptible populations, and life-stages. Details of the design and results of individual epidemiological studies and animal cancer bioassays are summarized in tables. Short tables that highlight key results are printed in Volume 100, and more extensive tables that include all studies appear on the *Monographs* programme website (<http://monographs.iarc.fr>).

It is hoped that this volume, by compiling the knowledge accumulated through several decades of cancer research, will stimulate cancer prevention activities worldwide, and will be a valued resource for future research to identify other agents suspected of causing cancer in humans.

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