Oral Microbiology Immunology 2004: 19: 217–223
Printed in Denmark. All rights reserved

Oral Microbiology and Immunology

Mini-review

Update on human cytomegalovirus in destructive periodontal disease

J. Slots
University of Southern California, School of Dentistry, Los Angeles, CA, USA

Slots J. Update on human cytomegalovirus in destructive periodontal disease. Oral Microbiol Immunol 2004: 19: 217–223. © Blackwell Munksgaard, 2004.

Aim: Human cytomegalovirus (HCMV), a herpesvirus, is discussed in this review as it relates to destructive periodontal disease in humans.
Results: HCMV genomic sequences, detected by polymerase chain reaction identification, occur with elevated frequency in severe adult periodontitis, localized and generalized aggressive (juvenile) periodontitis, Papillon–Lefèvre syndrome periodontitis, acute necrotizing ulcerative gingivitis, and periodontal abscesses.
Discussion: Herpesviruses establish lifelong persistent infections. HCMV infection involves an asymptomatic latent phase interrupted by periods of recrudescence where viral replication and possibly clinical disease become manifest. HCMV reactivation is triggered by a number of immunosuppressive factors, some of which have been shown also to be risk factors/indicators of periodontitis. HCMV periodontal infection may cause release of tissue-destructive cytokines, overgrowth of pathogenic periodontal bacteria, and initiation of cytotoxic or immunopathologic events.
Conclusions: A growing body of data supports the concept that HCMV contributes to severe types of periodontal disease. HCMV infection of the periodontium may alter the immune control of resident microorganisms and be important in a multistage pathogenesis of periodontitis involving viral activation, periodontopathic bacteria, and host immune responses. Understanding the significance of HCMV and other herpesviruses in the development of periodontal disease may have important therapeutic implications. Vaccines against HCMV, which are in various stages of development, need to be evaluated for their ability to decrease the incidence of destructive periodontal disease.

Key words: herpesvirus; human cytomegalovirus; pathogenesis; pathogenic bacteria; periodontal disease

Jørgen Slots, DDS, DMD, PhD, MS, MBA, University of Southern California, School of Dentistry – MC 0641, Los Angeles, CA 90089–0641, USA
Tel.: +1 213 740 1091 (voice);
fax: +1 213 740 2194
e-mail: jslots@usc.edu
Accepted for publication February 14, 2004

Important advances in understanding the infectious agents of periodontal disease have occurred in the past three decades (85, 89). Although before the 1970s bacterial plaque was considered the key etiologic factor of periodontal disease, no studies had shown a clear relationship between specific bacterial species and destructive periodontal disease (88). Following the boom in anaerobic microbiology in the 1970s, it was demonstrated that markedly different microfloras were associated with a healthy periodontium and periodontitis (73). *Actinobacillus actinomycetemcomitans* became implicated in the etiology of localized aggressive (juvenile) periodontitis and *Porphyromonas gingivalis* in the etiology of severe adult periodontitis (74). Since then, major inroads have been made into the microbiology, immunology and cause-related treatment of periodontal disease. In the past 9 years, various herpesviruses, including human cytomegalovirus (HCMV) and Epstein–Barr virus (EBV), have emerged as putative pathogens in destructive periodontal disease (76). It is now clear that periodontitis represents several disease entities, which exhibit a variety of clinical presentations, polymicrobial infections, and pathophysiologic mechanisms (29, 75, 79).

Reviews have recently appeared on the continually evolving field of herpesviral infections of the periodontium (35, 71, 76). It appears that HCMV contributes significantly to the etiopathogenesis of severe types of periodontal disease. HCMV DNA sequences occur frequently in adult periodontitis, localized and generalized aggressive periodontitis, Papillon–Lefèvre syndrome periodontitis, Down's syndrome periodontitis, HIV-associated periodontitis, acute necrotizing ulcerative gingivitis, and periodontal abscesses (68, 69, 76, 99). Active HCMV infection is also closely associated with symptomatic periapical pathosis (63, 65, 84, 86). The present

article reviews data on herpesviral infections of the human periodontium and proposes a model for severe periodontitis that is based upon a combined herpesviral–bacterial causation of the disease. Emphasis is placed on the role of HCMV in the development of human periodontitis.

HCMV biology and pathogenic potential

Membership in the family Herpesviridae is based on the structure of the virion. Herpesviruses contain a double-stranded DNA genome encased within an isosahedral capsid and an amorphous proteinaceous tegument. Surrounding the capsid and tegument is a lipid bilayer envelope derived from host cell membranes. The complete HCMV particle has a diameter of about 200 nm and has one of the largest genomes (230 kb) of any virus known to infect man. Herpesviral replication takes place in the nucleus of the host cell. Expression of the HCMV genome is controlled by a cascade of transcriptional events that involves the synthesis of three groups of viral proteins termed immediate-early, early, and late. During their life cycle, herpesviruses execute an intricate chain of events geared towards optimizing their replication.

Herpesviruses infect members of all groups of vertebrates, as well as some invertebrates. Of the more than 100 identified herpesviruses, only eight are known to infect humans, namely, herpes simplex virus (HSV) type 1 and 2, varicella-zoster virus, HCMV, EBV, human herpesvirus (HHV)-6, HHV-7, and HHV-8 (Kaposi's sarcoma virus). Acquisition of HCMV may occur in the uterus or at an early age by intimate contact with infected secretions. HCMV has been detected in saliva, gingival crevice fluid, urine, maternal milk, tears, stool, vaginal and cervical secretions, and semen. HCMV virions can be excreted in saliva of children for prolonged periods of time (45). Children frequently acquire HCMV from the mother (46) or from siblings (7), and they can infect the mother by HCMV acquired from other children (56). In addition, HCMV can be transmitted by blood transfusion and solid organ transplantation (91). In developed countries the HCMV overall seroprevalence is approximately 60% (20). Specific communities such as those comprising gay men, lower socioeconomic groups, and residents of developing countries have seroprevalence rates that can exceed 90% (22). A particularly high prevalence of HCMV infections in developing countries may partly explain a high occurrence of periodontitis in such populations (48), a notion which cautions against directly extrapolating epidemiologic findings on the 'natural history' of periodontitis in developing countries to populations in developed countries.

HCMV infection virtually always occurs without clinical symptoms in immunocompetent hosts, whereas the virus is an important medical pathogen in immunologically immature or immunocompromised populations. Clinical manifestations can be seen following primary infection, reinfection, or reactivation. HCMV infection is of great clinical significance in pregnant women, newborn infants with congenital infection, immunosuppressed transplant patients, and individuals with HIV-infection (28). In fact, HCMV represents the major infectious cause of birth defects, and is the most common life-threatening infection in transplant and HIV-infected patients (28). HCMV is the most important infectious pathogen in recipients of bone marrow or stem cell grafts (13). HCMV infection seems to be a significant risk factor for development of bacterial septic infections in liver transplant patients (47, 57) and causing colonization of the oropharynx by gram-negative bacilli in renal transplant patients (38). HCMV has also 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 (10, 87). Furthermore, HCMV has been associated with cervical carcinoma and adenocarcinomas of the prostate and colon (21). However, it should be cautioned that evidence of the presence of HCMV DNA in various disease entities does not prove causality by itself.

HCMV and other herpesviruses have been thought for almost 20 years to play a role in the development of three arterial diseases: primary atherosclerosis, post-angioplasty restenosis, and post-transplantation arteriosclerosis (50). HCMV DNA sequences have been detected in atheromatous plaques (30) and in the wall of atherosclerotic vessels (32, 70). Using polymerase chain reaction (PCR)-based identification, Shi & Tokunaga (70) found HCMV in 40%, EBV in 80%, and HSV-1 in 80% of atherosclerotic aortic tissue compared to 4%, 13%, and 13% of non-atherosclerotic control specimens, respectively. Also, cardiac transplant patients infected with HCMV 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 (24). Murine CMV has the potential to produce atherosclerosis in experimental mice (31). Although animal experiments on heart disease do not replicate exactly the human disease, they may provide valuable suggestions on causality. The possibility that HCMV gives rise to cardiovascular disease and periodontitis in an independent manner further complicates studies on the relationship between the two diseases, and raises a question about the notion of periodontitis being a direct risk factor of heart disease (82).

HCMV infections induce a strong antiviral immune response that nonetheless is incapable of eradicating the infection. The humoral immune response attempts to produce antibodies against proteinaceous surface molecules, thereby causing inactivation or clearance of the virus. The cellular immune response constitutes the specific host defense against an established HCMV infection. It attempts to eliminate virus-infected cells by means of cytotoxic $CD8^+$ T-lymphocytes that recognize viral peptides on the surface of infected cells in the context of major histocompatibility complex (MHC) class I molecules. Studies are needed to determine the periodontal level of HCMV-specific cytotoxic $CD8^+$ T-lymphocytes, cells that play a pivotal role in the control of HCMV infections.

An important characteristic of HCMV is the ability of the virus to establish a lifelong infection within the host following the initial infection. The persistence of HCMV for life is the reason the virus is endemic worldwide. However, the coexistence of HCMV with its host should be viewed as a precarious balance that can sometimes lead to reactivation of the virus. Reactivation may occur spontaneously or as a result of concurrent infection, fever, drugs, tissue trauma, emotional stress, or other factors impairing the host immune defense. Stressful events induce the release of corticosteroids, which have the potential to activate HCMV (66). Tobacco products may interact with and reactivate periodontal herpesviruses (53) and may decrease herpesvirus infectivity titers (6), which may partly explain the increased risk for periodontitis from tobacco usage (62). The ability of HCMV and other herpesviruses to cause latent infections that periodically reactivate has some similarity with the relapsing-remitting course of periodontitis.

HCMV and other herpesviruses exhibit marked tropism for cells of the immune system and, consequently, infection by herpesviruses may result in alterations of immune functions, leading at times to a state of immunosuppression. Monocytes/macrophages and endothelial cells seem to be major sites of HCMV persistence and latency. The molecular basis for herpesvirus tropism remains obscure. The display of tissue tropism of HCMV infections is in accordance with the segmental pattern of breakdown and the mirror-like bone destruction in contralateral teeth of many periodontitis patients.

During productive infection, HCMV DNA can be detected in neutrophilic leukocytes, probably as a result of phagocytosis, although active viral replication may also take place (41). HCMV infection may induce abnormalities in adherence, chemotactic, phagocytic, oxidative, secretory, and bactericidal activities of polymorphonuclear neutrophils (1). Neutrophils are key cells in controlling periodontal infections (96). Phagocytic and bactericidal capacities of periodontal neutrophils seem to be significantly impaired in subjects carrying herpesviruses in oral lymphocytes and epithelial cells, as compared to virus-free persons (51). The functional defects of neutrophils identified in localized aggressive periodontitis patients (95) may in part be due to a concurrent HCMV active infection in these patients (93).

HCMV interferes with innate and adaptive cellular and humoral immune effector mechanisms by activation and silencing of natural killer (NK)-cells, by downmodulating antigen presentation in the MHC class I and II pathways, and by impairing apoptosis (40). Herpesvirus infections also affect cytokine networks (43). Cytokines and chemokines play important roles in the first line of defense against human herpesvirus infections and also contribute significantly to regulation of acquired immune responses. However, by a diverse array of strategies, herpesviruses are able to divert potent antiviral cytokine responses or even interfere with cytokine production, which allow the viruses to be successful and survive (3, 94). The cytokine profile of an HCMV infection is typically pro-inflammatory, with production of interleukin-1β, interleukin-6, interleukin-12, tumor necrosis factor-α, interferon-α/β, and interferon-γ (43). Moreover, prostaglandin E_2 increases rapidly in response to exposure of cells to HCMV (42). Pro-inflammatory activities normally fulfill a positive biological role by aiming to overcome infection or invasion by infectious agents, but can also exert detrimental effects when a challenge becomes overwhelming or with a chronic pathophysiologic stimulus. Several of the HCMV-associated cytokines and chemokines occur in elevated levels in periodontitis sites and have the ability to induce bone resorption (27, 52). Comparison of levels of cytokines and chemokines in HCMV-infected and non-infected periodontal sites remains an important research topic.

PCR-based detection has dramatically changed diagnostic virology by providing a sensitive and specific tool to identify and quantify viral genomes in medical specimens (14). Parra & Slots (54) demonstrated that PCR diagnostics is highly useful in establishing HCMV presence in periodontal sites. Recent studies have shown that real-time PCR technique can be employed to quantify HCMV in periodontitis lesions (36).

HCMV in severe periodontal disease

The occurrence of HCMV and selected periodontal pathogenic bacteria has been studied in disease-active and disease-inactive aggressive periodontitis lesions (Table 1). HCMV, HCMV-EBV coinfection, *Dialister pneumosintes*, *P. gingivalis* and *D. pneumosintes–P. gingivalis* coinfection showed significant associations with disease-active periodontitis (Table 1). Each periodontitis site that demonstrated HCMV-EBV coinfection and all but one site showing *D. pneumosintes–P. gingivalis* coinfection revealed bleeding upon probing, a clinical sign of increased risk of progressive disease (37). In aggressive periodontitis lesions, subgingival specimens contain approximately 4000 HCMV copies/ml (36), but gingival tissue specimens may show 100 folds higher HCMV copy numbers (Saygun, personal communication). Moreover, HCMV, and no other study virus, was positively associated with the presence of *D. pneumosintes*, and the relationship was specific for individual periodontitis sites with no detectable subject effect (87). *D. pneumosintes* was in turn positively associated with periodontal pocket depth and disease-active periodontitis. When treating average percent alveolar bone loss of all teeth as a response, HCMV remained significant even after including *D. pneumosintes* in the statistical model, suggesting that both HCMV and *D. pneumosintes* affected bone loss or, alternatively, HCMV affected factors not studied that by themselves can induce bone loss. Periodontal HCMV was also a significant predictor of the presence of subgingival *P. gingivalis* (83). In turn, *P. gingivalis* was positively associated with periodontitis active disease, loss of probing attachment, probing pocket depth, gingival bleeding upon probing and patient age. The periodontitis disease risk associated with HCMV-*P. gingivalis* combination depended on both site-specific and subject-specific factors. Periodontal HCMV has also been related to the subgingival occurrence of other suspected periodontal pathogens, including *A. actinomycetemcomitans*, *Prevotella intermedia*, *Prevotella nigrescens*, *Tannerella forsythia*, *Treponema denticola* and *Campylobacter rectus* (18, 67, 93). In addition, HCMV active infection in periapical pathosis seems to be associated with elevated levels of anaerobic bacterial species (64). The available data are consistent with the notion that periodontal HCMV sets the stage for vigorous proliferation of periodontopathic bacteria and subsequent periodontal tissue breakdown.

Michalowicz et al. (39) determined the occurrence of HCMV, *P. gingivalis* and *A. actinomycetemcomitans* in subgingival plaque from 15 adolescents with localized aggressive periodontitis, 20 adolescents with incidental periodontal attachment loss, and 65 randomly selected healthy controls. All study subjects were Afro-Caribbeans living in Jamaica. The most parsimonious multivariate model for localized aggressive periodontitis included

Table 1. Human cytomegalovirus in periodontitis sites of 16 aggressive periodontitis patients. Adapted from Kamma et al. (34)

Items	32 disease-active periodontitis sites	32 disease-stable periodontitis sites	*P*-values (Chi-squared test)
Cytomegalovirus	19 (59%)*	4 (13%)*	<0.001
Cytomegalovirus and Epstein–Barr virus coinfection	9 (29%)	0 (0%)	0.004
D. pneumosintes	20 (63%)	6 (19%)	<0.001
P. gingivalis	23 (72%)	12 (38%)	0.01
D. pneumosintes and *P. gingivalis* coinfection	15 (47%)	0 (0%)	<0.001

*No. (%) positive sites.

Table 2. Human cytomegalovirus in deep and shallow periodontal sites of 11 localized aggressive (juvenile) periodontitis patients. Adapted from Ting et al. (93)

Items	5 active periodontitis lesions	11 shallow periodontal sites
Cytomegalovirus, No. (%) positive sites	5 (100%)*	2 (18%)*
Cytomegalovirus active infection, No. (%) positive sites	5 (100%)	0 (0%)
Epstein–Barr virus, No. (%) positive sites	3 (60%)	2 (18%)
Cytomegalovirus and Epstein–Barr virus coinfection, No. (%) positive sites	3 (60%)	2 (18%)
Presence of *A. actinomycetemcomitans*, No. (%) positive sites	5 (100%)	No data

*No. (%) positive sites.

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 probability of having localized aggressive periodontitis increased multiplicatively when both HCMV and *P. gingivalis* were present (Odds Ratio = 51.4; 95% confidence limits: 5.7, 486.5), when compared to the odds associated with having neither of the two infectious agents. Apparently, HCMV and *P. gingivalis* are independently and strongly associated with localized aggressive periodontitis in Jamaican adolescents, and HCMV and *P. gingivalis* act synergistically to influence the risk for both the occurrence and the extent of disease. Electron microscopy studies have previously detected virions in lesions of localized aggressive periodontitis (11, 12, 58).

Ting et al. (93) studied the occurrence of HCMV activation in disease-active periodontal sites in 11 localized aggressive periodontitis patients aged 10–23 years (Table 2). HCMV mRNA of the major capsid protein, indicative of viral activation, 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 pockets tested. HCMV activation was found exclusively in angular periodontal sites having no visible radiographic crestal alveolar lamina dura, a sign of likely periodontal disease progression (59). HCMV activation has also been detected in severe periodontitis lesions of adults (17). Furthermore, periodontal sites with active HCMV infection were more heavily infected with *A. actinomycetemcomitans* than sites with latent HCMV infection, maybe due in part to an HCMV-mediated increase in the adherence potential of the organism to epithelial cells (92). It can be hypothesized that during root formation of permanent incisors and first molars at 3–5 years of age, HCMV-active infection in tissues surrounding the tooth germ might alter the root surface structure and increase the susceptibility to future periodontal breakdown. HCMV infections of infants have the potential to cause changes in tooth morphology (26, 90) and teeth affected by localized aggressive periodontitis frequently show cemental hypoplasia (9). At the time of puberty, reactivation of periodontal HCMV or other herpesviruses due to hormonal changes may then give rise to overgrowth of periodontopathic bacteria and breakdown of tissue around teeth with a damaged periodontium.

HCMV has been detected in rare types of aggressive periodontitis in young individuals (Table 3). In a Hopi-Indian population, a single adolescent showed generalized aggressive periodontitis and was the only study subject revealing periodontal HCMV–EBV coinfection (72). One periodontitis patient with Papillon–Lefèvre syndrome also presented periodontal HCMV–EBV coinfection (97). One patient with Fanconi's anemia periodontitis demonstrated periodontal HCMV-active infection (49). The Papillon–Lefèvre syndrome patient and the Fanconi's anemia patient also harbored *A. actinomycetemcomitans* in deep periodontal lesions.

Acute necrotizing ulcerative gingivitis (ANUG) affects immunosuppressed, malnourished, and psychosocially stressed young individuals and may occasionally spread considerably beyond the periodontium and give rise to the life-threatening infection termed noma/cancrum oris (44). The distribution of herpesviruses has been studied in ANUG-affected and non-ANUG-affected children 3–14 years of age from Nigeria (16). A significantly higher occurrence of HCMV and other herpesviruses were detected in ANUG lesions of malnourished children than in non-ANUG, normal, or malnourished children (Table 4). In Europe and the USA, ANUG affects mainly young adults and HIV-infected individuals, and virtually

Table 3. Human cytomegalovirus in aggressive periodontitis of young individuals

Periodontal disease	Cytomegalovirus and Epstein–Barr virus coinfection	Cytomegalovirus active infection	*A. actinomycetemcomitans*
Generalized aggressive (juvenile) periodontitis; teenage patient (72)*	Yes	Not done	Yes
Papillon–Lefèvre syndrome periodontitis; 11-year-old patient (97)	Yes	Not done	Yes
Fanconi's anemia periodontitis; 11-year-old patient. (49)	No	Yes	Yes

*Eight of 75 adolescent Hopi-Indians showed various types of aggressive periodontal disease. The only Hopi-Indian with generalized periodontitis was also the only individual who revealed cytomegalovirus and Epstein–Barr virus periodontal coinfection.

Table 4. Human cytomegalovirus in acute necrotizing ulcerative gingivitis (ANUG) and normal periodontal sites of Nigerian children with malnutrition. Adapted from Contreras et al. (16)

Herpesviruses	ANUG + malnutrition (22 subjects)	Normal oral health + malnutrition (20 subjects)	*P*-values (chi-squared test)
Cytomegalovirus, No. (%) positive sites	13 (59%)	0 (0%)	<0.001
Epstein–Barr virus, No. (%) positive sites	6 (27%)	1 (5%)	0.13
Cytomegalovirus and Epstein–Barr virus coinfection, No. (%) positive sites	8 (36%)	0 (0%)	0.009

never young children. The occurrence of ANUG in children in Africa may be due to an acquisition of HCMV in early childhood (55), malnutrition that may promote herpesvirus reactivation (23), and the periodontal presence of virulent bacteria (25). A recent electronmicroscopic study detected large numbers of herpesvirus-like virions in ANUG lesions of HIV-infected patients in the USA (15).

Conclusion and perspectives

There are several lines of evidence that implicate HCMV as either a cofactor or a trigger of relapses in the etiopathogenesis of severe periodontal disease:

- high prevalence of HCMV nucleic acid sequences in periodontitis lesions of juveniles and adults;
- association between active HCMV infection and disease-active periodontitis;
- association between HCMV and ANUG in malnourished African children;
- increased frequency of periodontopathic bacteria in HCMV-positive periodontitis lesions;
- detection of HCMV nucleic acid sequences in periodontal inflammatory cells;
- ability of HCMV to induce the expression of tissue-damaging cytokines and chemokines in periodontal inflammatory and non-inflammatory cells.

It is hypothesized that some types of severe periodontal disease develop as a result of a series of interactions among periodontotropic herpesviruses, periodontopathic bacteria, and host immune reactions. Reactivation of herpesviruses in periodontal sites is suggested to constitute a particularly important pathogenic event in the development of periodontitis (80). Perhaps not coincidentally, the established risk factors/indicators of periodontitis, including tobacco smoking, HIV-infection, psychosocial stress, pregnancy, and hormonal changes (61), are also known triggers of herpesvirus reactivation.

Initially, bacterially induced gingivitis causes an influx of HCMV-infected macrophages and T-lymphocytes into gingival tissue (19). Subsequent HCMV reactivation diminishes the resistance of the periodontium, enabling overgrowth of pathogenic bacteria, and also enhances inflammatory mediator and cytokine responses in macrophages and other host cells. Lipopolysaccharide from resident gram-negative bacteria can also induce cytokine production in inflammatory cells and may act synergistically with HCMV in stimulating interleukin-1β gene transcription, resulting in a markedly increased interleukin-1β level at periodontitis sites (98). Triggering of pro-inflammatory cytokines may directly increase the severity of periodontal disease or, in a vicious circle, reactivate latent herpesviruses. Flare-up of periodontitis has been associated with increases in cytokines and other inflammatory mediators in periodontal sites (27, 52).

The different clinicopathologic features of periodontitis in young and older individuals may partly reflect the host immunoinflammatory response to a periodontal herpesvirus infection. Typically, the disease course in adolescents and young adults is aggressive and the period of tissue destruction relatively brief (4, 5). In older individuals, the course is more often slow and frequently associated with significant gingival inflammation and accumulations of plaque and calculus (4, 5). It seems that aggressive periodontitis in young patients requires less of an infectious agent stimulus to trigger a progressive disease response than the more chronic type of the disease in adults. Similar to primary herpetic gingivostomatitis and other acute herpesviral diseases (8), it can be hypothesized that aggressive periodontitis preferentially develops in immunologically immature people or in immunocompromised individuals unable to mount an adequate host response against the infectious agent(s). If so, some types of aggressive and chronic periodontitis may not constitute radically different disease entities but be merely a continuous spectrum of diseases whose clinical expression depends on the presence of a periodontal herpesvirus infection and the specific antiviral immune response, ranging from aggressive periodontitis in patients with inadequate immunity at one end, to chronic periodontitis in patients who are immunocompetent at the other end, with intermediary clinical disease types between these two extremes of immune function.

Since gingival inflammation in the herpesvirus–bacteria–periodontitis model constitutes the initiating event in the development of periodontitis, high priority is placed upon combatting gingivitis (78). Gingival inflammation in shallow and deep periodontal sites can be controlled by relatively simple means, employing professional debridement, subgingival irrigation, interdental cleaning, toothbrushing, and various effective, safe and affordable antimicrobial agents (33, 81). In the future, the development of anti-HCMV vaccines may help induce a protective immune response against destructive periodontal disease. However, the occurrence of multiple HCMV genotypes may complicate the development of a universally effective vaccine (60).

To distinguish between correlation and causality, the proposed etiopathogenic model of periodontitis needs to be verified in longitudinal clinical and microbiological studies and by research into molecular aspects of HCMV infections in periodontal disease. Nonetheless, based on the observed close association between HCMV and disease-active periodontitis, and the periodontopathic potential of HCMV active infection, it seems reasonable to propose adding human periodontitis to the list of infectious diseases that have HCMV and maybe other herpesviruses as putative contributory causes.

References

1. Abramson JS, Mills EL. Depression of neutrophil function induced by viruses and its role in secondary microbial infections. Rev Infect Dis 1988: **10**: 326–341.
2. Adam E, Melnick JL & DeBakey ME. Cytomegalovirus infection and atherosclerosis. Cent Eur J Public Health 1997: **5**: 99–106.
3. Alcami A & Koszinowski UH. Viral mechanisms of immune evasion. Trends Microbiol 2000: **8**: 410–418.
4. Armitage GC. Classifying periodontal diseases – a long-standing dilemma. Periodontol 2000 2002: **30**: 9–23.
5. Armitage GC. Periodontal diagnosis and classification of periodontal diseases. Periodontol 2000 2004: **34**: 9–21.
6. Bardell D. Exposure of herpes simplex virus type 1 and its host cells to cigarette smoke *in vitro*. Microbios 1987: **51** (208–209): 151–157.
7. Bello C, Whittle H. Cytomegalovirus infection in Gambian mothers and their babies. J Clin Pathol 1991: **44**: 366–369.
8. Birek C. Herpesvirus-induced diseases: oral manifestations and current treatment options. J Calif Dent Assoc 2000: **28**: 911–921.
9. Blomlöf L, Hammarström L, Lindskog S. Occurrence and appearance of cementum hypoplasias in localized and generalized juvenile periodontitis. Acta Odontol Scand 1986: **44**: 313–320.
10. Brogden KA, Guthmiller JM. Polymicrobial diseases, a concept whose time has come. ASM News 2003: **69**: 69–73.
11. Burghelea B, Serb H. Nuclear bodies and virus-like particles in gingival tissue of periodontopathic patients. Arch Roum Pathol Exp Microbiol 1990: **49**: 89–92.
12. Burghelea B, Serb H. Ultrastructural evidence of a Papova-type viral morphogenesis phenomenon in infiltrating cells from juvenile periodontal lesions. A case report. Arch Roum Pathol Exp Microbiol 1990: **49**: 253–267.
13. Clark DA, Emery VC, Griffiths PD. Cytomegalovirus, human herpesvirus-6, and

human herpesvirus-7 in hematological patients. Semin Hematol 2003: **40**: 154–162.
14. Clementi M. Quantitative molecular analysis of virus expression and replication. J Clin Microbiol 2000: **38**: 2030–36.
15. Cobb CM, Ferguson BL, Keselyak NT, Holt LA, MacNeill SR, Rapley JW. A TEM/SEM study of the microbial plaque overlying the necrotic gingival papillae of HIV-seropositive, necrotizing ulcerative periodontitis. J Periodontal Res 2003: **38**: 147–155.
16. Contreras A, Falkler WA Jr, Enwonwu CO, et al. Human Herpesviridae in acute necrotizing ulcerative gingivitis in children in Nigeria. Oral Microbiol Immunol 1997: **12**: 259–265.
17. Contreras A, Slots J. Active cytomegalovirus infection in human periodontitis. Oral Microbiol Immunol 1998: **13**: 225–230.
18. Contreras A, Umeda M, Chen C, Bakker I, Morrison JL, Slots J. Relationship between herpesviruses and adult periodontitis and periodontopathic bacteria. J Periodontol 1999: **70**: 478–484.
19. Contreras A, Zadeh HH, Nowzari H, Slots J. Herpesvirus infection of inflammatory cells in human periodontitis. Oral Microbiol Immunol 1999: **14**: 206–212.
20. de Jong MD, Galasso GJ, Gazzard B, et al. Summary of the II International Symposium on Cytomegalovirus. Antiviral Res 1998: **39**: 141–162.
21. Doniger J, Muralidhar S, Rosenthal LJ. Human cytomegalovirus and human herpesvirus 6 genes that transform and transactivate. Clin Microbiol Rev 1999: **12**: 367–382.
22. Emery VC. Cytomegalovirus and the aging population. Drugs Aging 2001: **18**: 927–933.
23. Enwonwu CO, Falkler WA Jr, Idigbe EO, et al. Pathogenesis of cancrum oris (noma): confounding interactions of malnutrition with infection. Am J Trop Med Hyg 1999: **60**: 223–232.
24. Fabricant CG, Fabricant J. Atherosclerosis induced by infection with Marek's disease herpesvirus in chickens. Am Heart J 1999: **138**: S465–S468.
25. Falkler WA Jr, Enwonwu CO, Idigbe EO. Microbiological understandings and mysteries of noma (cancrum oris). Oral Dis 1999: **5**: 150–155.
26. Fang F, Dong YS. Effects of cytomegalovirus hepatitis on growth, development and nervous system of infants. A follow-up study. Chin Med J (Engl) 1991: **104**: 138–141.
27. Gemmell E, Seymour GJ. Immunoregulatory control of Th1/Th2 cytokine profiles in periodontal disease. Periodontol 2000 2004: **35**: 21–41.
28. Griffiths PD, Emery VC. Cytomegalovirus. In: Richman DD, Whitley RJ, Hayden FG, eds. Clinical virology. New York: Churchill Livingstone, 1997: 445–470.
29. Haffajee AD, Socransky SS. Microbial etiological agents of destructive periodontal diseases. Periodontol 2000 1994: **5**: 78–111.
30. Haraszthy VI, Zambon JJ, Trevisan M, Zeid M, Genco RJ. Identification of periodontal pathogens in atheromatous plaques. J Periodontol 2000: **71**: 1554–1560.
31. Hsich E, Zhou YF, Paigen B, Johnson TM, Burnett MS, Epstein SE. Cytomegalovirus infection increases development of atherosclerosis in Apolipoprotein-E knockout mice. Atherosclerosis 2001: **156**: 23–28.
32. Hu W, Liu J, Niu S, Liu M, Shi H, Wei L. Prevalence of CMV in arterial walls and leukocytes in patients with atherosclerosis. Chin Med J (Engl) 2001: **114**: 1208–1210.
33. Jorgensen MG, Aalam A, Slots J. Periodontal antimicrobials – Finding the right solutions. Int Dent J 2004: in press.
34. Kamma JJ, Contreras A, Slots J. Herpes viruses and periodontopathic bacteria in early-onset periodontitis. J Clin Periodontol 2001: **28**: 879–885.
35. Kamma JJ, Slots J. Herpesviral–bacterial interactions in aggressive periodontitis. J Clin Periodontol 2003: **30**: 420–426.
36. Kubar A, Saygun I, Yapar M, Özdemir A, Slots J. Real-time PCR quantification of cytomegalovirus in aggressive periodontitis lesions using TaqMan technology. J Periodontal Res 2004: **39**: 81–86.
37. Lang NP, Joss A, Tonetti MS. Monitoring disease during supportive periodontal treatment by bleeding on probing. Periodontol 2000 1996: **12**: 44–48.
38. Mackowiak PA, Goggans M, Torres W, Dal Nogare A, Luby JP, Helderman H. Relationship between cytomegalovirus and colonization of the oropharynx by gram-negative bacilli following renal transplantation. Epidemiol Infect 1991: **107**: 411–420.
39. Michalowicz BS, Ronderos M, Camara-Silva R, Contreras A, Slots J. Human herpesviruses and *Porphyromonas gingivalis* are associated with early-onset periodontitis. J Periodontol 2000: **71**: 981–988.
40. Michelson S. Human cytomegalovirus escape from immune detection. Intervirology 1999: **42**: 301–307.
41. Mocarski ES Jr. Cytomegalovirus and their replication. In: Fields BN, Knipe DM, Howley PM, eds. Fields Virology, 3rd edn. Philadelphia: Lippincott-Raven Publishers, 1996: 2447–2492.
42. Mocarski ES Jr. Virus self-improvement through inflammation: no pain, no gain. Proc Natl Acad Sci USA 2002: **99**: 3362–3364.
43. Mogensen TH, Paludan SR. Molecular pathways in virus-induced cytokine production. Microbiol Mol Biol Rev 2001: **65**: 131–150.
44. Murayama Y, Kurihara H, Nagai H, Dompkowski D, Van Dyke TE. Acute necrotizing ulcerative gingivitis: risk factors involving host defense mechanisms. Periodontol 2000 1994: **6**: 116–124.
45. Murph JR, Bale JF Jr. The natural history of acquired cytomegalovirus infection among children in group day care. Am J Dis Child 1988: **142**: 843–846.
46. Murph JR, Souza IE, Dawson JD, et al. Epidemiology of congenital cytomegalovirus infection: maternal risk factors and molecular analysis of cytomegalovirus strains. Am J Epidemiol 1998: **147**: 940–947.
47. Mutimer D, Mirza D, Shaw J, O'Donnell K, Elias E. Enhanced (cytomegalovirus) viral replication associated with septic bacterial complications in liver transplant recipients. Transplantation 1997: **63**: 1411–1415.
48. Neely AL, Holford TR, Loe H, Anerud A, Boysen H. The natural history of periodontal disease in man. Risk factors for progression of attachment loss in individuals receiving no oral health care. J Periodontol 2001: **72**: 1006–1015.
49. Nowzari H, Jorgensen MG, Ta TT. Contreras A, Slots J. Aggressive periodontitis associated with Fanconi's anemia. A case report. J Periodontol 2001: **72**: 1601–1606.
50. O'Connor S, Taylor C, Campbell LA, Epstein S, Libby P. Potential infectious etiologies of atherosclerosis: a multifactorial perspective. Emerg Infect Dis 2001: **7**: 780–788.
51. Ongradi J, Sallay K, Kulcsar G. The decreased antibacterial activity of oral polymorphonuclear leukocytes coincides with the occurrence of virus-carrying oral lymphocytes and epithelial cells. Folia Microbiol (Praha) 1987: **32**: 438–447.
52. Page RC, Offenbacher S, Schroeder HE, Seymour GJ, Kornman KS. Advances in the pathogenesis of periodontitis: summary of developments, clinical implications and future directions. Periodontol 2000 1997: **14**: 216–248.
53. Park NH, Herbosa EG, Sapp JP. Effect of tar condensate from smoking tobacco and water-extract of snuff on the oral mucosa of mice with latent herpes simplex virus. Arch Oral Biol 1987: **32**: 47–53.
54. Parra B, Slots J. Detection of human viruses in periodontal pockets using polymerase chain reaction. Oral Microbiol Immunol 1996: **11**: 289–293.
55. Pass RF. Epidemiology and transmission of cytomegalovirus. J Infect Dis 1985: **152**: 243–248.
56. Pass RF, Little EA, Stagno S, Britt WJ, Alford CA. Young children as a probable source of maternal and congenital cytomegalovirus infection. N Engl J Med 1987: **28** (316): 1366–1370.
57. Paya CV, Wiesner RH, Hermans PE, et al. Risk factors for cytomegalovirus and severe bacterial infections following liver transplantation: a prospective multivariate time-dependent analysis. [Erratum in J Hepatol 1993 **19**: 325]. J Hepatol 1993: **18**: 185–195.
58. Preus HR, Olsen I, Namork E. The presence of phage-infected *Actinobacillus actinomycetemcomitans* in localized juvenile periodontitis patients. J Clin Periodontol 1987: **14**: 605–609.
59. Rams TE, Listgarten MA, Slots J. Utility of radiographic crestal lamina dura for predicting periodontitis disease-activity. J Clin Periodontol 1994: **21**: 571–576.
60. Rasmussen L, Geissler A, Winters M. Inter- and intragenic variations complicate the molecular epidemiology of human cytomegalovirus. J Infect Dis 2003: **187**: 809–819.
61. Rees TD. A profile of the patient with periodontal disease? Periodontol 2000 2003: **32**: 9–10.

62. Rivera-Hidalgo F. Smoking and periodontal disease. Periodontol 2000 2003: **32**: 50–58.
63. Sabeti M, Simon JH, Slots J. Cytomegalovirus and Epstein-Barr virus are associated with symptomatic periapical pathosis. Oral Microbiol Immunol 2003: **18**: 327–328.
64. Sabeti M, Slots J. Herpesviral-bacterial coinfection in periapical pathosis. J Endod 2004: **30**: 69–72.
65. Sabeti M, Valles Y, Nowzari H, Simon JH, Kermani-Arab V, Slots J. Cytomegalovirus and Epstein-Barr virus DNA transcription in endodontic symptomatic lesions. Oral Microbiol Immunol 2003: **18**: 104–108.
66. Sarid O, Anson O, Yaari A, Margalith M. Human cytomegalovirus salivary antibodies as related to stress. Clin Lab 2002: **48**: 297–305.
67. Saygun I, Kubar A, Özdemir A, Yapar M, Slots J. Herpesviral-bacterial interrelationships in aggressive periodontitis. J Periodontal Res 2004: **39**: 207–212.
68. Saygun I, Sahin S, Özdemir A, et al. Detection of human viruses in patients with chronic periodontitis and the relationship between viruses and clinical parameters. J Periodontol 2002: **73**: 1437–1443.
69. Saygun I, Yapar M, Özdemir A, Kubar A, Slots J. Human cytomegalovirus and Epstein-Barr virus type 1 in periodontal abscesses. Oral Microbiol Immunol 2004: **19**: 83–87.
70. Shi Y, Tokunaga O. Herpesvirus (HSV-1, EBV and CMV) infections in atherosclerotic compared with non-atherosclerotic aortic tissue. Pathol Int 2002: **52**: 31–39.
71. Simonian K. The role of herpesviruses in periodontal disease. J West Soc Periodontol Periodontal Abstr 2003: **51**: 5–9.
72. Skrepcinski FB, Tetrev S, Rams TE, Sutton B, Contreras A, Slots J. Periodontal disease in Hopi native American teenagers. J Dent Res 1997: **76** (Spec Iss): 439 (Abstract 3406).
73. Slots J. Subgingival microflora and periodontal disease. J Clin Periodontol 1979: **6**: 351–382.
74. Slots J. Update on *Actinobacillus actinomycetemcomitans* and *Porphyromonas gingivalis* in human periodontal disease. J Int Acad Periodontol 1999: **1**: 121–126.
75. Slots J. *Actinobacillus actinomycetemcomitans* and *Porphyromonas gingivalis* in periodontal disease: introduction. Periodontol 2000 1999: **20**: 7–13.
76. Slots J. Interactions between herpesviruses and bacteria in human periodontal disease. In: Brogden KA, Guthmiller JM, eds. Polymicrobial diseases. Washington DC: ASM Press, 2002: 317–331.
77. Slots J. Herpesviruses in human periodontal disease. In: Wilson TG Jr, Kornman KS, eds. Fundamentals of periodontics. Carol Stream, IL: Quintessence Publishing, 2003: 58–66.
78. Slots J. Update on general health risk of periodontal disease. Int Dent J 2003: **53**: 200–207.
79. Slots J, Chen C. The oral microflora and human periodontal disease. In: Tannock GW, ed. Medical importance of the normal microflora. London: Kluwer Academic Publishers, 1999: 101–127.
80. Slots J, Contreras A. Herpesviruses: a unifying causative factor in periodontitis? Oral Microbiol Immunol 2000: **15**: 277–280. [Translated to Spanish: '?Herpesvirus: un factor etiológico unificador en la periodontitis?'published in Acta Dent Int 2001: **2**: 11–16].
81. Slots J, Jorgensen MG. Effective, safe, practical and affordable periodontal antimicrobial therapy: where are we going, and are we there yet? Periodontol 2000 2002: **28**: 298–312.
82. Slots J, Kamma JJ. General health risk of periodontal disease. Int Dent J 2001: **51**: 417–427.
83. Slots J, Kamma JJ, Sugar C. The herpesvirus-*Porphyromonas gingivalis*-periodontitis axis. J Periodontal Res 2003: **38**: 318–323.
84. Slots J, Nowzari H, Sabeti M. Cytomegalovirus infection in symptomatic periapical pathosis. Int Endod J 2004: **37**: 519–524.
85. Slots J, Rams TE. Microbiology of periodontal disease. In: Slots J, Taubman MA, eds. Contemporary oral microbiology and immunology. St. Louis: Mosby-Year Book, 1992: 425–443.
86. Slots J, Sabeti M, Simon JH. Herpesviruses in periapical pathosis: an etiopathogenic relationship? Oral Surg Oral Medical Oral Pathol Oral Radiol Endod 2003: **96**: 327–331.
87. Slots J, Sugar C, Kamma JJ. Cytomegalovirus periodontal presence is associated with subgingival *Dialister pneumosintes* and alveolar bone loss. Oral Microbiol Immunol 2002: **17**: 369–374.
88. Socransky SS, Haffajee AD. Evidence of bacterial etiology: a historical perspective. Periodontol 2000 1994: **5**: 7–25.
89. Socransky SS, Haffajee AD. Dental biofilms: difficult therapeutic targets. Periodontol 2000 2002: **28**: 12–55.
90. Stagno S, Pass RF, Thomas JP, Navia JM, Dworsky ME. Defects of tooth structure in congenital cytomegalovirus infection. Pediatrics 1982: **69**: 646–648.
91. Stratta RJ. Clinical patterns and treatment of cytomegalovirus infection after solid-organ transplantation. Transplant Proc 1993: **25** (Suppl. 4): 15–21.
92. Teughels W, Van Ranst M, van Steenberghe D, Van Eldere J, Fives-Taylor P, Quirynen M. Epithelial infection with human cytomegalovirus enhances adhesiveness of *Actinobacillus actinomycetemcomitans* in 3 phases. IADR Abstract, 2003.
93. Ting M, Contreras A, Slots J. Herpesviruses in localized juvenile periodontitis. J Periodontal Res 2000: **35**: 17–25.
94. Tortorella D, Gewurz BE, Furman MH, Schust DJ, Ploegh HL. Viral subversion of the immune system. Annu Rev Immunol 2000: **18**: 861–926.
95. Van Dyke TE, Levine MJ, Genco RJ. Neutrophil function and oral disease. J Oral Pathol 1985: **14**: 95–120.
96. Van Dyke TE, Vaikuntam J. Neutrophil function and dysfunction in periodontal disease. Curr Opin Periodontol 1994: 19–27.
97. Velazco CH, Coelho C, Salazar F, Contreras A, Slots J, Pacheco JJ. Microbiological features of Papillon–Lefèvre syndrome periodontitis. J Clin Periodontol 1999: **26**: 622–627.
98. Wara-aswapati N, Boch JA, Auron PE. Activation of interleukin 1β gene transcription by human cytomegalovirus: molecular mechanisms and relevance to periodontitis. Oral Microbiol Immunol 2003: **18**: 67–71.
99. Yapar M, Saygun I, Özdemir A, Kubar A, Sahin S. Prevalence of human herpesviruses in patients with aggressive periodontitis. J Periodontol 2003: **74**: 1634–1640.