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Herpesviruses in periodontal diseases

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> '*…If, as is sometimes supposed, science consisted in nothing but the laborious accumulation of facts, it would soon come to a standstill, crushed, as it were, under its own weight…The suggestion of a new idea, or the detection of a law, supersedes much that has previously been a burden on the memory, and by introducing order and coherence facilitates the retention of the remainder in an available form…*' *Lord Rayleigh, University of Cambridge, 1884.*

Periodontitis is a disease attributable to multiple infectious agents and interconnected cellular and humoral host immune responses (60, 226, 238). However, it has been difficult to unravel the precise role of various putative pathogens and host responses in the pathogenesis of periodontitis. It is not understood why, in hosts with comparable levels of risk factors, some periodontal infections result in loss of periodontal attachment and alveolar bone while other infections are limited to inflammation of the gingiva with little or no discernible clinical consequences. Also, many periodontitis patients do not show a remarkable level of classical risk factors. Detection and quantification of periodontopathic bacterial species are useful for identifying subjects at elevated risk of periodontitis, but do not consistently predict clinical outcome. These uncertainties have galvanized efforts to find additional etiologic factors for periodontitis.

Even though specific infectious agents are of key importance in the development of periodontitis, it is unlikely that a single agent or even a small group of pathogens are the sole cause or modulator of this heterogeneous disease. Since the mid 1990s, herpesviruses have emerged as putative pathogens in various types of periodontal disease (43). In particular, human cytomegalovirus (HCMV) and Epstein-Barr virus (EBV) seem to play important roles in the etiopathogenesis of severe types of periodontitis. Genomes of the two herpesviruses occur at high frequency in progressive periodontitis in adults, localized and generalized aggressive (juvenile) periodontitis, HIV-associated periodontitis, acute necrotizing ulcerative gingivitis, periodontal abscesses, and some rare types of advanced periodontitis associated with medical disorders (212, 224). HCMV infects periodontal monocytes/macrophages and T-lymphocytes, and EBV infects periodontal B-lymphocytes (45). Herpesvirus-infected inflammatory cells elicit tissue-destroying cytokines and may exert diminished ability to defend against bacterial challenge. Herpesvirus-associated periodontal sites also tend to harbor elevated levels of periodontopathic bacteria, including *Porphyromonas gingivalis*, *Tannerella forsythia*, *Dialister pneumosintes/Dialister invisus*, *Prevotella intermedia*, *Prevotella nigrescens*, *Treponema denticola*, *Campylobacter rectus* and *Actinobacillus actinomycetemcomitans* (210, 224). Transcripts of HCMV and EBV have been identified in the great majority of symptomatic periapical lesions as well (231, 232). In the light of the close statistical relationship between herpesviruses and periodontitis, it is reasonable to surmise that some cases of the disease have a herpesviral component.

This chapter summarizes evidence that links herpesviruses, especially HCMV and EBV, to the development of severe types of periodontitis, and outlines potential mechanisms by which herpesviruses may contribute to periodontal tissue breakdown. It is suggested that the coexistence of periodontal HCMV, EBV and possibly other viruses, periodontopathic bacteria, and local host immune responses should be viewed as a precarious balance that has the potential to lead to periodontal destruction. Understanding the pathobiology of periodontal herpesviruses may help delineate molecular determinants that cause gingivitis to progress to periodontitis or stable periodontitis to convert to progressive disease. Evidence of a causal role of herpesviruses in periodontitis may

form the basis for new strategies to diagnose, prevent, and treat the disease.

Mammalian viruses

Viruses cause many acute and chronic diseases in humans. New viruses are continually being discovered and already known viruses are being implicated in clinical conditions with previously unknown etiologies.

Viruses occupy a unique position in biology. They are obligate intracellular agents, which are metabolically and pathogenically inert outside the host cell. Even though viruses possess some properties of living systems such as having a genome and the capability of replicating, they are in fact nonliving infectious entities and should not be considered microorganisms. The complete virus particle, called a virion, generally has a diameter of only 30–150 nm. Most mammalian viruses are also small in the genetic sense, having genomes from 7 to 20 kb in length, and a correspondingly small complement of virion proteins. Members of the herpesvirus family are larger, with virion diameters of 150–200 nm and with genome lengths of 125–235 kb. HCMV is the largest of the human herpesviruses. Reflecting their large genomic size, herpesviruses possess a high protein coding capacity, with estimates ranging from 160 to more than 200 open reading frames. The sequence of the HCMV genome has been known for over a decade.

More than 30,000 different viruses are known to infect vertebrates, invertebrates, plants or bacteria, encompassing all three domains of life – Eukaryotes, Archaea and Bacteria. Viruses are grouped into 3600 species, 71 families, and 164 genera. Fewer than 40 viral families and genera are identified to be of medical importance in humans (Table 1). Viruses are the cause of a large array of life-threatening infectious diseases and have been implicated in 15–20% of malignant neoplasms in humans. Viruses that have

Table 1. Examples of medically important virus families

DNA, double-stranded, enveloped viruses	
Herpesviridae	Herpes simplex virus 1 and 2, varicella-zoster virus, Epstein-Barr virus, cytomegalovirus, herpesvirus 8 (Kaposi's sarcoma virus)
Hepadnaviridae	Hepatitis B virus
Poxviridae	Smallpox virus (variola)
DNA, double-stranded, naked viruses	
Papovaviridae	Papillomaviruses (warts)
RNA, double-stranded, enveloped viruses	
Retroviridae	Human immunodeficiency virus (HIV), human T-cell lymphotropic virus
Orthomyxoviridae	Influenza virus type A, B and C
Paramyxoviridae	Mumps virus, measles virus
Coronaviridae	Severe acute respiratory syndrome (SARS)
Flaviviridae	Hepatitis C virus, yellow fever virus
Togaviridae	Rubella virus
Rhabdoviridae	Rabies virus
Filoviridae	Ebola virus
RNA, double-stranded, naked viruses	
Reoviridae	Rotavirus gastroenteritis (infantile diarrhea)
RNA, single-stranded, naked viruses	
Picornaviridae	Polioviruses, Coxsackie viruses, hepatitis A virus
Caliciviridae	Hepatitis E virus, Norwalk group of gastroenteritis viruses

been convincingly linked to various types of human cancer include human papillomaviruses (cervical carcinoma), human polyomaviruses (mesotheliomas, brain tumors), EBV (B-cell lymphoproliferative diseases and nasopharyngeal carcinoma), Kaposi's sarcoma herpesvirus (Kaposi's sarcoma and primary effusion lymphomas), hepatitis B and hepatitis C viruses (hepatocellular carcinoma), and human T-cell leukemia virus-1 (T-cell leukemias) (113). Most cancers associated with HIV infections are related to oncogenic virus infections, such as Kaposi's sarcoma herpesvirus, human papillomavirus and EBV. Viral gene functions that prevent apoptosis, enhance cellular proliferation, or help counteract the immune attack are likely to be important determinants of malignant transformations. Undoubtedly, future research will link an increasing number of known and yet unidentified viruses to human cancer.

All viruses consist of two basic components, nucleic acid (either DNA or RNA but not both) and a protective, virus-coded protein coat termed a capsid. The genome with its protein cover is referred to as the nucleocapsid. Some viruses have additional covering in the form of an envelope that consists of a lipid–protein bilayer derived from the cell membrane of the host. Viral glycoproteins, which extend from the surface of the virus envelope, act as viral attachment proteins for target mammalian cells and are major antigens for protective immunity. The viral envelope cannot survive the intestinal tract and can be disrupted by drying, detergents, solvents, and other harsh conditions, resulting in inactivation of the virus. To ensure infectivity, enveloped viruses must remain wet and are generally transmitted in fluids, respiratory droplets, blood or tissue. In contrast, nonenveloped (naked) viruses can survive the adverse conditions of the intestinal tract and may dry out while still retaining infectivity. Naked viruses can be transmitted easily, on fomites, hand-to-hand, dust, and small droplets. Table 2 lists important characteristics of enveloped and naked viruses.

Classification of viruses is based on the type of the nucleic acid genome (DNA or RNA), the strandedness of the viral nucleic acid (single-stranded or double-stranded genome), the presence or absence of an envelope (enveloped or naked), and other characteristics, such as the virion morphology, chemical composition, and mode of genomic replication (Table 1). Viral names may describe their characteristics, the diseases with which they are associated, or locations where they were first identified. The names picornavirus (pico, meaning small; rna, RNA) and togavirus (Greek for mantle, referring to the membrane envelope surrounding the virus) relate to the structure of the virus. The retrovirus name (retro, meaning reverse) conveys the virus-directed synthesis of DNA from an RNA template. Papovavirus is an acronym for members of the family (*pa*pilloma, *po*lyoma and *va*cuolating viruses). Reoviruses (*r*espiratory *e*nteric *o*rphan) are named for their first sites of isolation, but were not related to other classified viruses and were therefore designated orphans. Coxsackievirus is named after the town of Coxsackie in the state of New York, where the virus was first isolated. Herpesvirus (herpes, 'creeping') describes the nature of the pathologic lesion. 'Cytomegalovirus' refers to the increased cellular size of viral inclusion-bearing cells. The Epstein-Barr virus is named after the two individuals who first described the virus about 40 years ago.

Since viruses have no capacity to produce energy, reproduce their genomes or make their own structural proteins, their replication depends on their hosts to provide energy, substrates and machinery for replication of the viral genome and synthesis of viral proteins. Viruses acquire many of their functions for replication through piggybacking on cellular genes, thereby getting access to basic cellular machinery. Processes not provided by host cells must be encoded

Table 2. Characteristics of enveloped and naked viruses

Property	Enveloped viruses	Naked viruses
Surface structure	Lipid-protein membrane	Proteins
Virion stability	Environmentally labile	Environmentally stable
Virion release	Budding or cell lysis	Cell lysis
Virion transmissibility	Must stay wet	Readily
Predominant immunity	Cell-mediated response	Antibody response
Vaccine development	Complicated	Relatively easy

in the genome of the virus (e.g. the reverse transcriptase enzyme of the retroviruses).

Viral infection can lead either to a rapid replication of the agent and destruction of the infected cell, or to a prolonged period of latency. DNA viruses (except poxviruses) replicate in the nucleus and are more likely to persist in the host, whereas RNA viruses (except retroviruses) replicate in the cytoplasm. Viral replication starts with the virion particle recognizing and attaching to surface receptors of the mammalian cell. These events are followed by viral penetration into the cell, transcription of viral mRNA, viral protein synthesis, and replication of the viral genome. Viral receptor–ligand interactions and viral entry excite cellular responses, cytoskeletal rearrangement, and the induction of transcription factors, prostaglandins and cytokines. After assembling the viral genome and structural proteins, the virions are released from the cell by exocytosis or by cell lysis.

Key to an effective antiviral host response is the ability to recruit appropriate types and numbers of inflammatory cells and mediators to the site of infection. Suboptimal recruitment can lead to an inadequate inflammatory response, whereas overexuberant cell recruitment may result in damage to host tissues. Both cellular and humoral immunity responses are recruited in viral infections, but the pathogenic importance of the two arms of the immune system varies in different viral diseases. Enveloped viruses typically initiate cell-mediated inflammatory responses and delayed type hypersensitivity, which affect viral replication by killing mammalian cells that express viral proteins. Disease is often the result of inappropriate immune responses. Naked viruses are controlled mainly by antibody, and vaccines are generally effective. The role of humoral immunity is to produce antibodies against proteinaceous surface structures and thereby cause inactivation or clearance of the virus. Conversely, viruses have developed important means of escaping from immune detection, and have redirected or modified a normally protective host response to their advantage (256).

Viral diagnostics is a rapidly changing field in terms of assay principles and available diagnostic kits. Identification of viruses has traditionally been based on cell culture to detect characteristic cytopathic effects, morphologic determination of intracytoplasmic and intranuclear inclusion bodies, immunohistochemical techniques, immunoassays to identify viral antigens in clinical specimens, or the measurement of total or class-specific antibodies against specific viral antigens. In some viral infections, IgM antibodies are useful for determining primary infection, and IgG antibodies for assessing the susceptibility to primary infection and viral reactivation. Oral fluid collection may constitute a convenient and noninvasive method for serological surveillance of immunity to common viral infections (159).

Recently developed molecular technologies for detecting viral DNA or RNA in clinical specimens are now routinely used in virology laboratories. Viral nucleic acid can be measured directly by hybridization, or be detected after amplification by nucleic acid amplification methods (54). Polymerase chain reaction (PCR) offers a rapid and relatively inexpensive method of identifying viral nucleic acids in clinical specimens. Recent advances in quantitative real-time PCR techniques can provide additional insights into the natural history and disease associations of viral infections. Real-time PCR detection systems generally have a broad dynamic range and display high sensitivity, reproducibility and specificity. The use of PCR to monitor herpesvirus DNA load provides particularly high specificity (14). However, in order to evaluate the diagnostic utility of ultrasensitive PCR assays, correlations with clinical outcome are essential. The microarray-based detection assay provides a single-format diagnostic tool for the identification of multiple viral infections and will most likely become increasingly important in clinical virology. In the periodontal studies discussed below, PCR-based techniques were used to identify herpesviruses and bacterial species.

Herpesviruses

For a general introduction to herpesviruses, the reader is referred to a number of authoritative reviews (179, 195, 198). Because of the lack of effective therapeutics and vaccines, herpesvirus diseases continue to constitute a significant problem for public health. Herpesviral characteristics of potential importance in the pathogenesis of periodontitis are outlined below. Emphasis is placed on a description of HCMV and EBV because of these viruses' major suspected etiopathogenic role in human periodontitis (225).

Membership in the family *Herpesviridae* is based on a four-layered structure of the virion (Fig. 1). Herpesviruses have (i) a core containing a large double-stranded DNA genome encased within (ii) an isosapentahedral capsid containing 162 capsomers, (iii) an amorphous proteinaceous tegument and, surrounding the capsid and tegument, (iv) a lipid bilayer envelope derived from host cell membranes. The viral

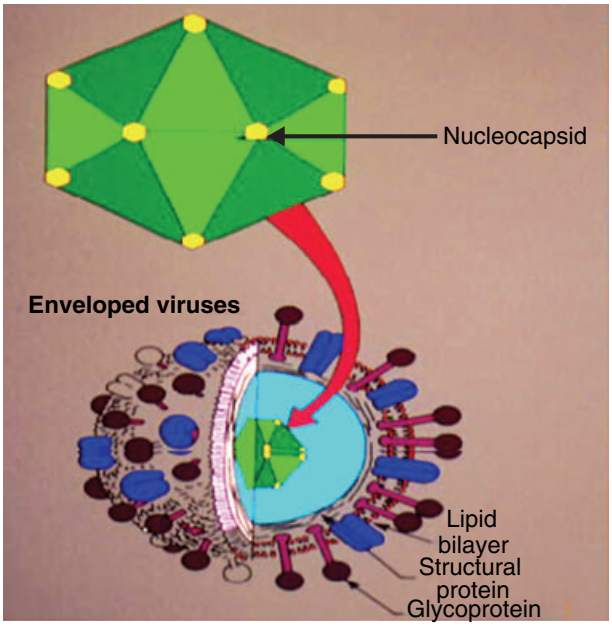

Fig. 1. Herpesvirus virion.

envelope contains viral-induced glycoproteins, which are ligands for cellular attachment and important targets for host immune reactions. Several herpesvirus proteins of the capsid, tegument, glycoprotein, replication, and immunomodulatory protein families have been identified and characterized.

Of the approximately 120 identified different herpesviruses, eight major types are known to infect humans, namely, herpes simplex virus (HSV) type 1 and 2, varicella-zoster virus, EBV, HCMV, human herpesvirus (HHV)-6, HHV-7, and HHV-8 (Kaposi's sarcoma virus). Research has identified more than 5000 different strains of herpesviruses. Humans are the only source of infection for these eight herpesviruses. Human herpesviruses are classified into three groups (α, β, γ) based upon details of tissue tropism, pathogenicity, and behavior under conditions of culture in the laboratory (Table 3). Alpha-herpesviruses are neurotropic, have a rapid replication cycle, and display a broad host and cell range. The β- and γ-herpesviruses differ in genomic size and structure, but replicate relatively slowly and in a restricted range of cells, mainly of lymphatic or glandular origin.

Herpesviruses can occur in a latent or a productive (lytic) state of replication. During latency, the herpesvirus DNA is integrated into and seems to behave like the host chromosomal DNA. In the viral productive cycle, the herpesvirus genome is amplified 100- to 1000-fold by the viral replication machinery. Figure 2 outlines the mode of the productive replication of herpesviruses. Herpesvirus transcription, genome replication, and capsid assembly occur in the host cell nucleus. The tegument and the envelope are acquired as the virion buds through the nuclear membrane. Herpesvirus virion genes are replicated in a specific order:

i) immediate-early genes, which encode regulatory proteins;
ii) early genes, which encode enzymes for replicating viral DNA;

Table 3. Human herpesviruses

Herpesviruses	Abbreviation	Herpes group	Major diseases
Herpes simplex virus type 1	HSV-1	α	Acute herpetic gingivostomatitis, keratitis, conjunctivitis, encephalitis, dermal Whitlow
Herpes simplex virus type 2	HSV-2	α	Herpes genitalis
Varicella-zoster virus	VZV	α	Varicella (chickenpox), zoster (shingles)
Epstein-Barr virus	EBV	γ	Classic infectious mononucleosis, Burkitt's lymphoma (Africa and New Guinea), Hodgkin's lymphoma, nasopharyngeal carcinoma, squamous carcinoma (Southern China), oral hairy leukoplakia, chronic fatigue syndrome (?)
Human cytomegalovirus	HCMV	β	Congenital symptomatic cytomegalovirus infection (growth retardation, jaundice, hearing defects, etc.), retinitis, encephalitis, mononucleosis-like syndrome, organ transplant rejection
Human herpesvirus 6	HHV-6	β	Exanthem subitum (roseola infantum) in young children and undifferentiated febrile illness
Human herpesvirus 7	HHV-7	β	Exanthem subitum (roseola)-like illness in young children
Human herpesvirus 8	HHV-8	γ	Kaposi's sarcoma in AIDS patients and intra-abdominal solid tumors

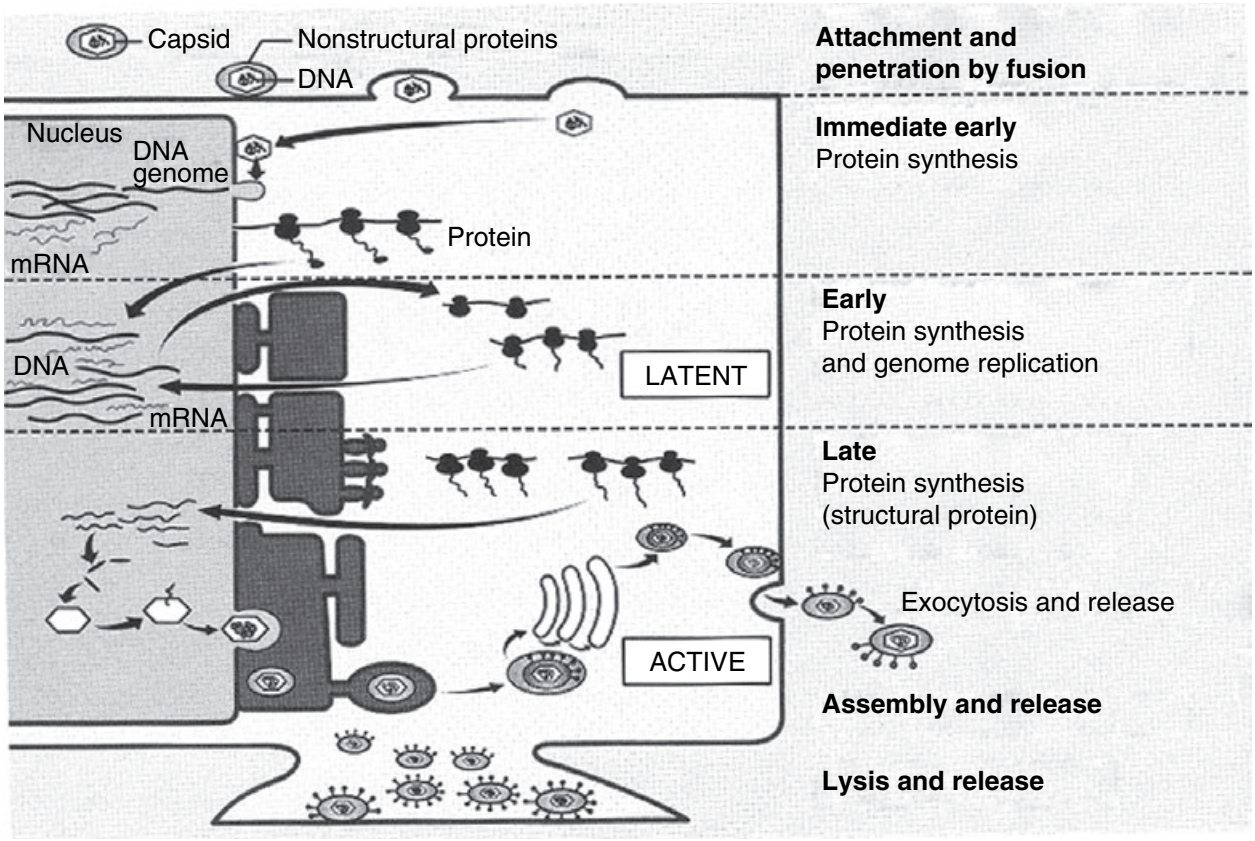

Fig. 2. Herpesvirus replication. Virion initiates infection by fusion of the viral envelope with plasma membrane following attachment to the cell surface. Capsid is transported to the nuclear pore where viral DNA is released into the nucleus. Viral transcription and translation occur in three phases: immediate early, early, and late. Immediate early proteins shut off cell protein synthesis. Early proteins facilitate viral DNA replication. Late proteins are structural proteins of the virus that form empty capsids. Viral DNA is packaged into preformed capsids in the nucleus. Virions are transported via endoplasmic reticulum and released by exocytosis or cell lysis.

iii) late genes, which encode structural proteins of the capsid of the virion.

Transcription of late genes can be used diagnostically to indicate active infection. Virions are transported to the cell membrane via the Golgi complex. The host cell dies with the release of mature virions or, alternatively, specific cell types may maintain herpesviruses in a latent state.

To survive, herpesviruses need to exploit macrophages, lymphocytes or other host cells for replication, while minimizing antiviral inflammatory responses of the host. Herpesviruses encode proteins that are specifically committed to subvert the immune defense of the host in order to evade virus elimination. To overcome viral immunoevasive proteins, the host in turn has evolved countermeasures to confine virus replication to below a harmful level. Herpesvirus diseases are generally limited to immunologically immature or immunocompromised individuals unable to mount an adequate host defense (189).

During their life cycles, herpesviruses execute an intricate chain of events geared towards optimizing their replication. The initial productive phase of infection is followed by a latent phase during which the viral genome integrates within the host cell's genome. Latency ensures survival of the herpesviral genome throughout the lifetime of the infected individual. From time to time, latent herpesviruses may undergo reactivation and re-enter the productive phase as a consequence of declining herpesvirus-specific cellular immunity. The balance between herpesvirus latency and activation involves the regulation of herpesvirus gene expression, but the genetic and biochemical mechanisms governing a herpesvirus latent infection and reactivation from latency are not fully understood. In general, the herpesvirus latent phase shows little tendency to transcription, whereas reactivation from latency results in a general viral gene expression (112). Nonetheless, expression of EBV-latency-associated genes has potent cell cycle-promoting activity of naive B-lymphocytes, which probably accounts for the growing panel of human cancers associated with the virus (67). During the active replication phase,

herpesvirus genomic transcription may induce changes in host cell expression of genes that encode proteins involved in immunity and host defense, cell growth, signaling, and transcriptional regulation (222). Psychosocial and physical stress, hormonal changes, infections, immunosuppressive medication, and other events impairing cellular immunity can trigger herpesviral reactivation. Transforming growth factor (TGF)-β1 in saliva seems also to have the potential to reactivate herpesviruses (164).

Herpesviruses are typically highly selective in regard to the specific tissues or organs they infect, reflecting their strong tendency to tissue tropism. Several herpesviruses reside in and may functionally alter cells of central importance for regulating the immune system (45, 156). HCMV infects monocytes/macrophages, T-lymphocytes, ductal epithelial cells of salivary glands, endothelial cells, fibroblasts and polymorphonuclear leukocytes, and establishes latent infection mainly in cells of the myeloid lineage. HCMV infection causes cytopathological effects that involve intranuclear and cytoplasmic inclusions ('owl's-eye' cells; large cells with enlarged nuclei containing violaceous intranuclear inclusions surrounded by a clear halo) in a characteristic enlargement of the host cells (cytomegaly). EBV infects relatively long-lived B-lymphocytes during primary infection and during latency, and can also infect the oropharyngeal epithelium. The molecular mechanism of tissue tropism of herpesviruses remains largely unknown.

Most herpesviruses are ubiquitous agents that often are acquired early in life and infect individuals from diverse geographic areas and economic backgrounds. An important exception is HHV-8, which is uncommon in the general population in the United States (less than 5% of the U.S. population is serologically positive for HHV-8) but is detected consistently in patients with AIDS-associated Kaposi's sarcoma and frequently in the eastern Mediterranean and sub-Saharan Africa, where Kaposi's sarcoma is endemic (34). Over the lifetime of the infected host, herpesvirus reactivation will lead to low-level infections that can be spread to acquaintances. The shedding of herpesvirus virions may take place without any detectable signs or symptoms of disease. Transmission of herpesviruses can happen vertically, either prenatally (HCMV) or perinatally, from mother to infant, or horizontally in children or adults by direct or indirect person-to-person contact. Infectious herpesviruses may be found in oropharyngeal secretions, urine, cervical and vaginal secretions, semen, maternal milk, tears, feces, and blood. Saliva of many immunocompetent and immunocompromised subjects contains several herpesvirus species and may frequently serve as a vehicle for viral transmission (72, 109). It is estimated that asymptomatic shedding of HCMV into saliva, cervical secretions, semen, and breast milk occurs in 10–30% of infected individuals (27). HCMV seroconversion, which is indicative of a recent active infection, can take place in all age groups between 18 and 60 years and, in Germany, occurs with elevated frequency in 30–35-year-old individuals (89).

Herpesvirus infections may be latent, subclinical or clinical. Herpesvirus colonization in most individuals is clinically unnoticeable, and activation of latent herpesviruses may cause both symptomatic and asymptomatic infection. Most serious clinical illness happens when primary infection occurs in adolescence or beyond. Clinical cases of herpesvirus infection are frequently the result of a reactivation of a latent infection, which is linked to the immune status of the patient. In immunocompetent hosts exhibiting protective antiviral immune responses, primary infection or reactivation of latent herpesvirus genomes is usually asymptomatic despite active virus replication and systemic dissemination. In immunocompromised patients, herpesvirus infection can produce a wide spectrum of outcomes, ranging from subclinical infection to disseminated fulminant disease having high mortality rates. Herpesvirus infections with associated immune impairment may also increase the risk or the severity of bacterial, fungal or other viral infections (24).

Herpesvirus infections are kept under control by various innate and immune responses that, although vigorous, are not capable of eliminating the viruses. The innate host response consists of a complex multilayered system of mechanical and secreted defenses, immediate chemokine and interferon responses, and rapidly recruited cellular defenses. Innate responses are the first line of defense during both primary and recurrent infection, and are essential during acute infection to limit initial viral replication and to facilitate appropriate adaptive immune responses. The humoral acquired immune response aims mainly at neutralizing and preventing initial herpesvirus infections. Gingiva of mice shows high resistance to infection by HSV, which may suggest the existence of a particularly efficacious antiherpesvirus defense in the murine periodontium (158).

The cellular immune response attempts to eliminate virus-infected cells by means of lymphocytes (86, 256). Cytotoxic T-lymphocytes and natural killer (NK) cells are the most important effector cells in immune suppression of herpesvirus replication and

in the maintenance of latency (160). Evidence for the importance of the cellular immunity in the control of herpesvirus infections comes from the observation that severe herpesvirus disease occurs almost exclusively in subjects with depressed cell-mediated immunity. Also, impaired cellular immunity leads to less efficient elimination of herpesvirus-infected host cells and to increased herpesvirus DNA replication. The T-lymphocyte response to herpesviruses changes over time from a predominantly $CD4^+$ response early in infection to a $CD8^+$ response during latent infection. $CD4^+$ cells contribute to expansion of cytotoxic $CD8^+$ T-lymphocytes. The antiviral cytotoxic T-lymphocyte response against herpesvirus is limited to a few proteins, with the predominant anti-HCMV response directed against the pp65 tegumental protein, which therefore represents a main target for cellular immunotherapy (19).

In response to antiviral host defenses, herpesviruses have devised a number of elaborate immunosubversive mechanisms to ensure persistent infections (241, 264). Herpesviruses can trigger dysregulation of macrophages and lymphocytes for the purpose of down-regulating the antiviral host immune response (24). HCMV can interfere with the immune functions of antigen-presenting monocyte-derived dendritic cells by impairing their maturation, antigen presentation and allostimulatory capacity (19). HCMV and other herpesviruses have also the ability to inhibit the expression of major histocompatibility complex (MHC) class I and II on the surface of macrophages (265), to evade cytotoxic T-cell recognition and attenuate induction of antiviral immunity (256), and to encode proteins that interfere with the presentation of viral peptide antigens to cytotoxic T-cells (256). The presence of genes that encode proteins that interfere with HCMV antigen presentation helps herpesvirus-infected cells escape $CD8^+$ and $CD4^+$ T-cell immunosurveillance. Cells that lack MHC class I molecules are normally recognized and eliminated by NK cells, but herpesvirus-infected cells have developed strategies to circumvent NK cell-mediated lysis (26, 265). The destruction of components of MHC class I and class II pathways within macrophages, which markedly impair their principal role in antigen presentation, together with the silencing of NK cells, help ensure the permanence of herpesvirus infections (152). HCMV has also the ability to inhibit the expression of macrophage surface receptors for lipopolysaccharide and thereby the responsiveness to gram-negative bacterial infections (101). Some herpesvirus genes protect cells from undergoing apoptosis to prolong the lives of infected cells (256, 265). One effect of the inhibition of apoptosis is the promotion of tumor cell survival, potentially interfering with anticancer chemotherapy (150). The large series of immune evasion molecules helps herpesviruses establish life-long latency interrupted by recurrent reactivations, despite an intact immune system of the host.

Herpesvirus infections affect cytokine–chemokine networks (156). Cytokines and chemokines play important roles in the first line of defense against human herpesvirus infections and also contribute significantly to the regulation of acquired immune responses. HCMV infection induces a proinflammatory cytokine profile, with production of interleukin (IL)-1β, IL-6, IL-12, tumor necrosis factor (TNF)-α, interferon (IFN)-α/β, and IFN-γ (156) and prostaglandin E_2 (PGE_2) (154). EBV infection stimulates the production of IL-1β, IL-1 receptor antagonist (IL-1Ra), IL-6, IL-8, IL-18, TNF-α, IFN-α/β, IFN-γ, monokine induced by IFN-γ (MIG), IFN-γ-inducible protein 10 (IP-10) and granulocyte-macrophage colony-stimulating factor (GM-CSF) (156). On primary HSV infection, the host responds by producing IL-1β, IL-2, IL-6, IL-8, IL-10, IL-12, IL-13, TNF-α, IFN-α/β, IFN-γ, GM-CSF, macrophage inflammatory protein 1α (MIP-1α) and MIP-1β, monocyte chemoattractant protein 1 (MCP-1) and regulated upon activation normal T-cells expressed and secreted (RANTES) (156). Proinflammatory cytokine and chemokine activities normally serve a positive biological goal by aiming to overcome infection or invasion by infectious agents. IFN-γ, TNF-α and IL-6 exert particularly high antiviral activity. However, by a diverse array of strategies, herpesviruses are able to interfere with cytokine production or divert potent antiviral cytokine responses (6, 155, 256). The extensive built-in redundancy of the cytokine system and the elaborate efforts by herpesviruses to undermine or exploit its function testify to the importance of cytokines in the antiviral host defense. It is of clinical significance that cytokines may exert detrimental effects when a challenge becomes overwhelming, or with a chronic pathophysiologic stimulus.

T-helper lymphocyte type 1 (Th1) proinflammatory immune responses aim to clear the host of intracellular pathogens, such as herpesviruses. Th1 cytokines favor the development of a strong cellular immune response, whereas Th2 cytokines favor a strong humoral immune response, and some of the type 1 and type 2 cytokines are cross-regulatory. In an effort to counteract ongoing inflammation, the initial proinflammatory response triggers the release of anti-inflammatory TGF-β and IL-10, a Th2 cytokine that

antagonizes Th1 proinflammatory responses (88). HCMV (123) and EBV (206) also encode unique homologs of IL-10 capable of inhibiting the production of TNF, IL-1 and other cytokines in macrophages and monocytes (254), and of preventing the activation and polarization of naive T lymphocytes towards protective gamma interferon-producing effectors (33). Moreover, herpesviruses can block the interferon signal transduction pathway, which limits the direct and indirect antiviral effects of the interferons (256). Viruses also display great inventiveness when it comes to diverting potent antiviral cytokine and chemokine responses to their benefit (256). PGE_2, which is a major mediator of the periodontal inflammatory response (73), increases rapidly in response to exposure of cells to herpesviruses, bacterial lipopolysaccharide, and IL-1β and TNF-α cytokines (261); however, PGE_2 may under certain circumstances serve to support HCMV replication (154, 278). In sum, herpesvirus infections induce a multiplicity of interconnected immunomodulatory reactions, and various stages of the infectious process may display different levels of specific inflammatory cells and mediators, underscoring the complexity of herpesvirus–host interactions.

Herpesviruses can cause serious infectious diseases and be tumorogenic (Table 3). Herpesvirus diseases occur primarily in individuals having an immune system that is immature or suppressed by drug treatment or coinfection with other pathogens. In immunocompetent persons, complications of an acute HCMV infection are rare, except in newborns, where HCMV represents the major infectious cause of pregnancy complications and birth defects (7). About 10% of HCMV-infected newborns may show low birth weight, jaundice, hepatosplenomegaly, skin rash, microcephaly or chorioretinitis (15). Congenital HCMV infection is the leading infectious cause of mental retardation and sensorineural deafness (194). In 1992, it was estimated that approximately 40,000 newborns annually in the USA were infected prenatally with HCMV and that up to 7000 of these newborns developed permanent central nervous damage as a result of the infection (69). Approximately one-third of newborns with symptomatic congenital HCMV infection born to mothers with recurrent HCMV infection or to mothers with primary HCMV infection during pregnancy may be premature (< 37 weeks' gestation) and small for their gestational age (25). In adolescents and young adults, primary HCMV infection causes about 7% of cases of the mononucleosis syndrome and may manifest symptoms almost indistinguishable from those of EBV-induced mononucleosis.

HCMV is capable of manifesting disease in nearly every organ system in immunocompromised patients. HCMV is the most common life-threatening infection in HIV-infected patients (82). Necrotizing retinitis is a relatively common HCMV-induced complication in untreated HIV-infected persons (248). Also, rather than *Helicobacter pylori*, HCMV may be the main causative pathogen of peptic ulcers in some AIDS patients (35). Salivary HCMV DNA occurs at an elevated rate with xerostomia in HIV-infected patients with low CD4 counts, suggesting HCMV may be a potential cause of salivary gland dysfunction in these patients (81). The introduction of the highly active antiretroviral therapy (HAART) has provided a means of reconstituting the immune system in HIV-infected individuals, allowing the HCMV infection to be controlled (242).

Organ transplantation has become a widely accepted treatment modality for end-stage diseases. With the escalation in the number of patients undergoing immunosuppressive therapy following solid organ or bone marrow transplantation, HCMV activation and resulting disease has become a major clinical problem in transplant recipients. HCMV is the most common infectious reason for transplant rejection, including bone marrow or stem cell grafts (37), and a relationship has been sought between periodontal HCMV and renal transplant complications (166). HCMV infection seems also to be a significant risk factor for the development of bacterial septic infection in liver transplant patients (163, 182), and for causing colonization of the oropharynx by gram-negative bacilli in renal transplant patients (138).

HCMV and HSV have for two decades been epidemiologically associated with the development of primary atherosclerosis, postangioplasty restenosis, and post-transplantation arteriosclerosis (169). Both vascular smooth muscle and endothelial cells are targets for HCMV primary infection and may serve as potential sites of HCMV latency. HCMV DNA sequences have been detected in atheromatous plaques (87) and in the wall of atherosclerotic vessels (104, 219). A PCR-based study identified genomes of HCMV in 40%, EBV in 80% and HSV-1 in 80% of atherosclerotic aortic tissue, compared to 4%, 13% and 13%, respectively, of nonatherosclerotic aorta controls (219). HCMV-infected cardiac transplant patients are prone to develop accelerated atherosclerosis (2). Animal research has shown the Marek's disease virus, an avian herpesvirus, to be capable of inducing atherosclerotic lesions in infected chickens (61). Murine CMV is able to produce atherosclerosis in experimental mice (103). Although animal

experiments on cardiovascular disease do not replicate exactly the human disease, they may provide valuable suggestions on causality. HCMV and HSV-1 may affect atherosclerosis directly or indirectly (121). Direct effects on vascular wall cells may include cell lysis, transformation, lipid accumulation, proinflammatory changes, and augmentation of procoagulant activity. Indirect systemic effects may involve induction of acute-phase proteins, establishment of a prothrombotic state, hemodynamic stress caused by tachycardia, increased cardiac output, or a regional inflammatory activation in response to systemic cytokinemia. It is theorized that herpesvirus infections, usually in combination with other risk factors, such as hypertension, smoking, hyperlipidemia, obesity, and family history, promote atherogenesis and trigger acute coronary events. The possibility that HCMV and other herpesviruses give rise to cardiovascular disease and periodontitis in an independent manner further complicates studies on the relationship between the two diseases, and raises questions about the notion of periodontitis being a direct risk factor of ischemic heart disease (223, 229). Similar reservations are applicable to the proposed relationship between periodontitis and atherosclerosis-associated ischemic craniovascular events (52).

HCMV and EBV appear with increased frequency in synovial fluid and tissue of autoimmune chronic arthritis, pointing to a possible viral factor in the disease (147). Furthermore, HCMV has been identified in diseases that have a bacterial component, including inflammatory bowel disease, enterocolitis, esophagitis, pulmonary infections, sinusitis, acute otitis media, dermal abscesses, and pelvic inflammatory disease (28, 224). Activation of HCMV and other herpesviruses may play roles in oral ulceration of the aphthous type (183, 191, 247). HCMV has also been associated with cervical carcinoma and adenocarcinomas of the prostate and the colon (51). However, it should be cautioned that the presence of herpesvirus DNA in various disease entities does not prove causality in itself. The difficulty in providing true causal evidence for the role of herpesviruses in disease lies in inadequate knowledge about molecular aspects of herpesviruses and the pathogenic mechanisms of herpesvirus-associated pathosis.

The primary route of EBV acquisition is through salivary exchange in the oropharynx (195). The virus is the main causative agent of infectious mononucleosis, which is a relatively common clinical manifestation of a primary EBV infection in adolescents and young adults. EBV has also been implicated in multiple sclerosis and various enigmatic syndromes, and seems to play a role in the development of oral hairy leukoplakia. Oral hairy leukoplakia is associated with EBV productive and nonproductive infection of tongue epithelial tissue (266), EBV-encoded nuclear antigen (EBNA)-2 protein function (268), and an EBV-related decrease in oral epithelial Langerhans cells (267). EBV can contribute to oncogenesis, as evidenced by its frequent occurrence in certain tumors arising in lymphoid or epithelial tissue, including B-lymphocyte neoplasms, such as Burkitt's lymphoma, post-transplant B-cell lymphoma and Hodgkin's disease, certain forms of T-cell lymphoma, and some types of epithelial tumors, including undifferentiated nasopharyngeal carcinoma and a portion of gastric carcinomas. EBV may also be involved in the pathogenesis of aggressive types of non-Hodgkin lymphomas affecting gingiva (277), particularly in HIV-infected individuals (213). Recently, EBV (141) and HCMV (200) have been associated with cases of breast cancer. EBV may induce tumors by influencing survival mechanisms of B-lymphocytes, but environmental, genetic, and iatrogenic cofactors are most likely also participants in EBV-related oncogenesis. That EBV may adopt different forms of latent infection in different tumor types is a reflection of the complex interplay between the virus and the host cell environment.

HSV is the cause of some of the most frequently encountered clinical infections in humans. HSV-1 usually causes orolabial disease, and HSV-2 is associated more frequently with genital and newborn infections. Most HSV clinical infections give rise to mild and self-limiting disease of the mouth and lips or at genital sites, but can be life-threatening when affecting neonatals and the central nervous system, especially in immunocompromised hosts (105, 270). Varicella-zoster virus (VZV) causes chickenpox (varicella), after which it establishes latency and can subsequently reactivate in adults to cause shingles (herpes zoster). Serious central nervous system complications can follow both primary infection and reactivation of VZV (77). Although HHV-6 is generally asymptomatic, the virus has been associated with exanthem subitum, febrile convulsions and encephalitis in infants and immunocompromised adults, and may play a role in multiple sclerosis, the Guillain-Barre syndrome, and acute disseminated encephalomyelitis (48). HHV-7 has not been shown to cause a specific disease, but is associated with febrile convulsions and has been implicated in a few cases of exanthem subitum and as a cause of encephalitis (48). HHV-8 is implicated in Kaposi's sarcoma, the plasma-cell variant of multicentric

Castleman's disease, and pleural effusion lymphoma (93). Herpesviruses can also give rise to other types of medical and orofacial infections and tumors, especially in immunocompromised hosts (205, 207).

Treatment of herpesvirus infections can be difficult because few options exist (127). Presently available antiherpesvirus drugs can produce clinical improvement, but suffer from poor oral bioavailability, low potency, development of resistance, and dose-limiting toxicity. Nucleic acid molecules are emerging as new antiviral tools in antisense therapy, in which an antisense oligonucleotide to mRNA of genes involved in pathogenesis selectively modulates gene expression. Conventional vaccination with attenuated herpesviruses or herpesviral proteins fails to prime efficient immunologic protection, presumably because critical antigens are not presented effectively *in vivo*. Development of novel herpesviral vaccines and vaccination technologies are of high priority, and several promising herpesviral vaccine candidates are currently in clinical trials (180, 273). The prime goal of a vaccine should be to prevent primary infection, but vaccines may also be used to modify the course of established persistent herpesvirus infections by so-called postinfective immunization or therapeutic vaccination.

Herpesviruses in periodontal disease

Studies during the past 10 years have associated herpesviruses with human periodontitis. Table 4 describes the distribution of herpesviruses in biopsy specimens from clinically healthy and inflamed gingiva of adult (chronic) periodontitis patients living in Los Angeles. DNA of 2–6 herpesviruses was demonstrated in all 14 biopsies from periodontitis sites. In contrast, HCMV only occurred in two and EBV-type 1 (EBV-1) in three biopsies from 11 healthy gingival sites. HSV, HCMV, EBV-1, EBV-type 2 (EBV-2) and HHV-7 showed significant associations with periodontitis. HHV-6 and HHV-8 were only detected in biopsies from periodontitis lesions. Three of four biopsies yielding HHV-8 originated from patients with confirmed HIV infection; the HIV-status of the fourth HHV-8-positive subject was unknown.

Table 4. Herpesviruses in gingival biopsies from periodontitis and clinically healthy sites in adults[a]

Herpesviruses	Periodontitis (14 subjects)	Healthy periodontium (11 subjects)	*P*-values (chi-squared test)
HSV	8 (57)[b]	1 (9)	0.04
EBV-1	11 (79)	3 (27)	0.03
EBV-2	7 (50)	0 (0)	0.02
HCMV	12 (86)	2 (18)	0.003
HHV-6	3 (21)	0 (0)	0.31
HHV-7	6 (43)	0 (0)	0.04
HHV-8	4 (29)[c]	0 (0)	0.17

[a]Adapted from Contreras et al. (42).
[b]No. (%) of virally positive samples.
[c]Three patients were confirmed HIV-positive.

Table 5 lists the occurrence of subgingival HCMV, EBV and HSV DNA in periodontitis patients from different countries. In Turkey, HCMV was detected in 44% of chronic periodontitis lesions and in 14% of healthy periodontal sites ($P < 0.05$), EBV-1 in 17% of periodontitis lesions and in 14% of healthy sites, and HSV in 7% of periodontitis lesions but in no healthy study site (211). Another study from Turkey identified HCMV in 68% of chronic periodontitis lesions and in 33% of gingivitis lesions (252). In 62 Chinese patients, Li et al. (132) found EBV in 58% of disease-active periodontitis sites, but only in 23% of quiescent periodontitis sites and in 19% of gingivitis sites. In Japan, Idesawa et al. (108) detected EBV in 49% of chronic periodontitis lesions and in 15% of healthy periodontal sites. Studies of periodontitis in Taiwanese adult patients showed subgingival HSV monoinfection and HSV-HCMV coinfection to be associated with increased periodontal pocket depth and attachment loss, and elevated frequency of gingival bleeding but relatively little dental plaque (133). In Italy, HSV-1 (208) and HHV-7 (31) have been related to periodontal disease. Israeli subjects revealed HSV antigens in 39% of biopsies from clinically healthy gingiva (8). In France, Madinier et al. (139) detected EBV DNA in eight of 20 gingival specimens but, despite the potential of EBV to replicate in oral mucosa (9), only in one specimen from nasal, laryngeal, and oral mucosa, suggesting inflamed gingiva serves as a reservoir for EBV. Even though herpesvirus carriage varies by age, country, region within country, and population subgroups (235), studies from the various countries all report on a high prevalence of herpesvirus DNA in periodontitis lesions, attesting to the robustness of the herpesvirus–periodontitis association.

Kamma et al. (114) investigated the occurrence of DNA of HCMV, EBV-1 and selected periodontal pathogenic bacteria in 16 patients with aggressive periodontitis from Greece (Table 6). In each patient,

Table 5. Prevalence of herpesvirus DNA in periodontitis patients from various countries

Study	Country	Periodontal status	Herpes simplex virus type 1	Epstein-Barr virus[a]	Cytomegalovirus
Contreras et al. (42)	USA	Advanced chronic periodontitis	57% (periodontitis) 9% (healthy or slight gingivitis)	79% (periodontitis) 27% (healthy or slight gingivitis)	86% (periodontitis) 18% (healthy or slight gingivitis)
Ting et al. (255)	USA	Aggressive localized periodontitis	55% (periodontitis) 9% (healthy)	64% (periodontitis) 18% (healthy)	73% (periodontitis) 18% (healthy)
Michalowicz et al. (151)	Jamaica	Localized periodontitis	No data	33% (aggressive) 45% (incipient) 17% (healthy/gingivitis)	73% (aggressive) 40% (incipient) 22% (healthy/gingivitis)
Kamma et al. (114)	Greece	Generalized periodontitis	35% disease-(active) 9% (disease-stable)	44% (disease-active) 13% (disease-stable)	59% (disease-active) 13% (disease-stable)
Saygun et al. (210)	Turkey	Generalized periodontitis	78% (aggressive) 0% (healthy)	72% (aggressive) 6% (healthy)	72% (aggressive) 0% (healthy)
Kubar et al. (124)	Turkey	Generalized periodontitis	No data	89% (aggressive) 46% (chronic)	78% (aggressive) 46% (chronic)
Ling et al. (133)	Taiwan	Chronic periodontitis	31%	4%	52%
Li et al. (132)	China	Chronic periodontitis	No data	58% (disease-active) 23% (quiescent) 19% (gingivitis)	No data
Idesawa et al. (108)	Japan	Chronic periodontitis	No data	49% (saliva of periodontitis patients) 15% (saliva of healthy subjects)	No data

[a]Most studies report on EBV type 1.

Table 6. Occurrence of human cytomegalovirus (HCMV) and Epstein-Barr virus type 1 (EBV-1) in progressing and stable periodontitis sites of 16 patients with aggressive periodontitis patients[a]

Items	32 disease-active periodontitis sites	32 disease-stable periodontitis sites	*P*-values (chi-squared test)
Mean pocket probing depth in mm	5.9 ± 0.8	5.2 ± 1.0	Not significant
Bleeding upon probing, *n* (%) positive sites	31 (96.9%)	19 (59.4%)	< 0.001
% teeth exhibiting alveolar bone loss	41.3 ± 6.3	43.9 ± 6.2	Not significant
HCMV, *n* (%) positive sites	19 (59.4%)	4 (12.5%)	< 0.001
EBV-1, *n* (%) positive sites	14 (43.8%)	4 (12.5%)	0.01
HCMV and EBV-1 coinfection, *n* (%) positive sites	9 (28.7%)	0 (0%)	0.004
D. pneumosintes, *n* (%) positive sites	20 (62.5%)	6 (18.8%)	< 0.001
P. gingivalis, *n* (%) positive sites	23 (71.9%)	12 (37.5%)	0.01
D. pneumosintes and *P. gingivalis* coinfection, *n* (%) positive sites	15 (46.9%)	0 (0%)	< 0.001

[a]Adapted from Kamma et al. (114).

subgingival samples were collected from two progressing and two stable periodontitis sites with similar depth and gingival inflammation. The study revealed that herpesviruses can be detected in some but not in other periodontitis lesions of the same individual. HCMV, EBV-1 and HCMV-EBV-1 coinfection were statistically associated with disease-active periodontitis. All periodontitis sites that demonstrated HCMV-EBV-1 coinfection and all but one site that showed *P. gingivalis-D. pneumosintes* coinfection revealed bleeding upon probing (114), a clinical sign of elevated risk for disease progression (128). Some of the *Dialister* strains may have belonged to the new species *D. invisus* (53). Patients with an HCMV-EBV-1 periodontal coinfection exhibited, on average, a more rapid progression of periodontitis than patients with a herpesvirus monoinfection. Other studies have also demonstrated a strong association between subgingival *P. gingivalis*, *D. pneumosintes* and *P. gingivalis-D. pneumosintes* co-occurrence, and disease-active periodontitis (114, 230, 234). In experimental mice, a murine CMV-*P. gingivalis* combined infection produced distinct liver and spleen damage and a higher mortality rate than monoinfections by either MCMV or *P. gingivalis*, pointing to an important pathogenic interaction between MCMV and *P. gingivalis* (245). In parallel control *Escherichia coli*-MCMV coinfection experiments, the mortality and pathological findings were similar to those observed in mice infected with MCMV only (245). The ability of herpesviruses to induce immunosuppression may set the stage for enhanced proliferation of subgingival *P. gingivalis*, *D. pneumosintes* and other periodontopathic bacteria, and increase the risk of periodontal disease progression.

Herpesviruses do not appear to be only passive bystanders to gingival inflammation in periodontitis lesions. Kamma et al. (114) showed that, even if no difference was observed in the level of gingival inflammation, herpesviruses occurred more frequently in actively progressing than in stable periodontitis sites. Kubar et al. (125) found increased periodontal pocket depth and attachment loss in aggressive periodontitis sites with HCMV presence, compared to periodontitis sites with similar degree of clinical inflammation but with no detectable HCMV.

Yapar et al. (275) described a close relationship between herpesviruses and aggressive periodontitis, detecting HCMV in 65%, EBV-1 in 71% and HCMV-EBV coinfection in 47% of the deep lesions studied. In aggressive periodontitis lesions, subgingival specimens averaged 4000–10,000 HCMV copies/ml (124, 125) and gingival tissue specimens yielded up to 750,000 HCMV copies (124). The same research group from Ankara, Turkey, detected a lower qualitative and quantitative occurrence of herpesviruses in chronic periodontitis lesions (124, 211). The predilection of herpesviruses for aggressive periodontitis emphasizes the need for a careful assessment of the periodontal disease status in clinical studies of periodontal herpesviruses.

Michalowicz et al. (151) studied the presence of subgingival HCMV, EBV-1, *P. gingivalis* and *A. actinomycetemcomitans* in 15 adolescents with localized aggressive periodontitis, 20 adolescents with incidental periodontal attachment loss, and 65 randomly selected healthy controls. The study subjects were Afro-Caribbeans living in Jamaica. The most efficient multivariate model for localized aggressive periodontitis included HCMV (Odds Ratio = 6.6; 95% confidence limits: [1.7, 26.1]) and *P. gingivalis* (Odds Ratio = 8.7; 95% confidence limits: [1.7, 44.2]). The odds of having localized aggressive periodontitis increased multiplicatively when both HCMV and *P. gingivalis* were present compared to harboring neither of the two infectious agents (Odds Ratio = 51.4; 95% confidence limits: [5.7, 486.5]). Apparently, HCMV and *P. gingivalis* are independently and strongly associated with localized aggressive periodontitis in Jamaican adolescents, and the two infectious agents seem to act synergistically to influence the risk for both the occurrence and the severity of the disease.

Ting et al. (255) studied the relationship between HCMV activation and disease-active vs. disease-stable periodontitis in 11 patients with aggressive juvenile periodontitis between the ages of 10 and 23 years living in Los Angeles (Table 7). The presence of mRNA of the HCMV major capsid protein, which is an indication of an active HCMV infection, was detected in deep pockets of all five HCMV-positive patients with early disease (aged 10–14 years), but only in one of three HCMV-positive patients older than 14 years, and not in any shallow test sites. The study found HCMV reactivation in some and HCMV latency in other periodontal sites of the same patient, pointing to site-specificity in oral HCMV transcription state. HCMV activation was exclusively identified in periodontal sites showing no visible radiographic alveolar crestal lamina dura, a sign of possible periodontal disease progression (188). Gingiva of aggressive periodontitis lesions tends to show high levels of T-suppressor cells (148) and Langerhans cells (149), which are potential carriers of the HCMV

Table 7. Occurrence of human cytomegalovirus (HCMV) and Epstein-Barr type 1 (EBV-1) in deep and shallow periodontal sites of 11 localized aggressive periodontitis patients[a]

Items	5 disease-active periodontitis sites *n* (%) viral-positive sites	4 disease-stable periodontitis sites *n* (%) viral-positive sites	11 shallow periodontal sites *n* (%) viral-positive sites
HCMV	5 (100%)	2 (50%)	2 (18%)
HCMV active infection	5 (100%)	0 (0%)	0 (0%)
EBV-1	3 (60%)	3 (75%)	2 (18%)
HCMV and EBV-1 coinfection	3 (60%)	1 (25%)	2 (18%)
Presence of *A. actinomycetemcomitans*	5 (100%)	0 (0%)	Not done

[a]Adapted from Ting et al. (255).

Table 8. Occurrence of human cytomegalovirus (HCMV) and Epstein-Barr type 1 (EBV-1) in ANUG sites and normal periodontal sites of Nigerian children with and without malnutrition[a]

Herpesviruses	ANUG + malnutrition (22 subjects) *n* (%) viral-positive sites	Normal oral health + malnutrition (20 subjects) *n* (%) viral-positive sites	*P*-values (chi-squared test)
HCMV	13 (59.0%)	0 (0%)	< 0.001
EBV-1	6 (27.3%)	1 (5.0%)	0.13
HCMV and EBV-1 coinfection	8 (36.4%)	0 (0%)	0.009

[a]Adapted from Contreras et al. (40).

genome. Infiltrating cells of aggressive periodontitis lesions in juveniles have revealed a viral morphogenesis phenomenon by electron microscopic examination (29). Periodontal sites demonstrating HCMV reactivation also tend to exhibit elevated levels of *A. actinomycetemcomitans*, a major pathogen of the disease (233). Apparently, HCMV activation together with *A. actinomycetemcomitans* constitutes an important pathogenetic feature of localized aggressive periodontitis lesions in U.S. patients.

To explain the discrete nature of tissue breakdown in localized aggressive periodontitis, it is hypothesized that an active HCMV infection in tissue surrounding the tooth germs damages the root surface structure during the time of root formation of permanent incisors and first molars at 3–5 years of age. HCMV infections of infants are known to have the potential to cause changes in tooth morphology (63, 243), and teeth affected by localized aggressive periodontitis frequently show cemental hypoplasia (23). Also, DNA virus particles within odontogenic cells of developing teeth in hamsters have been related to fibrolytic and osteolytic lesions in the periodontal ligament and adjacent alveolar bone (71). It is further hypothesized that localized aggressive periodontitis patients experience reactivation of periodontal herpesviruses due to puberty-related hormonal changes, the effect of which may be overgrowth of resident periodontopathic bacteria and subsequent tissue breakdown around teeth with weakened periodontium.

Acute necrotizing ulcerative gingivitis (ANUG) affects immunocompromised, malnourished and psychosocially stressed young individuals, and the disease may occasionally spread considerably beyond the periodontium and give rise to the life-threatening infection termed noma/cancrum oris (161). It is estimated that 770,000 people are currently afflicted by noma sequelae (16). Table 8 shows the distribution of herpesviruses in ANUG-affected and non-ANUG-affected children 3–14 years of age from Nigeria (40). A significantly higher occurrence of DNA of HCMV and other herpesviruses was detected in ANUG lesions of malnourished children than in non-ANUG, normal, and malnourished children. In Europe and the U.S.A., ANUG affects mainly adolescents, young adults, and HIV-infected individuals, and virtually never young children. The occurrence of ANUG in children in Africa may be due to an acquisition of herpesviruses in early childhood (178), malnutrition that may promote herpesvirus

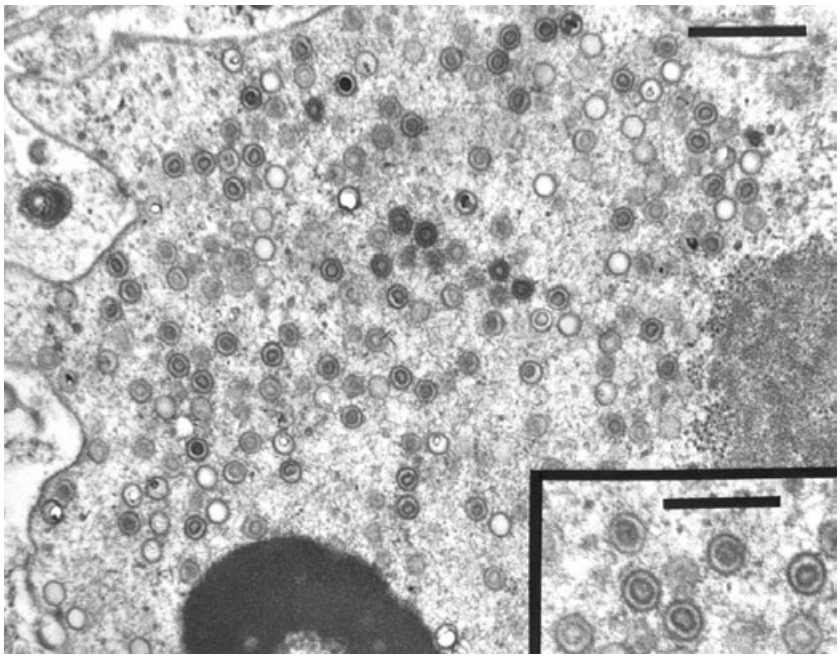

Fig. 3. Transmission electron microscopic view of herpesvirus-like virions in gingival epithelial cells of HIV-associated necrotizing ulcerative periodontitis. Bar = 0.5 μm; inset bar = 0.25 μm. Obtained from Cobb et al. (39) with the permission of the author.

activation (59), and the presence of particularly virulent periodontal bacteria (62). Maxillary osteonecrosis and severe periodontal destruction have also been described in middle-age American individuals who were systemically healthy but positive for the varicella-zoster virus (153, 185).

Periodontitis in HIV-infected patients may resemble that of periodontitis of non-HIV-infected individuals, or may be associated with profuse gingival bleeding or necrotic gingival tissue (100). HIV-induced immunosuppression is known to facilitate herpesvirus reactivation (64). Electron microscopic examination has revealed herpesvirus-like particles in 57% of biopsies from necrotic gingival papillae of HIV-associated periodontitis (39) (Fig. 3). Also, significantly more herpesvirus species have been detected in gingival specimens from HIV-periodontitis lesions than from periodontitis lesions of non-HIV patients (41). HCMV occurred in 81% of the HIV-associated periodontitis lesions and was the most common herpesvirus species identified (41). In HIV-positive individuals, HCMV has also been implicated in acute periodontitis (50), periodontal abscess formation and osteomyelitis (20), and refractory chronic sinusitis (258). EBV DNA has been detected in gingival papillae (137, 139), and EBV reactivation has been related to rapid gingival recession in HIV-infected patients (174). Contreras et al. (41) identified EBV-2 DNA in 57% of biopsies from HIV-periodontitis lesions, which agrees with previous findings of an unusually high incidence of EBV-2 in HIV-infected patients (213, 274). Moreover, Contreras et al. (41) found HHV-8 DNA, the Kaposi sarcoma virus, in periodontitis lesions of 24% of HIV-infected individuals having no clinical signs of Kaposi sarcoma, but not in periodontitis sites of non-HIV-infected individuals. Kaposi sarcoma lesions in gingiva have been linked to severe alveolar bone loss (39). Triantos et al. (257) have identified HHV-8 in the oral mucosa of HIV-infected and immunosuppressed oncologic patients from Greece. HHV-8 has tropism for and is able to infect and replicate *in vitro* in cultured oral epithelial cells (55). In HIV-infected patients, HCMV, EBV, HSV and HHV-8 DNA can be found in saliva (22, 68), and have been related to widespread gingival and mucosal inflammation (66) and oral ulcerative lesions (66, 110, 192, 249).

HCMV and EBV-1 are present in a variety of other types of severe periodontal disease, including Papillon–Lefèvre syndrome periodontitis (262), Fanconi's anemia periodontitis (167), and periodontal abscess formation (212). Down's syndrome patients demonstrate high prevalence of HCMV infection (49) and periodontitis lesions of these patients usually harbor several herpesviruses (85). In renal transplant patients, active HCMV replication has been detected in sites with gingival overgrowth and increased pocket depth (166). EBV has been identified in hyperplastic gingiva of cardiac transplantation patients with a history of cyclosporine use (170), and in odontogenic and nonodontogenic tumors (111). An acute HSV-1 infection can give rise to gingival recession, as observed in a 26-year-old male patient who suddenly developed severe gingival inflammation and vesicle formation and, within a few hours, experienced a marked destruction of the gingival

tissue (186). In patients with acute myeloid leukemia, HSV may be an important pathogen of oral mucosal ulcerations (214). Viruses other than herpesviruses can also reside in the human periodontium, but their relationship to destructive periodontal diseases remains unclear (18, 30, 65, 116, 140, 143, 144, 176, 199, 250). Hormia et al. (102) suggested that the periodontium serves as a reservoir for human papillomavirus. Viruses have also been related to periodontal disease in primates (236), cats (99, 136, 193), mice (220), and hamsters (71).

Herpesviruses may interfere with periodontal healing. In guided tissue regeneration, Smith MacDonald et al. (237) recorded an average gain in clinical attachment of 2.3 mm in four periodontal sites that revealed either HCMV or EBV DNA, compared with a mean clinical attachment gain of 5.0 mm in 16 virus-negative sites ($P = 0.004$). By infecting and altering the function of fibroblasts and other periodontal cells, herpesviruses may compromise the regenerative potential of the periodontal ligament. Undiagnosed herpesvirus infections in the human periodontium may help explain why barrier membrane-associated treatment is unsuccessful in some patients. Moreover, 11 of 15 (73%) HSV-1 seropositive patients, but only 7 of 15 (47%) matched controls experienced dry socket complications after tooth extraction (92). Tooth extraction in experimental rats can reactivate a latent HSV-1 infection, resulting in delayed healing of the extraction socket (90, 91). In order to mimic the human situation, studies on periodontal regeneration and healing may have to be performed in herpesvirus-infected animals.

Data are available on means of controlling periodontal herpesviruses. Saygun et al. (210–212) and Pacheco et al. (172) reported that antimicrobial periodontal therapy can greatly reduce the herpesviral load in the periodontium, probably because the persistence of periodontal herpesviruses depends on the presence of gingival inflammatory cells. HCMV infects periodontal monocytes/macrophages and T-cells, and EBV infects B-cells (45), and since inflammatory cells have a lifespan of up to a few months (177), an extended periodontal presence of herpesviruses may require repeated influx of infected cells or, possibly, a herpesvirus-mediated inhibition of apoptosis (279). The ability of thorough antimicrobial therapy to markedly reduce or eliminate periodontal herpesviruses may in part be responsible for a positive therapeutic outcome. However, the extent to which eradicating periodontal herpesviruses may translate into healing beyond that obtained by controlling the periodontopathic bacteria needs to be established. Moreover, Saygun et al. (209) and Idesawa et al. (108) showed that periodontal treatment and oral hygiene follow-up reduced periodontal as well as salivary HCMV and EBV counts, sometimes to undetectable levels, which may help control herpesviral transmission from individual to individual and associated oral and nonoral diseases.

Herpesviruses are also involved in the pathogenesis of periapical symptomatic lesions (201–204, 231, 232). Symptomatic periapical lesions exhibit a significantly higher frequency of HCMV and EBV active infections than asymptomatic lesions of similar radiographic size (202, 232). Although HCMV appears to be the more important endodontopathogenic herpesvirus, HCMV and EBV may often serve as copathogens in severe cases of periapical disease (231). It has been suggested adding HCMV and probably EBV to the list of putative pathogenic agents in symptomatic periapical pathosis (231).

Pathogenesis of herpesvirus-associated periodontal disease

It seems clear that periodontal tissue breakdown occurs more frequently and progresses more rapidly in herpesvirus-infected than in herpesvirus-free periodontal sites. Herpesviruses may cause periodontal pathosis as a direct result of virus infection and replication, or as a consequence of virally induced impairment of the periodontal immune defense, resulting in heightened virulence of resident bacterial pathogens (43). It is assumed that the ability of herpesviruses to express cytopathogenic effects, immune evasion, immunopathogenicity, latency, reactivation from latency, and tissue tropism is of relevance for the development of periodontitis.

Herpesviruses may cause a direct cytopathic effect on fibroblasts, keratinocytes, endothelial cells, inflammatory cells, and possibly bone cells. Ongradi et al. (171) found that phagocytic and bactericidal capacities of periodontal neutrophils, cells of key importance in the periodontal defense (260), were significantly impaired in subjects who carried herpesviruses in oral lymphocytes and epithelial cells, as compared to virus-free persons. In addition, herpesvirus infection of fibroblasts and other key periodontal cells may hamper tissue turnover and repair following regenerative periodontal therapy (237). Also, herpesvirus infection and damage of periodontal pocket epithelium may contribute to gingival bleeding, as suggested by a high prevalence of HCMV

and EBV DNA in periodontal sites exhibiting bleeding upon probing (108, 114). However, herpesviruses can also occur with minimal gingival bleeding, as seen in localized aggressive periodontitis (255) and in some chronic periodontitis lesions (133).

Periodontal herpesvirus infections may lead to overgrowth of periodontopathic bacteria. In adult periodontitis, the presence of subgingival HCMV or EBV-1 DNA is related to an elevated occurrence of the periodontal pathogens *P. gingivalis*, *T. forsythia, D. pneumosintes*, *P. intermedia*, *P. nigrescens*, *C. rectus* and *T. denticola* (44, 210, 230, 234). Localized aggressive periodontitis lesions with active HCMV infection tend to yield elevated *A. actinomycetemcomitans* counts (255). Studies in Finland and Russia found positive associations between serum antibodies against HSV and serum antibodies against *P. gingivalis* and *A. actinomycetemcomitans* (263). Herpesviruses may perturb inflammatory cells involved in the periodontal defense, thereby predisposing to bacterial superinfection, or may affect the adhesion potential of periodontopathic bacteria, possibly in a species-specific manner. Teughels et al. (253) found that *A. actinomycetemcomitans* strains showed 70% (52–107%) higher and *P. gingivalis* strains 39% (36–42%) lower ability to adhere to and invade HCMV-infected HeLa epithelial cells compared to HeLa cells not infected by HCMV.

Proinflammatory cytokines play both beneficial and harmful roles in viral diseases. Herpesviruses can induce altered and, maybe, overzealous inflammatory mediator and cytokine responses in host cells attempting to counter the viral attack (156). HCMV infection can up-regulate IL-1β, TNF-α and other cytokine expression of monocytes and macrophages (156, 256, 269). Lipopolysaccharide from resident gram-negative bacteria can also induce cytokine responses in inflammatory cells and may act synergistically with HCMV in stimulating IL-1β gene transcription, resulting in markedly increased IL-1β levels at periodontal sites (269). Increased gingival concentration of proinflammatory cytokines has been associated with enhanced susceptibility to destructive periodontal disease (173). EBV may act as a potent polyclonal B-lymphocyte activator, capable of inducing proliferation and differentiation of immunoglobulin secreting cells, features associated with the progression of some types of periodontal disease (74).

Herpesviruses may produce tissue injury as a result of immunopathologic responses. HCMV can modulate antigen-specific T-lymphocyte functions, resulting in relative increases in $CD8^+$ suppressor cells, which in turn may lead to an impairment of cell-mediated immunity (215, 271). Consistent with immune responses of a herpesvirus infection, aggressive periodontitis has been related to low $CD4^+/CD8^+$ ratios (120, 142) and, within the $CD8^+$ lymphocytes, a shift towards cytolytic T (Tc) lymphocytes (184). The Tc effector cells, which execute their function by direct cytotoxicity or by releasing antiviral cytokines, comprise the first order response of the adaptive immune system in the recovery from primary viral infections. Depending on individual circumstances, the action of cytolytic effector functions can be beneficial, detrimental or neutral to host tissue.

Figure 4 proposes an infectious disease model for the development of periodontitis based on herpesvirus-bacteria–host interactive responses. Herpesvirus infection of periodontal sites may be important in a multistage pathogenesis by altering local host responses. Initially, bacterial infection of the gingiva causes inflammatory cells to enter gingival tissue, with periodontal macrophages and T-lymphocytes harboring latent HCMV and periodontal B-lymphocytes harboring latent EBV (45). IgA antibodies against HCMV, EBV, and HSV in gingival crevice fluid seem to originate mainly from local plasma cell synthesis rather than from passive transudation from serum, which is a further indication of a gingival herpesvirus presence (96–98). Reactivation of herpesviruses from

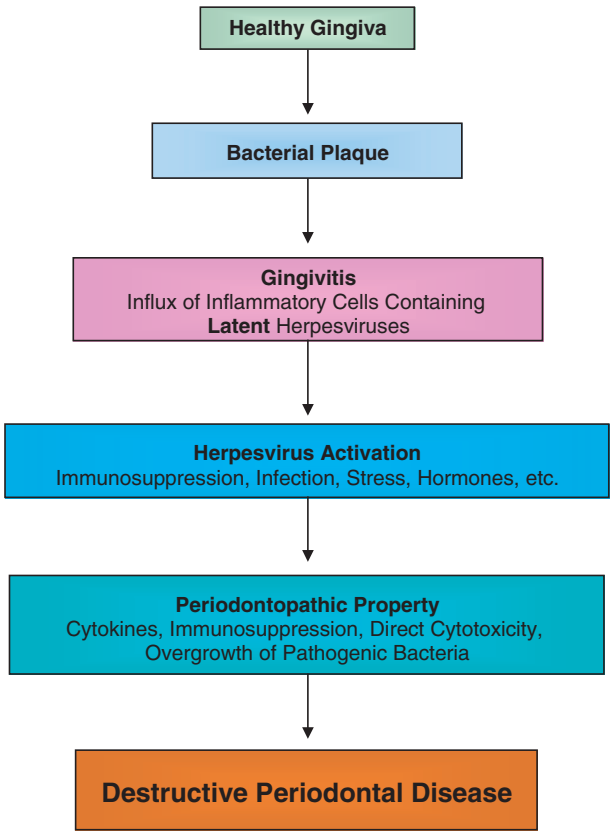

Fig. 4. Herpesviruses in destructive periodontal disease.

latency may occur spontaneously or during periods of impaired host defense, resulting from immunosuppression, infection, physical trauma, hormonal changes, etc. Herpesvirus-activating factors are also known risk factors/indicators for periodontal disease (168, 190). Herpesviral activation leads to increased inflammatory mediator responses in macrophages, and probably also in connective tissue cells within the periodontal lesion. After reaching a critical virus load, activated macrophages and lymphocytes may trigger a cytokine/chemokine 'storm' of IL-1β, TNF-α, IL-6, prostaglandins, interferons, and other multifunctional mediators, some of which have the potential to propagate bone resorption (118, 126). Several of the herpesvirus-associated cytokines and chemokines are prominent in periodontal lesions (80). Herpesvirus-induced immune impairment may also cause an upgrowth of resident gram-negative anaerobic bacteria (203, 224), whose lipopolysaccharide together with HCMV, as discussed above, can induce cytokine and chemokine release from various mammalian cells, and may act synergistically in stimulating IL-1β gene transcription (269). In a vicious circle, the triggering of cytokine responses may activate latent herpesviruses, and in so doing may further aggravate periodontal disease. Similarly, medical infections by HCMV can lead to increased susceptibility to bacterial and fungal infections and enhance the severity of existing microbial infections (24). It is conceivable that herpesviruses rely on coinfection with periodontal bacteria to produce periodontitis and, conversely, periodontopathic bacteria may depend on viral presence for the initiation and progression of some types of periodontitis.

A periodontal herpesvirus infection may partly explain the more rapidly advancing type of periodontitis that is detected in young than in adult individuals. Typically, the periodontal disease course in adolescents and young adults is aggressive with a relatively short period of tissue destruction (12). In adults, the disease course is more often slow and frequently associated with significant gingival inflammation and accumulations of plaque and calculus (12). These observations may suggest that aggressive periodontitis in young patients requires less infectious agent stimulus to trigger a progressive disease response than the more chronic type of adult periodontitis. However, progressive periodontitis may appear in HIV-infected adults (162, 244) and in aging individuals (134), probably because of suppressed cellular immunity by virtue of illness, therapies, or simply old age (58, 145).

It is common for primary and recurrent episodes of herpesvirus clinical infections to exhibit considerably different signs and symptoms. Pathosis occurring at primary infection tends to be severe in immunologically immature young people and in immunocompromised individuals, and mild-to-moderate in adults with preexisting herpesvirus immunity from past infection. For example, the varicella-zoster virus causes chickenpox during primary infection and shingles during an endogenous relapse of the primary varicella infection. Herpes simplex virus may cause acute gingivostomatitis during primary infection and epidermal or mucosal ulcers during viral recrudescence, and EBV and HCMV can give rise to mononucleosis during primary infection and a variety of relatively mild diseases during viral recrudescence (21). Similar to acute herpesvirus diseases, aggressive periodontitis may preferentially occur in immunologically immature young people, or in immunocompromised or aging individuals who are unable to mount an adequate host response against established herpesviruses and therefore will experience frequent or long-lasting herpesvirus reactivation (58). The subsequent latency period then represents the time required for the herpesviruses to overcome antiviral responses of the periodontium. In agreement with this hypothesis, virtually all established risk factors/indicators of periodontitis are immunosuppressive with potential to activate latent herpesviruses (168). If so, some types of aggressive and chronic periodontitis are basically not different diseases but merely a continuous spectrum of diseases, whose clinical expression depends on the presence of a periodontal herpesvirus infection and the specific immunity of the host, ranging from aggressive periodontitis in patients with inadequate immune response at one end, to chronic, nonprogressing periodontitis in patients who are immunocompetent at the other end, with intermediary clinical disease types between these two extremes of immune function.

Herpesvirus infections can cause both cytopathogenic and immunopathogenetic effects (227), and although the relative contribution of the two pathogenic mechanisms to destructive periodontal disease is unknown, it is likely that the early stages of periodontitis in immunologically naive hosts mainly comprise cytopathogenic events, whereas most clinical manifestations in immunocompetent individuals are secondary to cellular or humoral immune responses. Clustering of aggressive periodontitis in families (11) may arise from a transmission of herpesviruses among individuals in the same household rather than from a genetic predisposition, although the disease development may involve both pathogenetic components.

Periodontitis =

High herpesvirus load (inflammatory level) at periodontal sites +
Activation of herpesviruses in the periodontium +
Inadequate anti-viral T-cytotoxic cell response +
Presence of specific periodontal pathogenic bacteria +
Inadequate protective anti-bacterial antibody response +
A time period sufficiently long to produce tissue breakdown

Fig. 5. Pathogenic determinants of severe periodontitis.

One of the greatest challenges in confirming or refuting a role for herpesviruses in human periodontitis is their ubiquitous nature and the relatively rare occurrence of progressive periodontitis. This dilemma is apparent not only for periodontitis but also for the expanding spectrum of human diseases with which herpesviruses have been associated (224). It is likely that periodontal breakdown has a polymicrobial causation and depends upon the simultaneous occurrence of a number of infectious disease events, including at least (i) herpesvirus presence at periodontal sites, (ii) reactivation of latent periodontal herpesviruses, (iii) inadequate antiviral cytotoxic T-lymphocyte response, (iv) presence of specific pathogenic bacteria, and (v) insufficient level of protective antibacterial antibodies (Fig. 5). Reactivation of herpesviruses in periodontal sites may comprise a particularly important pathogenetic event (227). Presumably, the pathogenic determinants of periodontitis cooperate with each other in destructive constellations relatively infrequently and primarily during periods of impaired host defense. Also, the periodontal pathogenic determinants have to interact for a period of time that is sufficiently long to produce clinical breakdown.

Conclusion and perspectives

Even though bacteria are recognized to be indispensable for the development of periodontitis, and although current hypotheses on the etiopathogenesis of periodontitis correctly emphasize the importance of assessing bacterial and host factors collectively, bacterial–host interaction alone seems insufficient in explaining important clinical characteristics of the disease. It is not understood why periodontitis tends to progress in a localized pattern in many patients, the propensity to bilateral symmetry of tissue breakdown, and the intermittent exacerbation of the disease in individual teeth. It is particularly troubling that no detailed explanation exists as to the pathogenic events that trigger the conversion of a gingivitis lesion to periodontitis or a stable periodontitis site to a disease-active lesion. No unequivocal association has been established with cytokine polymorphisms or HLA haplotypes and periodontitis, although HLA-DR4 carriers may be at elevated risk for the disease (165). Variation in clinical manifestations of periodontal disease is almost certainly the result of differences in type and load of infectious agents and associated host responses. In that regard, the simultaneous occurrence of periodontal herpesvirus infection and progressive periodontitis is probably not a fortuitous event. Herpesvirus periodontal infections may cause direct damage to periodontal tissues, or impair the resistance of the periodontium, thereby permitting subgingival overgrowth of pathogenic bacteria (227).

Henle-Koch postulates of disease etiology address monocausal infectious diseases and are not readily applicable to multicausal infectious diseases such as periodontitis, which may result from a synergistic interaction among different pathogenic agents that individually may not lead to disease. The question of coincidence or a causal nexus between herpesviruses and periodontitis can be appraised on the basis of Hill's criteria of causality (94). The measures for strength of association, consistency, temporal sequence, biologic plausibility, and analogy seem to be met (94). Amongst the many arguments for a herpesvirus involvement in human periodontal disease are the following observations:

- PCR amplification of nucleic acid sequences of HCMV, EBV and other herpesviruses in severe periodontitis lesions of adolescents and adults has been robustly reported by independent laboratories in various countries.
- Herpesvirus-positive periodontitis lesions harbor increased levels of periodontopathic bacteria.
- There exists an apparent association between HCMV active infection and progressing periodontitis.
- An association between herpesviruses and acute necrotizing gingivitis has been demonstrated in malnourished children in Nigeria.
- Periodontal inflammatory cells contain nucleic acid sequences of herpesviruses.
- Herpesvirus infection of periodontal inflammatory cells has the potential to profoundly alter the host defense.
- Herpesviruses have the potential to increase the expression of tissue-damaging cytokines and chemokines in periodontal inflammatory and connective tissue cells.

Table 9 summarizes pathomorphologic characteristics of periodontitis that may be explained by a combined herpesvirus–bacteria etiological model, but

Table 9. The likelihood of herpesviruses and bacteria explaining the disease characteristics of periodontitis

Periodontitis features	Herpesviruses + Bacteria	Bacteria alone
Dental plaque amount and level of dental care not commensurate with disease severity. (36, 46)	Yes (herpesvirus active infection in the periodontium is not related to dental plaque amount). (255)	Yes (increased occurrence of specific species of bacterial pathogens in certain plaques). (226)
Localized and bilateral symmetry of tissue breakdown. (157)	Yes (herpesvirus infection exhibits tissue tropism, and tissue around similar teeth may show similar propensity to attract herpesviruses).	No.
Intermittent exacerbation of disease. (78, 239)	Yes (alterations between periods of herpesvirus latency and reactivation [240], which may correspond to disease stability and progression, respectively).	Maybe (temporary increase of periodontopathic bacteria due to nonherpesviral effects).
Cemental hypoplasias in teeth with aggressive juvenile periodontitis. (23)	Yes (active HCMV infection at the time of root development, which may cause alterations in the tooth surface). (224)	No.
Familial predisposition to disease. (17)	Yes (transmission of herpesviruses within a family). (4)	Yes (transmission of pathogenic bacteria within a family). (13)
Increased disease prevalence in lower socioeconomic groups. (56, 60)	Yes (higher rates of herpesvirus infection in individuals in lower socioeconomic groups). (32)	Maybe (individuals in lower socioeconomic groups may harbor increased levels of periodontopathic bacteria). (221)
Increased alveolar bone loss in institutionalized compared to noninstitutionalized mentally retarded individuals. (70)	Yes (high rate of herpesvirus transmission in institutionalized individuals). (216)	Unlikely (poor oral hygiene in institutionalized individuals). (129)
Occlusal trauma as a risk indicator of disease. (84)	Yes (trauma may induce herpesvirus reactivation).	Unlikely (slightly increased occurrence of periodontopathic bacteria with increased mobility). (79)
Immunodeficiency predisposes to increased incidence/prevalence of disease. (181)	Yes (immunosuppression is an important event in herpesvirus reactivation). (240)	Unlikely (some pathogenic bacteria possess immunosuppressive properties). (217, 218)
Old age as a risk indicator of disease. (134, 244)	Yes (reduced immune capacity [8] and increased herpesvirus occurrence with increasing age). (4)	Maybe (increased acquisition of pathogenic bacteria over time). (228)
HIV-infection as a risk indicator of disease. (244)	Yes (most HIV-infected patients harbor several periodontal herpesviruses that have the potential to reactivate frequently due to the immunosuppression). (41)	Unlikely (HIV and non-HIV patients harbor similar periodontopathic microbiota). (187)
Psychosocial stress as a risk indicator of disease. (131, 244)	Yes (stress can induce herpesvirus reactivation). (119, 246)	Maybe (host-derived nutrients in gingival crevice fluid of stressed individuals may stimulate[or inhibit] the growth of selected bacterial species). (135, 197)
Hormonal influences on periodontal disease. (146)	Yes (hormones and progesterone may increase the susceptibility to herpesvirus infections). (117)	Maybe (sex hormones may serve as growth factors for some periodontopathic bacteria). (122)

Table 9. Continued

Periodontitis features	Herpesviruses + Bacteria	Bacteria alone
Cigarette smoking as a risk indicator of disease. (106, 196)	Yes (tobacco products can interact with and possibly reactivate periodontal herpesviruses [175] or act synergistically with HCMV to enhance the sensitivity of peripheral blood lymphocytes to genetic damage). (5)	Unlikely (some anaerobic periodontal bacteria may occur at increased levels in smokers). (83)
Disease progression in the presence of elevated antibacterial antibodies. (57)	Yes (herpesvirus active infection is not controlled by antibacterial antibodies).	Possibly (if antibodies are directed against noncritical antigens, or against nonaccessible bacteria in biofilms, or are part of immunopathologic mechanisms of tissue destruction).
Predominance of T-lymphocytes in relatively stable and B-lymphocytes in progressive periodontitis lesions. (75)	Yes (HCMV and HSV reside in T-lymphocytes and EBV resides in B-lymphocytes). (45)	Unlikely, if not an immunopathologic mechanism of tissue breakdown is postulated. (276)
Defective neutrophil functions associated with aggressive disease. (47)	Yes (herpesviruses may infect and perturb neutrophils). (1, 76, 171)	Unlikely (some bacterial species may perturb neutrophils). (259)
Occurrence of $CD8^+$ and Th1-type lymphocytes in periodontitis. (251)	Yes (herpesvirus active infection leads to increased level of cytotoxic $CD8^+$ cells). (3, 74)	Unlikely (a few bacterial species may stimulate T-suppressor cells). (218)
Possible relationship between periodontal disease and major medical disorders (coronary heart disease, cerebrovascular disease, low birth weight infants). (272)	Yes (herpesviruses may induce both periodontitis and medical disorders; if so, periodontitis and medical disorders may not exhibit a direct causal relationship). (223)	Still to be resolved. (229)

probably not by a model based solely on a bacterial causation of the disease. Prolonged periods of latency interspersed with periods of activation of herpesvirus infections may in part be responsible for the burst-like episodes of periodontitis disease progression. Tissue tropism of herpesvirus infections may help explain the localized pattern of tissue destruction in most types of periodontitis. Frequent reactivation of periodontal herpesviruses may account for the rapid periodontal breakdown in some patients showing little dental plaque. The absence of a herpesvirus infection or of viral reactivation may explain why some individuals carry periodontopathic bacteria while still maintaining periodontal health.

The apparent importance of herpesviruses in periodontal disease may have practical consequences in addition to theoretical interest. As discussed above, effective treatment of gingival inflammation can reduce gingival (172, 212) and salivary (108, 209) herpesvirus loads, and may help diminish the risk of transmitting herpesviruses to other individuals. On the other hand, antiviral chemotherapeutics have a limited, short-term effect on oropharyngeal herpesvirus shedding and are probably ineffective in treating periodontitis. Vaccination is on the horizon as a means of preventing colonization or reactivation of human herpesviruses (10, 180). The impact of antiherpesvirus vaccines on destructive periodontal disease constitutes a future research topic of great interest. As new antiherpesvirus interventions become available, dental professionals may be able to significantly enhance the outcome of periodontal prevention and therapy. Also, although of limited usefulness in the routine diagnosis of uncomplicated periodontal disease, tests to monitor the state and level of viral replication may serve a valuable diagnostic purpose in severe periodontal infections in immunocompromised patients.

In summary, destructive periodontal disease is a heterogeneous group of pathoses characterized by a predominance of specific infectious agents in the face of inadequate local host defenses. Predisposing factors of periodontal tissue destruction are becoming better understood, but the magnitude of the effects of the most commonly reported risk factors

has not been adequately quantified in population-based studies. Resolving the many questions about the etiopathogenesis of periodontal diseases may require a readiness to give up bacteria as a single-cause of periodontitis development. The frequent occurrence of herpesviruses in various types of severe periodontal disease makes the participation of herpesvirus species in the etiology of periodontitis a distinct possibility. It is theorized that herpesvirus-associated periodontitis has its most severe course during the time of inadequate antiherpesvirus immunity at the initial disease phase, and then tapers off after the establishment of effective herpesvirus-specific cellular immune responses. The sooner the host develops adequate immunity against periodontal herpesviruses, the more localized the periodontal destruction may become. Periodontal disease relapses may preferentially occur in individuals with diminishing antiherpesvirus immunity. Synergistic interactions between periodontal herpesviruses and bacteria may enhance the risk of tissue breakdown. Mammalian viruses other than herpesviruses may also be involved in destructive periodontal disease. Recognizing a pathophysiologic relationship between mammalian viruses and periodontal disease has the potential to extend our insight into mechanisms of periodontal tissue breakdown and bridge the knowledge gap, on the molecular level, between gingivitis and periodontitis and between stable and progressive periodontitis. As we move into an era of thinking of a network of causation in periodontitis, the need is growing for well-designed studies to delineate the relative importance of the various types of infectious agents, the multiple and complex pathogenic pathways, and the genetic and environmental factors contributing to the disease. Based on current information, it seems reasonable to add human periodontitis to the list of infectious diseases that have HCMV, EBV, and maybe other viruses as probable contributory causes.

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