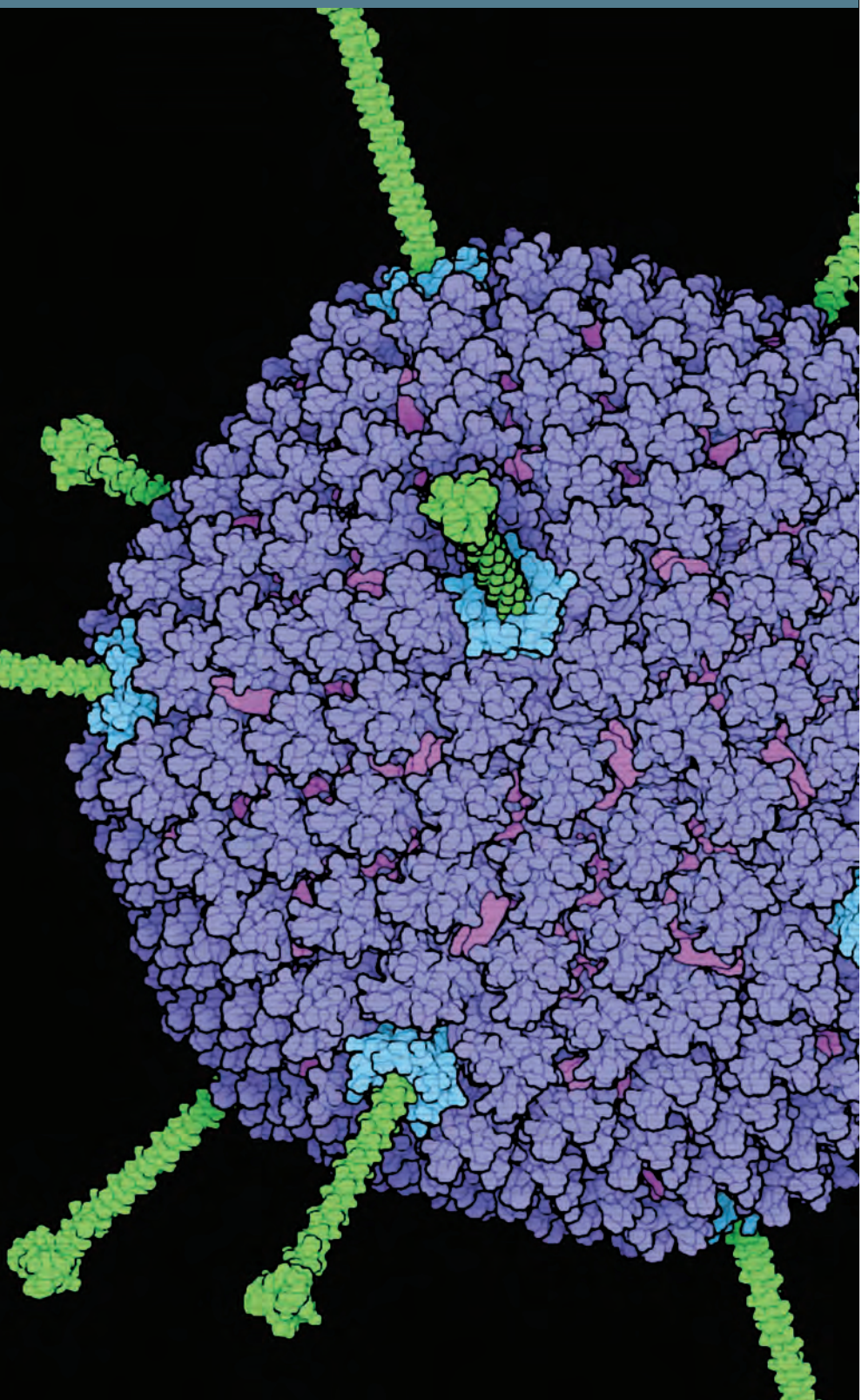
CHAPTER 7

Beneficial Use of Viruses



INTRODUCTION

While viruses are primarily known as pathogens, they can also be used for the benefit of humans. The most obvious is in their role in increasing our understanding of biology. The first genome sequences to be revealed were those of viruses (RNA bacteriophage MS2 and DNA bacteriophage ΦX174). The basic technology of genetic manipulation was developed from studies with bacterial viruses, and viral elements are still widely used in such work (described in more detail in Chapter 9). And the reverse flow of genetic information (RNA to DNA) was discovered from work with the *Retroviridae*.

About the chapter opener image
Adenovirus
(Courtesy of the Research Collaboratory for Structural Bioinformatics Protein Data Bank and David S. Goodsell, The Scripps Research Institute, USA.)

There are also a number of ways in which viruses may produce direct benefits for human health. The most obvious of these is as vaccines and vaccine vectors, which are covered in detail in Chapter 5. Vaccines do not simply protect against infection with the same virus. Relatively harmless viruses are often used to provide protection from their more dangerous relatives (for example, the use of vaccinia virus to protect against smallpox, or Shope fibroma virus to protect against myxomatosis) and viral vectors may be used to develop candidate vaccines against a range of diseases both viral and nonviral in nature.

This chapter will describe other ways in which viruses can be beneficial:

- Gene therapy
- Cancer prevention and control
- Control of harmful or damaging organisms, in both agriculture and medicine

7.1 GENE THERAPY

Viruses are routinely used in the genetic modification of model organisms for research purposes. The production of transgenic plants and animals in agriculture has also been established, but germ-line modification of humans has not been attempted for technical and ethical reasons. However, genetic manipulation of somatic cells of individuals has been under investigation for many years. This is known as **gene therapy**.

The key element of gene therapy is the introduction of functioning genes into the cells of a human patient, to express desired functions or to correct defective or non-operational genes within those cells. The original concept behind gene therapy was the treatment of individuals with an inherited genetic disorder, but applications in this area have been limited. The most common target has been cancers, accounting for almost two-thirds of all clinical trials to date (see also Section 7.2). It is also possible to target infectious diseases by introducing specific inhibitory genes, including those producing antisense or small interfering (si) RNAs (see Chapter 6).

The type of condition or disease amenable to gene therapy is one where a single gene (or, potentially, a very few genes) is able to correct the disorder, since it is not possible to introduce enough functioning genes to modify a complex condition resulting from the interaction of many different genetic elements. Another essential requirement is to have an identified and available therapeutic gene (often a functional version of a defective cellular gene), along with some way to deliver it to the cells where it is required.

Delivery systems must be able to introduce DNA into appropriate target cells (**Figure 7.1**). *Ex vivo* gene therapy involves removal of the cells from the body, followed by their treatment and reintroduction. *In vivo* gene therapy is more challenging, and involves targeting of target cells within the body. Examples of current approaches include the lung cells of cystic fibrosis sufferers where a defect in an ion transport protein produces excess mucus and damages the lung surface, and the hematopoietic cells of sufferers from various forms of severe combined immunodeficiency (SCID), which causes profound immunosuppression by preventing the production of lymphocytes. Hematopoietic cells are removed from the patient for treatment before being reintroduced (*ex vivo* gene therapy), while the lung cells of cystic fibrosis sufferers are readily accessible and can be treated *in vivo*. For both of these cases, chemical/mechanical systems such as fusogenic liposomes containing the therapeutic DNA may be adequate. With such systems, once the DNA is within the cell it may integrate into the cellular genome, although this occurs at very low efficiency.

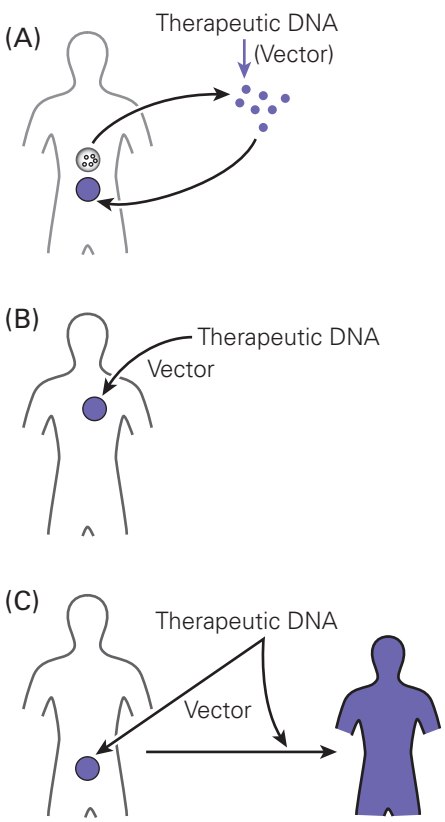


Figure 7.1
Approaches to gene therapy. (A) *Ex vivo* somatic: gene introduced into cells outside the body before reintroduction into the patient. (B) *In vivo* somatic: gene introduced into cells inside the body. (C) Germ line: gene introduced into cells that will produce the next generation. This is currently not used for human therapy.

Virus vector systems

Virus vectors are used for *ex vivo* gene therapy, but are particularly useful where the target cells are in less accessible areas of the body. Viruses provide highly efficient systems for getting foreign nucleic acid into cells, and they are also highly suited to protecting a nucleic acid while transporting it to the required area of the body. Unsurprisingly, the use of viruses in gene therapy has a long history.

Viruses naturally exhibit *cell tropism*, where the requirements of the virus for specific receptors (see Chapter 3) along with other factors can be used to ensure that specific types of cells are infected. While most viruses used for gene delivery will infect many types of cell, careful selection (and, where appropriate, genetic modification) of the virus vector can favor the delivery of the therapeutic gene to the required location.

The main types of viruses being evaluated for use as gene therapy vectors in clinical trials are summarized in **Tables 7.1** and **7.2**. Adenoviruses are widely used as vectors, and can be engineered both to enhance specificity and to minimize unwanted effects. Enhancement of specificity can involve either altering the surface receptors of the virus, or using cell type-specific promoters to control the expression of inserted genes. Approval of the first gene therapy product in Europe (Colybera® for pancreatitis) may occur in 2011. This uses an adeno-associated virus vector.

Complications

While the principles are apparently straightforward, there are many issues that complicate the successful use of gene therapy.

Table 7.1 Viral vectors in use for clinical trials of gene therapy

Vector	Virus family	Number of trials
Adenovirus	*Adenoviridae*	372
Flavivirus	*Flaviviridae*	8
Herpes simplex virus	*Herpesviridae*	51
Measles virus	*Paramyxoviridae*	3
Newcastle disease virus	*Paramyxoviridae*	1
Sendai virus	*Paramyxoviridae*	2
Adeno-associated virus	*Parvoviridae*	67
Poliovirus	*Picornaviridae*	1
SV40 virus	*Polyomaviridae*	1
Vaccinia virus	*Poxviridae*	95
Poxvirus	*Poxviridae*	64
Lentivirus	*Retroviridae*	21
Retrovirus	*Retroviridae*	326
Vesicular stomatitis virus	*Rhabdoviridae*	2
Semliki Forest virus	*Togaviridae*	1
Venezuelan equine encephalitis virus	*Togaviridae*	2
Multiple viruses		37

The remaining 483 trials used nonviral vectors.

Table 7.2 Characteristics of viral vector systems

Virus	Advantages	Disadvantages
Adenovirus type 5 and others (*Adenoviridae*)	Efficient nuclear entry, high levels of expression possible, specialized vectors available, insert size up to 8 kbp (36 kbp in some systems)	May be cytotoxic, immunity to adenovirus may prevent use, narrow host range with some types, safety concerns from previous clinical trials
Adeno-associated virus (*Parvoviridae*)	Nonpathogenic if helper virus not present, infects a broad range of cell types, easy to manipulate ssDNA genome, low immunogenicity, can produce long-lasting expression (vectors necessarily unable to replicate), efficient integration into host genome at defined site	Very limited insert size (5 kb), may be high levels of preexisting immunity
Herpesviruses (*Herpesviridae*)	Well characterized, large viruses, wide choice of insertion sites, inserts up to 10 kb (larger in amplicon or episomal vectors	May be pathogenic, cytotoxic, concerns over latency, may transform cells, limited availability of vectors
Vaccinia (*Poxviridae*)	Wide choice of insertion sites, large inserts possible (25 kbp), some systems allow high-level expression, wide availability	May be pathogenic for humans, risk of early termination with some inserts, introns can be problematical
Moloney murine leukemia virus, Lentiviruses (*Retroviridae*)	High efficiency of gene transfer, efficient integration into host genome, multiple systems available	Concerns over safety and oncogenicity (including leukemia induction in clinical trials), integration at variable sites, limited insert size (8–10 kbp maximum), requirement for actively dividing cells (except Lentiviruses)
Simian virus 40 (*Polyomaviridae*)	Stable high-level expression, low immunogenicity, infects a broad range of cell types, inserts up to 18 kb possible using pseudovirion (viral particle produced *in vitro* with no viral DNA sequences)	Small genome may restrict insert size, concerns over transformation and possible oncogenicity (if viral sequences present)
RNA viruses (*Coronaviridae, Flaviviridae, Paramyxoviridae, Picornaviridae, Reoviridae, Rhabdoviridae, Togaviridae*)	Capability to target specific cell types, high levels of gene expression	Small genomes restrict insert size, high mutation rate from RNA genome, no defined route of insertion into host genome

Specialization of cells (differentiation) may result in variant gene expression. For example, lung cells (targeted in attempts to treat cystic fibrosis) are highly specialized. It is also important to note that the routine elimination of cells by the body (the rate of which varies enormously between cell types) can require relatively frequent repetition of gene therapy since cells expressing the therapeutic protein are not immune to routine replacement by new, untreated cells. Such replacement is relatively frequent in the case of lung cells, but appears to be low enough to permit some therapeutic benefit. However, the extremely high rate of turnover of lymphocytes makes them more difficult targets. By delivering the therapeutic gene into the hematopoietic stem cells which actually produce all of the cell types in the blood, this problem is avoided while the number of cells produced which contain the therapeutic gene is greatly increased. Unsurprisingly, hematopoietic stem cells represent a major target of gene therapy.

Treatment of germ-line cells would overcome some of the problems encountered with attempts to genetically modify differentiated cells of specific tissues. But even the possibility of establishing foreign DNA in the human germ line, and (at least potentially) in every copy of the human genome descended from the treated individual, is something that requires thorough knowledge along with extremely careful consideration. The expanding field

of bioethics is concerned with the acceptability of such procedures, among many others.

The limited number of cases where a single gene defect produces serious illness and is amenable to correction using such methods are likely to provide the first examples of human use. However, the humans in question would be permanently altered by these procedures, as yet with little understanding of the long-term effects of such changes. While the technology has been established and transgenic animals produced, as of 2010 there are no serious proposals for human **germ-line gene therapy**.

Once the therapeutic gene is inside the target cell, it must be expressed at an appropriate level. Once again, viruses can provide a route to achieve this. Many viruses, such as retroviruses (*Retroviridae*) or adeno-associated virus (AAV; *Parvoviridae*), have a highly efficient integration step in their life cycle, and foreign genes introduced into the viral nucleic acid may use viral mechanisms to become integrated into the cellular DNA in order to permit stable expression. However, this integration is not without its problems for the *Retroviridae* (see below), while the very small size of the AAV genome limits its utility.

Other viruses (*Herpesviridae*, *Polyomaviridae*) may be maintained relatively stably as extrachromosomal genetic elements, while others (*Adenoviridae*) may produce more transient expression.

Problems

The potential for gene therapy was originally seen as immense and immediate, but as with genomics progress to useful medicines has been far slower than originally thought. As one commentator noted, "speculation that gene therapy would quickly revolutionise medicine has clearly been wrong … there was an overoptimistic view of the pace of progress and an underestimation of the problems remaining to be overcome."

In 1999, a patient being treated with an adenovirus vector for an ornithine transcarbamylase deficiency died, with multiple organ failure and a pathology indicating a reaction to the adenovirus vector with which he had been treated. There were also reports of serious side effects in three patients in a related study.

Then, problems arose in trials against X-linked severe combined immunodeficiency disease (X-SCID) in France and in the United Kingdom, using a vector derived from a murine retrovirus. Of eleven patients in France, nine responded to treatment, and seven apparently had long-term positive benefits. But within a few years four patients developed leukemia. Of ten British patients, one developed leukemia. The main cause seemed to be insertional mutagenesis caused by the retroviral vector, with insertion near the proto-oncogene LMO2 promoter in four of the five cases, leading to uncontrolled clonal proliferation of mature T cells. Four patients responded to therapy, but one death was reported.

X-SCID is a major and life-threatening illness, justifying higher levels of intervention than does a milder illness such as that in the adenovirus trial, but this was enough to stop trials using this approach in the USA. Even more worryingly, studies carried out at the Salk Institute in California seemed to indicate that the therapeutic gene in use (IL2RG, the γ chain of the interleukin 2 receptor) could itself promote leukemia in mice. While these data have been challenged, it remains a worrying issue.

After these results, progress was more cautious. Adenovirus vectors were modified to restrict their ability to replicate, while retrovirus vectors were used in more limited ways. It is undeniable that these findings were a major setback for work on gene therapy.

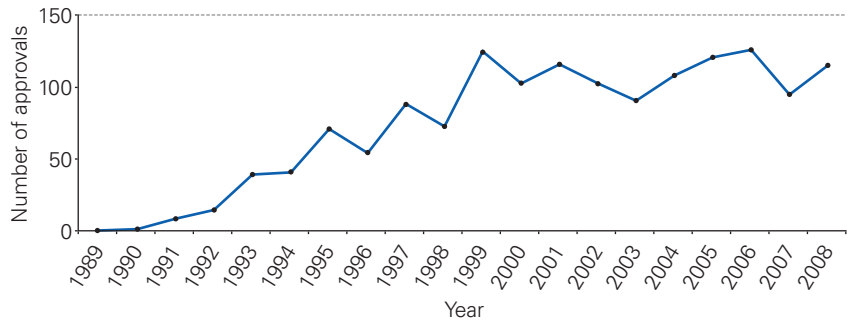


Figure 7.2
Number of gene therapy trials approved by year.

Another potential setback emerged in 2009, when a xenotropic murine leukemia virus–related virus (XMRV) related to the original virus used to create the murine retrovirus vector used in trials was identified, and was suggested to be linked to both chronic fatigue syndrome and, perhaps more worryingly, prostate cancer.

Where are we now?

As will be appreciated, any work with recombinant DNA requires careful assessment of the risks and benefits and of the ethical issues involved. Codes of conduct and supervisory bodies have been established in many countries. Gene therapy, where the intention is to introduce and express recombinant DNA in humans, is one of the most controversial areas. Despite this, by 2009, 1537 protocols had been approved worldwide, rising from one trial in 1989 to 117 in 2006 (**Figure 7.2**); 975 of these trials were in the United States and 348 in the European Union.

Over two-thirds of these trials used viral vectors (Table 7.1), indicating the central role of this approach in gene therapy.

This is an impressive number of trials, but only 65 of these (4.2% of the total) were the large, late-stage phase 3 trials that precede drug approval (see Box 6.1 for an explanation of the phases of clinical trials). Even more worryingly, only one gene therapy product (Gendicine, see below) has been approved for use against cancer—and that only in China. Advexin, a similar product delivering p53 via an adenoviral vector, encountered very serious delays in the United States, eventually resulting not in approval of the product but rather in the failure of the company developing it.

In theory, there is a huge range of biomedical problems which could be addressed by gene therapy. That promise is still there, but has yet to deliver.

7.2 CANCER PREVENTION AND CONTROL

A number of viruses are associated with cancer in humans (see Chapter 4) and these have provided the first instances of the prevention of cancers by vaccination. However, viruses can also have beneficial applications in the control of cancer. Some viruses are innately able to target and destroy cancer cells, while other methods use molecular approaches based on viral vector systems to create specific therapeutics. Approaches which are in use or under investigation are listed in **Table 7.3**.

Vaccines

The most direct approach to using viruses to prevent cancers is simply that of vaccination against viruses that are associated with cancer. Vaccines

Table 7.3 Use of viruses in the prevention or control of cancers

Approach	Mode of action	Examples
Prophylactic vaccine*	Stimulation of immune system to prevent a cancer, typically one associated with a virus infection	Existing subunit vaccines for hepatitis B virus, human papillomaviruses
Therapeutic vaccine*	Stimulation of the immune system to control an existing cancer	Experimental approaches under evaluation, e.g. using adenovirus vectors or papillomavirus DNA
Replication-competent virus	Preferential killing of cancer cells by virus	Adenovirus, Newcastle disease paramyxovirus
Modified replication-competent virus	As above, with enhanced killing of cancer cells	Adenovirus with enhanced receptor binding
Nonreplicating virally derived vector	Transfer into cancer cells of a cytotoxic gene	Rexin-G (retrovirus core with cytocidal cyclin G gene)
Virus-directed enzyme prodrug therapy (VDEPT)	Virus-mediated delivery of enzyme combined with systemic administration of prodrug	Recombinant adenovirus expressing herpes simplex enzyme, plus treatment with ganciclovir

* Vaccine may be any of the current types, including live virus, subunit, vector, or DNA, as discussed in Chapter 5.

for hepatitis B virus (*Hepadnaviridae*; associated with hepatocellular carcinoma) and human papillomavirus (*Papillomaviridae*; associated with cervical cancer) are already available and are in widespread use. Both use selected proteins of the virus (*subunit vaccines*, see Chapter 5). Although many studies have been carried out, these are the only vaccines approved to date by the US Food and Drug Administration (FDA) for the prevention of cancer. Of course, few forms of cancer involve identified viruses.

Therapeutic vaccination is also under evaluation, where vaccines are used to stimulate an immune response in an attempt to control or to eliminate an existing cancer by stimulating specific immunity to the cancerous cells. Cancer cells are derived from and very similar to the cells of the host, and so it is important to avoid stimulating immunity that targets normal host cells. Fortunately, many cancers express characteristic proteins on the surface of malignant cells. These may be normal cell surface proteins that are overexpressed or mutated, markers of the particular cell type present, or proteins associated with embryonic cells (see **Table 7.4**). Where viruses are associated with the formation of the cancer, viral proteins or MHC-associated peptides may be present on the cell surface. It is possible to select any marker of the type of cancer cell that is present and use it to target the immune response whether using a vector virus or by any other route.

Cancers of all types are subject to the effects of the immune system at all stages of growth, and it is only those that can circumvent this (as well as maintain themselves in a viable state) that are able to develop. Thus immunological control of tumors is a challenging prospect. Although a wide range of clinical trials have been carried out, as of 2009 no FDA-approved therapeutic vaccine exists for any cancer.

Virotherapy

It is also possible to use the cell-killing effects of viruses directly, rather than relying on the immune system. A range of viruses have been used in efforts to produce targeted killing of cancerous cells, and the approach as a whole

Table 7.4 Cancer-associated cellular antigens

Surface antigen	Type	Locations
Seen in multiple types of cancer		
Carcinoembryonic antigen (CEA)	Glycoprotein	Fetal tissues and some cancers, e.g. breast, colorectal, pancreatic and stomach cancer, pancreatic cancers as well as some lung cancers
Cancer/testis antigens (e.g. NY-ESO-1)	Multiple proteins	Sperm cells, melanomas, cancers of the brain, breast, colon, lung, ovary, pharynx, and tongue
Gangliosides (e.g. GM3 and GD2)	Glycolipid	Melanomas, neuroblastomas, soft tissue sarcomas, some lung cancers
HER2/neu protein (ERBB2)	Overproduced protein	Breast, ovarian, and other cancers (targeted by Herceptin)
Mucin-1 (MUC-1)	Glycoprotein	Mucus-producing epithelial cells, cancers including breast, colon, pancreatic, prostate, and some lung cancers
p53 protein	Mutated forms of tumor suppressor protein	All types of cell
Restricted to a single type of cancer		
Idiotype (Id) antibodies	Unique antibodies	Produced by B-cell cancers (used as markers for diseases including multiple myeloma and lymphomas)
Mutant epidermal growth factor receptor (EGFRvIII)	Mutated protein	Glioblastoma multiforme
Melanocyte/melanoma differentiation antigens (e.g. gp100, MART1, and tyrosinase)	Multiple proteins	Mature melanocytes and in melanomas
Prostate-specific antigen (PSA)	Glycoprotein, proteinase	Released into semen and (at lower levels) blood, greater production by prostate cancer cells compared to normal prostate cells leads to higher blood levels (used as a marker for prostate cancer)

Adapted from, Cancer Vaccines - National Cancer Institute. http://www.nci.nih.gov/cancertopics/factsheet/Therapy/cancer-vaccines

is known as **virotherapy**. Some of the earliest evidence for virotherapeutic approaches resulted from the observation of beneficial effects in cancer patients associated with naturally occurring virus infections, apparently due to the induction of *innate immune responses* (see Chapter 4). More recent studies have shown that virus infection of cancer cells may enhance both innate and *adaptive immune responses*. In the latter case, responses to both viral and cancer cell antigens may be associated with beneficial effects.

Virotherapeutic approaches have used either natural, unmodified viruses or genetically modified viruses or virus vectors, either of which can produce selective killing of cancer cells (oncolytic activity). Typically, viruses are introduced directly into the cancerous tissue. Systemic administration has also been evaluated, but removal of viruses by the immune system can be a significant problem.

A number of RNA viruses have innate anti-cancer activity, producing higher levels of cytotoxicity in cancerous cells. Several types of RNA virus have been investigated as potential therapeutic agents, including reovirus (*Reoviridae*), vesicular stomatitis virus (VSV, *Rhabdoviridae*), and Newcastle disease virus (NDV, *Paramyxoviridae*). For example, NDV produces severe disease (fowl pest) in chickens but only limited and localized disease in humans. It also grows better in transformed human cells compared to normal (diploid) human cells. This led to its evaluation as a virotherapeutic agent in a number of clinical trials, with apparently promising results. Naturally selected variants with enhanced cytotoxic activity in cancer-derived cells have also been used. However, no RNA virus has yet been approved for use as an anti-cancer therapy.

With DNA viruses, genetically modified viruses have been used. The modification may be to reduce replication, to increase specificity of infection for cancer cells, or to increase targeted cytotoxicity. A range of viruses have been used, including adenoviruses (*Adenoviridae*), herpes simplex virus (*Herpesviridae*), and vaccinia (*Poxviridae*). Some studies have shown limited effects, for example the 0–14% local tumor regression rates seen in trials with Onyx-015 adenovirus (see below). There is evidence that such responses can be increased by the simultaneous use of chemotherapy against the cancer under treatment.

Many different genetic modifications have been evaluated. Two products using this approach are approved and in clinical use in China, although some commentators believe such approval is premature.

Oncorine (H101) (approved in November 2005) is an adenovirus with deletions in the E1B-55 and E3 regions. This makes the virus replicate preferentially in cancer cells.

Interestingly, Oncorine (and the very similar Onyx-015) was originally intended to function by removal of the p53 suppressor activity of the adenoviral E1B-55K protein, preventing its replication in cells with functional p53 (and thus allowing replication in cancer cells, where this is often disrupted). However, more recent studies have shown that p53 inhibition is not linked to the cancer cell-specific activity of these mutants, the molecular basis of which remains unclear.

Gendicine (approved in October 2003) is an E1-deleted nonreplicating adenoviral vector expressing a functional p53 tumor suppressor gene. The virus is not oncolytic (though immunological responses to infection may have this effect), and is actually a form of gene therapy since the introduction of a functional p53 gene to cancers where the existing gene is nonfunctional provides the therapeutic basis for the effect.

Other modifications include the use of selective promoters that favor gene expression in cancerous cells, changes to receptor binding to favor infection of such cells, or the inclusion of effector genes such as interleukin 18 or CD40 ligand to enhance their anti-cancer effect (see Chapter 4). The latter approach is seen as necessary by many commentators, given the limited efficacy of adenoviruses without such enhancements.

Since the use of any live virus in an immunosuppressed patient carries with it an element of risk, other approaches bypass the use of replicating viruses entirely. These use gene vectors which lack functional viral nucleic acid. One example that is currently in clinical trials is Rexin-G, a murine retroviral core that delivers a cytocidal form of the cyclin-G1 protein.

Virus-directed enzyme prodrug therapy (VDEPT)

Viruses may also be used in **virus-directed enzyme prodrug therapy** (VDEPT) to insert into target cells an enzyme that can activate an inactive precursor of a cytotoxic drug (a prodrug) that is administered systemically. Thus, the active, cytotoxic form of the drug is only produced where the relevant enzyme is present and active. For example, an adenovirus expressing the thymidine kinase (TK) enzyme of herpes simplex virus can be combined with systemic administration of ganciclovir, which is converted by the TK to its active form only in cells where this enzyme is present. Alternatively, antibody can be used to provide the cellular targeting function (ADEPT), as described in Section 6.6. A related technique, GDEPT (gene-directed enzyme prodrug therapy) may use virally derived vectors that are incapable of replication.

Table 7.5 Viruses used as pest control agents

Virus type	Number in use	Target
Baculoviruses (various)	13	Caterpillars, sawflies
Oryctes rhinoceros virus	1	Rhinoceros beetle
Myxoma poxvirus	1	Rabbit
Rabbit hemorrhagic disease calicivirus	1	Rabbit

7.3 BIOLOGICAL PEST CONTROL

The use of biological organisms to control damaging pests is broadly known as **biological control**, or biocontrol. Traditionally this has been used in agriculture, but applications exist in the control of agents important to human health as well. There are four basic approaches:

- Predators, which prey on the target species
- Parasites or parasitoids (insects that lay their eggs inside or on the host)
- Pathogens, which cause disease in the target species
- Competing species (antagonists)

Of these, only pathogens are viral in nature. Although they account for a small amount of total pesticide use, viruses are used for the control of multiple species of insects (and have been evaluated for other arthropods such as mites), and also for the control of rabbits (**Table 7.5**).

Biological agents can produce long-lasting effects and in some cases are able to spread among the target population. They have also been recognized as inherently less toxic than conventional pesticides by the US Environmental Protection Agency. However, they account for less than 2% of total agricultural pesticide usage, with total sales in the region of $500 million, compared to more than $25 billion for conventional pesticides (**Figure 7.3**). The limited (though still expanding) use of biological control agents is due to a number of factors, including:

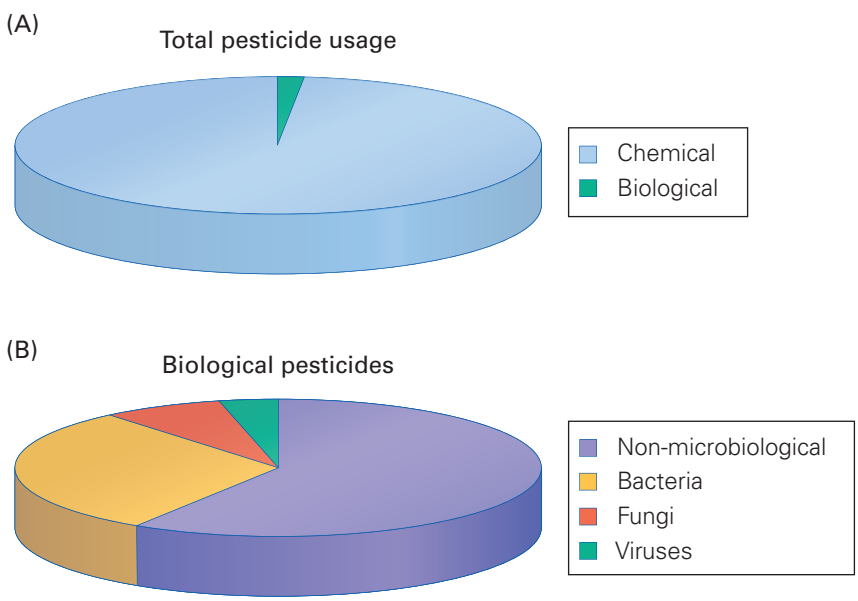


Figure 7.3
Usage of biological pesticides.
(A) Biological pesticides (green) as a proportion of total pesticides used.
(B) Viral pesticides (green) as a proportion of biological pesticides.

- High specificity, limiting the range of insects that can be controlled and often requiring identification of the pest insect before use
- Relatively slow effects, compared to chemical agents, allowing crop damage from an infestation to continue for some days after treatment
- Relatively high cost for the initial treatment, although control may then be long lasting
- Low environmental stability, particularly in sunlight
- Lack of support for their use from large pesticide companies

Microbiological agents account for approximately 40% of expenditure on biological pesticides. The most widespread use for biological pest control is the control of crop-damaging insects. For such applications, the most commonly used microbiological agent is *Bacillus thuringiensis* (BT), which produces a crystalline toxin that is insecticidal across a wide range of species. Some varieties of BT may be also used for control of mosquitoes, and may thus help to prevent viral diseases.

Fungi and viruses are also used, but BT alone accounts for the majority of expenditure on microbiological control agents in agriculture, while viral agents account for less than 10%. Thus it can be seen that the market for virus-based biological controls in agriculture in all applications is limited, and is in total worth only a few tens of millions of dollars each year. This is further divided between more than 20 products.

Within this sector, the main products are baculovirus insecticides (**Table 7.6**).

Table 7.6 Commercial baculovirus insecticides

Strain	Trade names*	Target insects
Anagrapha falcifera NPV	*CLV LC*	Celery looper
Autographa californica NPV	Gusano Biological Pesticide	Alfalfa looper (and other lepidopteran species)
Cabbage army worm NPV	Mamestrin	American bollworm, cabbage moth, diamondback moth, grape berry moth, potato tuber moth
Codling moth GV	*Carpovirusine*, *Cyd-X*, Madex, Granupom, *Virosoft CP4*	Codling moth
Helicoverpa zea NPV	(*Biotrol VHZ*), *Gemstar LC*, *Elcar*, *Heliothis NPV*, *NPH*, *Stellar LC*, *Viron H*	Cotton bollworm, tobacco budworm, tomato fruitworm
Heliothis armigera NPV	Ness-A	Old world bollworm
Lymantria dispar NPV	(*Gypchek*, *Gypsy moth NPV*)	Gypsy moth
Neodiprion lecontei NPV	Lecontvirus	Redheaded pine sawfly
Neodiprion sertifer NPV	(*Neochek-S*, *Preserve*)	European pine sawfly
Orgyia pseudotsugata NPV	*TM Biocontrol 1*, Virtuss	Douglas fir tussock moth
Plodia interpunctella NPV	*Nutguard-V*, *Fruitguard-V*	Indian meal moth
Spodoptera exigua NPV	Ness-E, *Spod-X LC*	Beet army worm
Spodoptera littoralis NPV	Spodopterin	Egyptian army worm

* Trade name in italic where listed by US Environmental Protection Agency. Strain names follow the naming conventions for baculoviruses, where each virus is named for the target insect followed by general type (genus) of the infecting baculovirus; nucleopolyhedrosis virus (NPV) or granulovirus (GV).

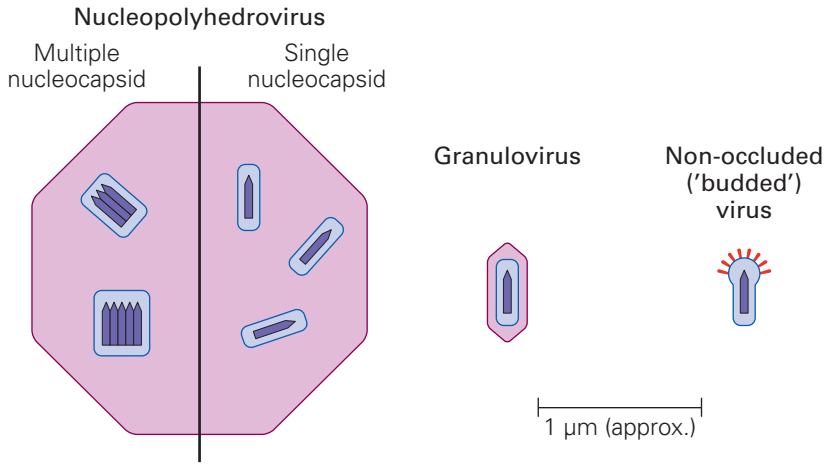


Figure 7.4
Baculovirus structure.
Nucleopolyhedroviruses have a single occlusion body (OB) that contains multiple infectious units while in *Granuloviruses*, each OB contains a single infectious unit. During infection of the host, a non-occluded or 'budded' form of the virus is produced which lacks the occlusion body. From, Harper D (2006) Biological control by microorganisms. In Encyclopedia of Life Sciences. With permission from John Wiley & Sons, Inc.

Viruses to control insect pests

Baculoviruses (*Baculoviridae*) are a large group of viruses that infect insects and other arthropods. All tend to be quite specific in the species that they will infect. Baculoviruses show a high level of environmental stability due to their formation of thick protein shells, know as **occlusion bodies** (OBs), around the nucleocapsid that contains the viral DNA genome (**Figure 7.4**). The family *Baculoviridae* is subdivided into two genera. For the nucleopolyhedroviruses (NPVs), a single occlusion body contains multiple nucleocapsids packed singly or in groups, and is formed of the viral polyhedrin protein. For the granuloviruses (GVs), each OB contains a single nucleocapsid and is formed of the viral granulin protein. A non-occluded form of the virus, referred to as the budded form, is formed during infection of insect hosts, in which it spreads the infection from cell to cell. The OBs also have an outer, carbohydrate-rich coat that helps to protect them from degradation, particularly if eaten by larger animals.

As part of their natural infectious cycle, baculoviruses are eaten by insect larvae. They then infect the cells of the gut and grow there. From these cells, the virus can then spread throughout the body of the insect, destroying it and releasing a new generation of virus from the liquefied remains of the killed larva (**Figure 7.5**).

This pattern of infection is seen in the larvae of butterflies and moths (members of the family *Lepidoptera*), and usually results in death in 4–5 days, although this can take longer under field conditions. In sawfly larvae (suborder *Symphyta*) the infection, while still lethal, is localized to the gut, and virus is shed by defecation and vomiting. Virus is released more rapidly, although less is produced overall.

Oryctes rhinoceros virus (OrV) infects the coconut rhinoceros beetle, a destructive tropical pest. This virus was formerly considered to be a baculovirus, but does not form OBs and is now classified separately. Virus infection is restricted to the midgut of adult beetles, but generates high levels of infectious virus. The beetles have been referred to as flying virus factories, and this helps with the effectiveness of the control process. The virus results in a generalized infection of larvae, which are killed in 9–25 days after infection. Since it does not form an OB, it is less stable than occluding viruses. However, it is capable of long-term persistence in the environment, aided by virus production in the midgut of adult beetles. It has been in use as a control agent since 1967 and appears to produce long-term control. In one field study, OrV reduced the numbers of beetles over a two-year period, then kept numbers low for a further two years.

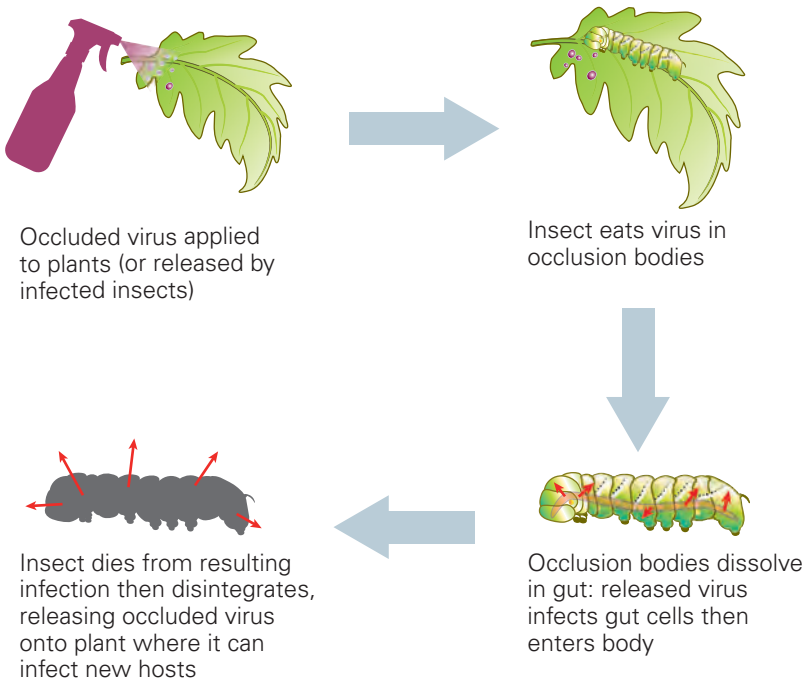


Figure 7.5
Insecticidal effect of generalized baculovirus infection. From, Harper D (2006) Biological control by microorganisms. In Encyclopedia of Life Sciences. With permission from John Wiley & Sons, Inc.

The entomopox viruses (family *Poxviridae*) and the cytoplasmic polyhedrosis viruses (family *Reoviridae*) also infect insects and produce OBs. However, neither of these has as yet been developed as a commercial biological control agent. Some work (including field trials) has taken place with other insect viruses, including the *Parvoviridae*, *Picornaviridae*, and *Tetraviridae*. Some of these approaches target urban rather than agricultural pests, including cockroaches, ants, and termites, but this is still at an early stage.

Viruses to control rabbits

It is not only insects that are controlled with viral agents. These have also provided the most successful approach to controlling the devastating numbers of European rabbits infesting much of Australia.

European rabbits were released into Australia in 1859 as part of a very unsuccessful attempt at improvement of the ecosystem by early settlers. With no effective predator and very limited competition, they rapidly became a highly destructive pest. Attempts to control them were unsuccessful, and the arrival of one potential biological control agent, the domestic cat, also proved an ecological disaster.

The myxoma virus is a member of the *Poxviridae*, and was originally observed as a cause of a mild skin disease in American rabbits (genus *Sylvilagus*) in Uruguay in the late nineteenth century. However, in European rabbits (genus *Oryctolagus*), it causes a hugely damaging systemic infection, apparently due to a different interaction with the immune system. With European rabbits between 90% and 100% are killed by the resultant infection, which is known as myxomatosis.

From 1938 onward it was evaluated as a possible control agent for introduced European rabbits in Australia. It was finally released in 1950 and proved highly effective, reducing the rabbit population by an estimated 500 million (around 85%) in two years. This provides an excellent example of the self-propagating nature of an effective biological agent.

However, in Australia, myxoma virus is spread by mosquitoes. During winter the lack of suitable insects to spread the disease meant that viruses that killed rabbits rapidly died out, while those that did not kill their host rapidly, permitting it to survive until the insect vector was again available, had an evolutionary advantage. In addition, small numbers of rabbits had some resistance to the disease, and these tended to be the ones that survived to breed. These two evolutionary pressures meant that, within a few years, rabbit numbers were rising once more. By 1957, only 25% of rabbits in Australia were killed by myxomatosis, and evidence has now appeared that the virus has established long-term infections in some rabbits. This is a clear example of antagonistic co-evolution, whereby pathogen and host co-evolve, changing the nature of their relationship. It also illustrates some of the limits of biological control in such systems.

The virus was introduced into Europe in 1952 in an attempt to control rabbits on an estate in France. As might have been expected (but apparently was not, at the time) it then spread across the continent (with the rabbit flea as its main vector) and is still active today.

Despite the evolution of resistance, myxoma virus still represented the most successful attempt to control rabbits in Australia. So, when a new and lethal rabbit disease (rabbit hemorrhagic disease, caused by a member of the *Caliciviridae*) was identified in China in 1984, there was pressure to repeat the experiment. The Australian government instituted a testing program on Wardang Island, three miles from the South Australian coast, to determine whether the virus would harm native Australian wildlife. However, during testing, the virus spread to the mainland which was perhaps not very surprising given the limited distance it had to cover. The cause of the escape was never confirmed, but evidence that it can be carried by flying insects provides one possible answer.

Within three months of the escape, 20 million rabbits had died. While the virus did not appear to harm native Australian species, hungry predators denied their usual food found alternatives from native wildlife. With the virus now released, the Australian government then undertook a program of controlled releases. The virus spread throughout the country, but while up to 90% of rabbits were eliminated in some dry areas, in other, wetter areas there was much less effect.

In 1997, the virus was introduced illegally into New Zealand, in what one commentator called "an act of biological warfare." Faced with the reality of the virus releases, the New Zealand government also authorized its use. However, the release had occurred at the wrong stage of the breeding cycle for maximum effect (since very young rabbits are unaffected) and rabbit numbers have now risen once again.

There have also been signs that, as with myxomatosis, virus and host are adapting to reduce the virulence of the circulating virus. Within three years of the accidental release of the virus on to the Australian mainland, there were calls for more effective versions to be developed.

Resistance

In contrast to the resistance to viruses developed by rabbits, resistance to viral insecticides in susceptible forms of the host insect is very rare, which may reflect the more limited (and typically non-adaptive) immune system present in insects. Many insects acquire increasing resistance as they age, with adult forms often very highly resistant. This process is known as developmental resistance and reflects changes in the maturing insect body; it is not transmitted to the next generation of larvae.

This development of resistance in insects is very limited compared with the rapid development of resistance to chemical pesticides, demonstrating a significant advantage for biological control.

Integrated pest management

The term **integrated pest management** (IPM) refers to the use of multiple, often low-potency, controls that together can reduce pest numbers to acceptable levels. Biological control agents are often used as part of an IPM strategy. This may include introducing or maintaining habitats for natural enemies, such as hedgerows or suitable plants, along with the reduction or elimination of plants or conditions that favor the pest organism. In such programs, predators may actually serve to spread infection—baculoviruses survive passage through the gut with high efficiency and can be spread in the droppings. Even at the microbiological level, it is possible to use multiple biological control agents simultaneously. For mosquitoes, for example, fungal and bacterial agents can be used together.

Chemical pesticides can also be used in combination with other approaches, including biological agents, provided that the biological agent itself is not harmed by the chemical treatments used. Since viruses are relatively unaffected by these chemical treatments they are well suited to such combined uses, and additive effects have been observed where both virus and chemical treatments exerted controlling effects.

A similar term, integrated pathogen management, has been suggested for the combined use of biological and other approaches in preventing and controlling infectious disease.

7.4 BACTERIOPHAGE THERAPY

Any cellular agent that causes human disease will be infected by its own pathogens, which can theoretically be developed as a control agent. Most progress in this area has been made with viruses that infect bacteria.

Bacteriophages

Bacteriophages are highly specific viruses that can target, infect, and (if correctly selected) destroy pathogenic bacteria. Antibacterial activity was first observed in the waters of the Ganges and Jumna rivers by Ernest Hankin in 1896. The causative agent was discovered independently in 1915 by Frederick Twort and in 1917 by Felix d'Herelle. It was d'Herelle who named them bacteriophages (devourers of bacteria) and who then expanded on his initial finding to establish many of the techniques that form the basis of modern virology.

Bacteriophages are believed to be the most numerous type of viruses accounting for the majority of the 10^{31} viruses present on Earth. They can be found at high concentrations in water, with over 10^8 per milliliter being recorded from some sources. More than 90% of characterized bacteriophages are classified in the order *Caudovirales* (**Figure 7.6**). These are the tailed bacteriophages, with a large double-stranded DNA genome in the range of 33,000 to 170,000 bp or even larger. Other families of bacteriophages also exist, with a range of morphologies, genome types, and genome sizes (**Table 7.7**).

From the 1940s onward, bacteriophages became one of the basic tools of molecular biology. Along with an understanding of the processes of the cell came a vastly increased understanding of the nature and activities of the bacteriophages themselves.

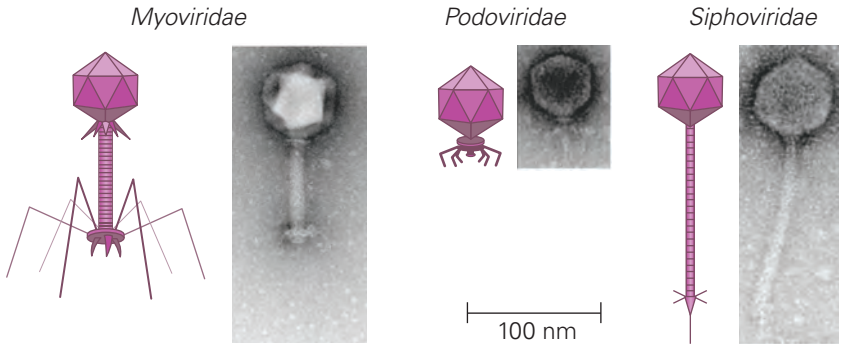


Figure 7.6
Tailed bacteriophages (*Caudovirales*).
Courtesy of Biocontrol Limited.

It soon became clear that very large numbers of bacteriophages existed, and that the vast majority were specific to a single host species (and indeed usually restricted to a limited range of strains within that species). While many bacteriophages produced rapid lysis of the host cell (**Figure 7.7**), others could integrate into the host chromosome, entering a latent state known in this context as lysogeny. The inserted bacteriophage DNA is then known as a prophage. Reactivation may occur in response to a variety of stimuli, and is directly analogous to the chromosomal insertion/reactivation cycle seen with the *Retroviridae* in eukaryotic cells.

The ability to enter the lysogenic state is associated with a range of characterized genetic functions within the bacteriophage genome including

Table 7.7 Families of bacteriophages

Virus family	Genome type	Genome size (kbp)	Structure	Example
Caudovirales				
Myoviridae	dsDNA	33.6–170	Non-enveloped, icosahedral head (50–110 nm, may be elongated) with long contractile tail	*Enterobacteria* phage T4
Podoviridae	dsDNA	40–42+	Non-enveloped, icosahedral head (60 nm) with short, non-contractile tail	*Enterobacteria* phage T7
Siphoviridae	dsDNA	48.5	Non-enveloped, icosahedral head (60 nm) with long, non-contractile tail	*Enterobacteria* phage λ
Other families				
Tectiviridae	dsDNA	147–157	Icosahedral, contains lipid, 63 nm with 20 nm spikes	*Enterobacteria* phage PRD1
Corticoviridae	dsDNA	9–10	Icosahedral, contains lipid, 60 nm+	*Pseudoalteromonas* phage PM2
Plasmaviridae	dsDNA	12	Enveloped, spherical/pleomorphic, 80 nm	*Acholeplasma* phage L2
Inoviridae	ssDNA	4.4–8.5	Non-enveloped, filamentous, 6–8 nm × 760–1950 nm	*Enterobacteria* phage M13
Microviridae	ssDNA	4.4–5.4	Non-enveloped, icosahedral, 25–27 nm	*Enterobacteria phage* ΦX174
Leviviridae	ssRNA	3.4–4.2	Non-enveloped, icosahedral, 26 nm	*Enterobacteria* phage MS2
Cystoviridae	dsRNA (segmented)	13.4 (3 segments)	Enveloped, spherical, 86 nm with 8 nm spikes	*Pseudomonas* phage Φ6

The *Caudovirales* account for more than 90% of all characterized bacteriophages. Other viruses (including members of the *Myoviridae* and the *Siphoviridae*) infect the Archaea.

a repressor of lytic gene function (that prevents killing of the host bacterial cell) and DNA integrases (that insert the viral DNA into the bacterial genome).

When lysogenic bacteriophages emerge from their latent state they may pick up and transfer bacterial DNA as part of this process. In some cases these may be associated with bacterial virulence, but this is not universal. The two cycles, lytic and lysogenic, are summarized in **Figure 7.8**.

Bacteriophages as therapeutic agents

Bacteriophages were discovered before effective antibiotics and so it was hoped that they would be able to control bacterial disease. In 1919, there were reports of the apparently successful treatment of typhoid in chickens and of dysentery in five humans. In 1921, bacteriophages were used against *Staphylococcus* in skin disease.

During the 1920s, both localized and large-scale experiments were undertaken in many countries, including the treatment of over a million patients in India. A wide range of commercial preparations were sold in Europe and the USA. The novel (1925) and film (1931) *Arrowsmith* presented a fictionalized account of their use.

Unfortunately, understanding of the nature of bacteriophages was extremely limited at that time. It was not until 1939 that use of early electron microscopes helped to settle an ongoing argument as to whether they were viruses or some form of chemical toxin. In consequence, much of the early work using bacteriophages was deeply flawed:

- Bacteriophages were used against diseases with no bacterial component (e.g. herpes, urticaria).
- It was assumed that bacteriophages were able to destroy a wide range of bacteria, whereas they are highly specific. They were thus used against inappropriate bacterial targets.
- Inappropriate growth conditions or preservatives were used that limited or prevented the inclusion of infectious bacteriophages.
- Methods of administration were used that inactivated any bacteriophages present (some new evidence shows this may not destroy them).

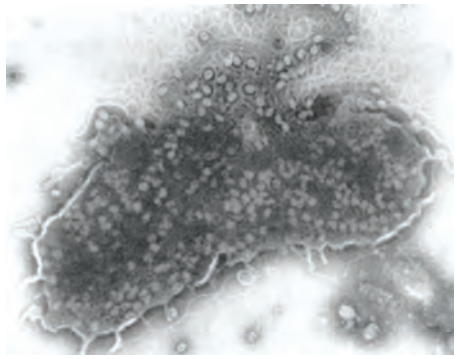


Figure 7.7
Bacteriophage lysis of the host cell. From Brown JC (2003) Virology. In Encyclopedia of Life Sciences. With permission from John Wiley & Sons, Inc.

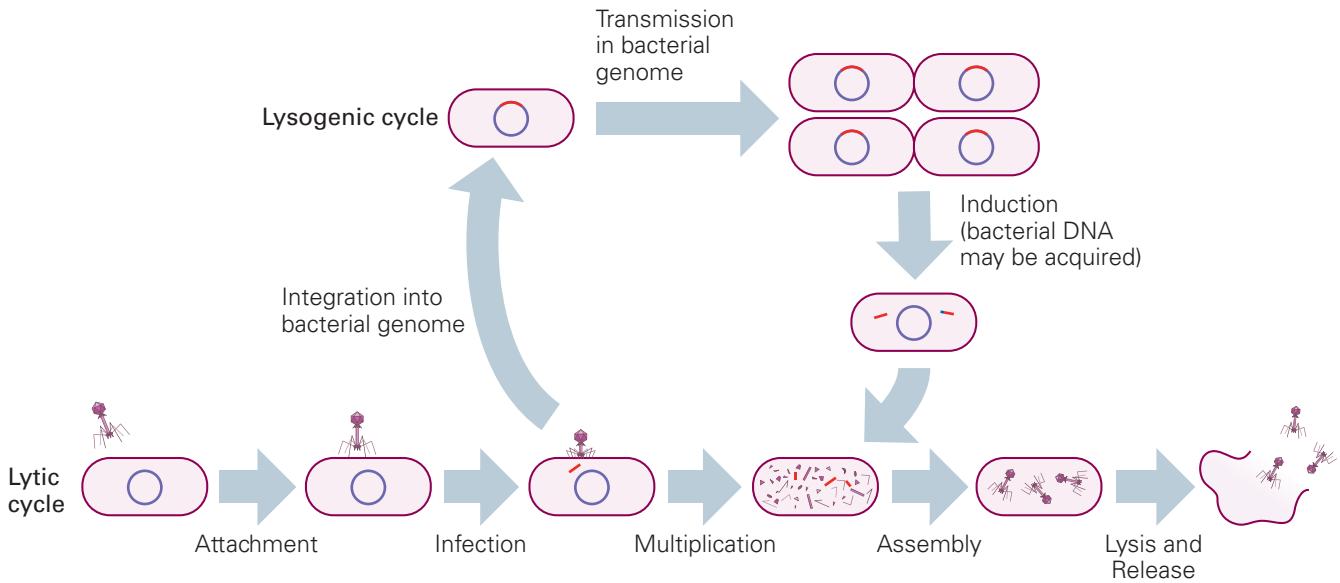


Figure 7.8
The lytic and lysogenic life cycles of a bacteriophage. Courtesy of Biocontrol Limited.

In 1934, the *Journal of the American Medical Association* published the results of a large study carried out by the US Council on Pharmacy and Chemistry. It concluded that, apart from a few restricted uses, there was no good evidence that bacteriophages actually worked in the therapeutic uses that had been evaluated. It further noted that most of the work carried out was deeply flawed, lacking proper controls, adequately purified therapeutic substance, or sufficient numbers of patients.

Despite this, the therapeutic use of bacteriophages continued through World War II. The German and Soviet armies used many bacteriophage preparations, notably against dysentery.

The antibiotic age

With the introduction of antibiotics, the popularity of bacteriophages declined. In 1932, Prontosil, the first sulfonamide drug, had been manufactured. In 1941, the first clinical use of penicillin showed the promise of this new class of drugs. Antibiotics began to appear in increasing numbers. They had broader activity, were simpler to use, and were supported by properly conducted clinical studies.

Some work with therapeutic bacteriophages continued, notably in Soviet Georgia, but the antibiotic era had arrived and was to last for over half a century. Work with bacteriophage therapeutics did continue in Western Europe, but on a very small scale, until the 1960s in France and even until the 1980s in Switzerland. However, the main locations for such work were the countries of the Soviet bloc.

In 1926, Georgiy Eliava, a bacteriologist from Soviet Georgia, visited Felix d'Herelle in Paris. On his return he began to work on bacteriophages. During the 1930s d'Herelle visited Georgia and started to work with Eliava to establish a centralized institute for bacteriophage research in Tbilisi, the capital of Soviet Georgia. This was derailed when Eliava was executed in 1937 during the Stalinist purges, but work continued and at its height the Institute produced millions of doses of a range of bacteriophage mixtures, with multiple targets and specific indications for their use. Alongside this, work also proceeded in Poland and Russia. Given the limited availability of antibiotics in the Soviet bloc, bacteriophage drugs became an accepted part of medical practice.

Meanwhile, in the West, the triumph of antibiotics continued. In 1969, US Surgeon General William Stewart was reported as saying in a report to the US Congress, "It is time to close the book on infectious diseases. The war against pestilence is over." He is on record as denying ever having said this, but it was a commonly held attitude at the time. However, antibiotic resistance was already a significant problem and was about to get a lot worse.

By the 1990s, rising numbers of cases were occurring where antibiotics could no longer cope. Resistance to all available antibiotics was by then apparent for a range of pathogenic bacteria, including methicillin-resistant *Staphylococcus aureus* (MRSA), vancomycin-resistant *Enterococcus* (VRE), and a range of others.

The situation was made worse by a lack of novel antibiotics. Drug companies had by this time moved away from research into antibiotics, where there were seen to be plenty of drugs available, into areas where the potential for long-term use of "lifestyle" drugs (such as those to lower cholesterol or to control heart disease) made them more profitable. In the five years from 1983 to 1987, fifteen new antibiotics were approved by the FDA. Twenty years later, from 2003 to 2007, it was just three. To add to the problem, even when a new type of antibiotic was finally developed, resistance appeared almost immediately.

Now, with resistance approaching crisis levels and few new drugs in the pipeline, interest turned once again to the potential of bacteriophages.

A renewal of interest in bacteriophages

In the 1980s, a British bacteriologist, William Smith, had conducted a series of experiments in both mice and farm animals. Bacteriophages were shown to be more effective than a variety of antibiotics in reducing the mortality of mice with generalized and intracerebral *Escherichia coli* infections, and bacteriophages were shown to be multiplying in the tissues. Very low doses of phage were found to be effective. Smith and his co-worker, Michael Huggins, evaluated the treatment of experimentally induced diarrhea in calves, piglets, and lambs due to several different strains of *E. coli*, and were able successfully to treat and to prevent infections, even when low doses of bacteriophages were used. However, some caution was warranted since these were experimental infections rather than trials against natural disease.

These demonstrations of the potential of bacteriophage therapy, combined with the growing urgency of the antibiotic crisis, led to renewed interest in this approach in the 1990s. The British clinician James Soothill showed that bacteriophages could prevent the destruction of skin grafts in guinea pigs and could protect mice against lethal levels of bacterial infection.

Combined with the continuing increase in antibiotic resistance, work showing that bacteriophages could be effective led several groups to look at the possibility of bringing therapeutic bacteriophage technology back from the former Soviet bloc, which was now accessible following the fall of the old Communist regimes several years earlier.

Unfortunately, despite the apparent promise of this technology, and despite widespread use including a very large body of work involving the treatment of many thousands of patients in local studies, the work which had been done was not enough to prove the case for bacteriophage therapy for Western use. Under the different pressures that applied in the Soviet era, particularly the desperate need for treatment options, this work had not involved the detailed documentation, procedures, and double-blind controls required for progress within a modern regulatory framework. The evidence was supportive, but not sufficient.

As a result, much of the initial excitement over access to former Soviet science in the mid-1990s proved unsupportable. While clinics in Poland and Georgia offered bacteriophage treatments to those motivated enough to travel to them for treatment, these could not be used in Western Europe or the United States. Those companies that managed to survive in the West generally did so on the basis of other kinds of applications, in particular the somewhat less complex areas of food and agricultural applications.

By 2007, five products were licensed in the United States. One was to prevent bacterial infections of tomatoes and peppers, two to destroy bacteria associated with food poisoning on food animals before slaughter, and two for the control of *Listeria monocytogenes* on ready-to-eat food. In this latter use, exposure of humans to the bacteriophages was accepted by the FDA—an important step forward.

Clinical work in humans was slower to develop, not least due to the relatively high cost of such work. The range of issues relating to the use of bacteriophages as antibiotics are summarized in **Box 7.1**.

Some groups have focused on investigating the use of genetically modified (GM) bacteriophages, often with enhanced abilities to destroy their bacterial target but with reduced ability to replicate—essentially a gene vector

Box 7.1 Issues relating to the use of bacteriophages as antibiotics

Advantages:

- Bacteriophages are highly specific, helping to avoid side effects. This is in line with an increasing trend in antibiotic usage to avoid drugs with broad specificity.
- The use of replicating bacteriophages also allows for the use of very low input levels that can multiply up at need. This can reduce both cost and toxicity.
- Bacteriophages are unaffected by antibiotic resistance, and there is evidence that they may be able to work synergistically with some conventional antibiotics.
- Bacteriophage preparations appear free of gross toxicity. Potential complications arising from the release of bacterial endotoxins from lysed cells (the Herxheimer effect, as seen with some antibiotics) do not seem to be an issue at the dosing levels now in use.
- Bacteriophages are relatively simple to manufacture (although the highly regulated manufacturing process required for clinical use adds to costs).
- Bacteriophages themselves may be able to adapt to counter bacterial resistance.

Disadvantages:

- High specificity means that it is important to identify the pathogenic bacteria present and to ensure that they are responsible for the disease. Multiple bacteriophages may be required to obtain useful levels of coverage, though this can in turn reduce the chance of resistance.
- Bacteriophages have limited suitability for systemic use since they can generate immune responses which can reduce efficacy. This can be avoided by use on infected sites on the body surface or oral administration. The potential for systemic use remains to be assessed in clinical trials.
- The mobilization of bacterial genetic material that may be able to moderate virulence is to be avoided. For this reason, only lytic (rather than lysogenic) bacteriophages are used, and regulators require careful monitoring of all forms of transduction (transfer of bacterial genes).

Regulatory and commercial issues:

- The attitudes of the EU and US regulators to this novel therapy have proven to be less of an issue than many had predicted, and it is clear that trials can be undertaken and completed successfully.
- Patentability of bacteriophages has been cited as an issue, since the basic technology is well established and there are a huge number of bacteriophages waiting to be isolated. However, monoclonal antibodies are now a commercial success despite occupying a very similar position.

approach. However, any use of GM technology brings with it very significant additional regulatory requirements.

In addition, many researchers consider the ability of bacteriophages to replicate and thus to expand their numbers at precisely those points where they are needed to be a major strength of the approach, and thus question the use of nonreplicating bacteriophages or bacteriophage-derived vectors (**Box 7.2**). Unlike gene therapy vectors, where replication appears to have the potential to cause problems, bacteriophages can only replicate in their prokaryotic target cells. To eukaryotic cells they are effectively inert, since

Table 7.8 Modern clinical trials of bacteriophage therapeutics

Phase	Location	Target	Condition
Phase 1, safety only			
Completed 1999	UK	*Enterococcus*	Gut infection
Completed 2005	Switzerland	*E.coli*	Diarrhea
Completed 2008	USA	*Pseudomonas*, MRSA, *E. coli*	Leg ulcers
Completed 2009	Belgium	*Pseudomonas*, MRSA	Burns
Phase 2, safety and efficacy			
Completed 2007	UK	*Pseudomonas*	Ear infection
Under way (with earlier Phase 1)	Bangladesh	*E. coli*	Diarrhea

Planned: MRSA (nasal carriage), *P. aeruginosa* (lung infections, ear infections, ulcers).

Box 7.2 Active and passive bacteriophage therapy

In the first phase 2 trial, completed in 2007 (against *Pseudomonas aeruginosa*), the input dose of bacteriophages was 600,000 infectious units, equating to only 2.4 ng of protein. This approach, known as *active bacteriophage therapy*, relies on bacteriophage multiplication to generate therapeutic doses of the bacteriophages against the far larger numbers of bacteria that are present.

Use of higher doses sufficient to produce antibacterial effects without relying on bacteriophage replication is known as *passive bacteriophage therapy*, and many approaches using genetically modified agents may use this since the modified bacteriophages are unable to replicate.

their controls for gene expression are specific for prokaryotic cells. While properly configured genes may be expressed if eukaryotic controlling elements are added, this does require the deliberate insertion of such controls from other sources.

It should be noted that all trials reported to date have used unmodified, replication-competent bacteriophages.

Into the clinic

The first modern clinical trial of a bacteriophage therapeutic was carried out in 1999 in London, though this used healthy volunteers without the targeted infection, and thus only addressed safety issues. The intended target was vancomycin-resistant *Enterococcus* infections. After this, additional safety trials were carried out (**Table 7.8**), but it was not until 2007 that the first modern clinical trial of the efficacy of a bacteriophage therapeutic was begun, targeting *Pseudomonas aeruginosa* infections of the ear. The trial completed in 2007 and larger, phase 3 trials are now planned. It will be necessary for these trials to be completed successfully and approved by the relevant regulators—the European Medicines Agency (EMA) in Europe and the Food and Drugs Administration (FDA) in the USA—before any bacteriophage therapeutic can proceed to market in these areas. However, it seems that progress is being made.

Key Concepts

- Viruses may be used in a variety of beneficial ways, including their use as model organisms in biological research.
- Viruses can be used in the delivery of therapeutic genes, known as gene therapy. Originally seen to have great potential for the control of inherited genetic diseases, progress in gene therapy has been much slower than was initially anticipated and its focus has switched to anti-cancer applications.
- Viruses may be used to treat cancers (virotherapy). Such treatment originally used the natural properties of specific viruses, but is now usually based on genetic modification and the use of viral and gene vectors.
- Biological control in agriculture only accounts for a small section of the pest control market. Viruses account for less than 0.1% of expenditure on agricultural pesticides, although this is expanding.
- The use of viruses of bacteria (bacteriophages) to control damaging bacterial infections following their discovery in the early part of the twentieth century was rapidly replaced by the use of chemical antibiotics, but recent developments are reviving interest in this approach.

DEPTH OF UNDERSTANDING QUESTIONS

Hints to the answers are given at http://www.garlandscience.com/viruses

Question 7.1: Why has gene therapy not delivered on its original promise for inherited genetic disease?

Question 7.2: Gendicine, a gene therapy product delivering a functional p53 gene into cancer cells, has been approved for use in China. Is it likely that the US and European regulators will now approve this approach?

Question 7.3: Why did farmers in New Zealand release rabbit Calicivirus, even though this has been classed as bioterrorism? What are the consequences of this act?

Question 7.4: Is it necessary to use genetic modification techniques to develop bacteriophage-based antibiotics?

FURTHER READING

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INTERNET RESOURCES

Much information on the internet is of variable quality. For validated information, PubMed (http://www.ncbi.nlm.nih.gov/pubmed/) is extremely useful.

Please note that URL addresses may change.

Biological control: a guide to natural enemies in North America. http://www.nysaes.cornell.edu/ent/biocontrol/

Gene Therapy Clinical Trials Worldwide, provided by the Journal of Gene Medicine. http://www.abedia.com/wiley/

Human Genome Project gene therapy resource. http://www.ornl.gov/sci/techresources/Human_Genome/medicine/genetherapy.shtml