Oral Diseases (2005) 11, 219–229

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REVIEW ARTICLE

Viruses in periodontal disease – a review

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The purpose of this review was to evaluate the evidence supporting the hypothesis that viral infection plays a role in the development of periodontitis. An involvement in periodontal diseases has been suspected specifically for human immunodeficiency virus (HIV) and herpes viruses. An association has been demonstrated between HIV infection and some distinct forms of periodontal infection, i.e. necrotizing lesions. Furthermore, reports of increased prevalence and severity of chronic periodontitis in HIV-positive subjects suggests that HIV infection predispose to chronic periodontitis. Several studies, most of them from the same research group, have demonstrated an association of herpesviruses with periodontal disease. Viral DNA have been detected in gingival tissue, gingival cervicular fluid (GCF) and subgingival plaque from periodontaly diseased sites. In addition markers of herpesviral activation have been demonstrated in the GCF from periodontal lesions. Active human cytomegalovirus (HCMV) replication in periodontal sites may suggest that HCMV re-activation triggers periodontal disease activity. Concerns regarding sampling, methods and interpretation cast doubts on the role of viruses as causes of periodontal disease.
Oral Diseases (2005) **11**, 219–229

Keywords: human immunodeficiency virus; cytomegalovirus; Epstein–Barr virus; herpes simplex virus; periodontitis/pathogenesis

Introduction

Many bacterial infections in humans occur as superinfections of viral diseases. A well known example is the bacterial complication in influenza outbreaks: most deaths in influenza epidemics occur in elderly people and are frequently attributed to a secondary bacterial pneumonia mainly due to *Staphylococcus aureus*, *Streptococcus pneumoniae* or *Haemophilus influenzae* (Cate, 1998; Sethi, 2002). This example could be applied to other situation of viral-bacterial interactions, for example in the oral cavity. Recently, it was suggested that certain viruses might also influence the development and severity of periodontal disease.

It is generally believed that both gingivitis and periodontitis are caused by bacteria colonizing the tooth surfaces, and that the major mechanisms of periodontal destruction are initiated by bacteria. This view is based on a large number of studies essentially demonstrating an association between bacterial plaque and clinical signs of gingivitis and periodontitis. Cross-sectional studies of human populations have shown a positive correlation between the amount of plaque and the severity of gingivitis (O'Leary and Prignace, 1962) as well as bone loss (Schei *et al*, 1959). In addition, experimental short-term studies have shown that, once an individual abstains from mechanical tooth-cleaning and microorganisms start to colonize the tooth surfaces, clinical signs of gingivitis appear within a few days (Löe *et al*, 1965). The inflammatory alterations are resolved or reversed when the bacterial deposits are again removed from the tooth surfaces. Other studies have indicated that antiseptic agents such as chlorhexidine are able to suppress the bacterial colonization and the development of gingivitis (Löe *et al*, 1965; Corbet *et al*, 1997a,b), and that antibiotics could reduce plaque scores and improve gingival conditions in subjects with periodontal disease (Ciancio *et al*, 1980, 1982). The benefit of adjunctive antibiotic therapy to mechanical debridement procedures in controlling several forms of periodontitis further strengthens the argument for a major etiological role of bacteria in human periodontal disease (Herrera *et al*, 2002).

While the role of bacterial plaque in general seems to be evident, the following observations indicate that other functions may contribute to the development of periodontal diseases. Although all subjects with poor oral hygiene develop gingivitis, not every gingivitis lesion invariably leads to attachment loss. Despite a large variation in general levels of oral hygiene and gingivitis in different societies, and a high prevalence of potential bacterial pathogens in certain populations (Eisenmann *et al*, 1983; Dahlén *et al*, 1989; McNabb

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Received 15 June 2004; revised 30 November 2004; accepted 20 December 2004

et al, 1992; Al-Yahfoufi *et al*, 1994; Gmür and Guggenheim, 1994; Mombelli *et al*, 1999), severe periodontal destruction seems to be limited to only 7–15% subjects worldwide (Baelum *et al*, 1986, 1996; Schürch *et al*, 1988; Dahlén *et al*, 1989). Global epidemiological data allow the inference that the progression of destructive periodontitis is subject related and comparatively few individuals in the population show advanced periodontal breakdown (Papapanou, 1996).

Although constantly colonized by varying numbers and species of bacteria (Haffajee *et al*, 1991) even established periodontal lesions do not invariably progress, i.e. show further loss of supporting periodontal tissue. Episodic disease activity at specific sites has been documented (Goodson *et al*, 1982).

The above observations lead to the hypothesis that factors beyond dental plaque are important in the pathogenesis of periodontitis. Exogenous factors, such as tobacco smoking (Bergström, 1989; Brochut and Cimasoni, 1997a,b) as well as endogenous factors, such as genetically determined variations in inflammatory response patterns (Kornman *et al*, 1997) have in fact been shown to influence the clinical course of periodontal disease significantly.

Table 1 shows the main viral infections that may be associated with an oral manifestation. Almost all infections described in this table can develop lesions on the gingiva. Except for human immunodeficiency virus (HIV) and enteroviruses, most viruses potentially involved in periodontal disease are DNA viruses. An involvement in periodontal diseases has been suspected specifically for HIV and herpes viruses. In the following we will focus on these two groups of viruses.

The purpose of this review is to give an overview of the viruses involved in oral pathology, to highlight specifically those viruses that may be potentially involved in periodontal diseases, and to evaluate the evidence supporting the hypothesis that viral infection plays a role in the development of periodontal diseases.

HIV

Oral lesions related to HIV are often the first clinical signs of this infection and can play a specific role in the diagnosis of patients with unknown HIV serostatus (Greenspan, 1994; Patton *et al*, 1999; Patton and van der Horst, 1999). Oral candidiasis and hairy leukoplakia have consistently been reported as the most prevalent HIV associated oral disease (Shiboski *et al*, 2001; Holmes and Stephen, 2002; Patton *et al*, 2002). Neoplasia such as Kaposi's sarcoma and non-Hodgkin's lymphoma are other oral lesions strongly associated to HIV infection which, together with oral candidiasis, are able to affect the periodontum (Langford, 1994). In addition, HIV is associated with the following periodontal conditions: linear gingival erythema (LGE), necrotizing gingivitis (NG), necrotizing periodontitis (NP), and chronic periodontitis.

Linear gingival erythema is defined as a distinct erythematous band of marginal gingiva with either diffuse or punctuate erythema of the attached gingiva (Holmstrup and Westergaard, 1998). In early studies it

Table 1 Main viral infections and their oral manifestations

DNA-Viruses	*Genus*	*Pathology*	*Oral manifestation*
Herpesviruses	Herpes simplex viruses (HSV)	Primary herpetic gingivostomatitis	Visiculous ulceration
		Herpes labialis	
		Recurrent herpetic gingivostomatitis	
		Chronic herpetic gingivostomatitis	
	Varicella–Zoster virus (VZV)	Varicella (chicken pocks)	Visiculous ulceration
		Herpes zoster (shingles)	
	Epstein–Barr virus (EBV)	Infectious mononucleosis	Ulcerations and palatal petechiae
		Hairy leukoplakia	White lesion
		Lymphomas	
	Cytomegalovirus (CMV)	Infectious mononucleosis	Visiculous ulceration
		Reactivation	
	Human herpes virus 6 (HHV 6)	Unknown	Unknown
	Human herpes virus 7 (HHV 7)	Unknown	Unknown
	Human herpes virus 8 (HHV 8)	Kaposi's sarcoma	
Papovaviridae	Papilloma viruses	Focal epithelial hyperplasia (Heck's disease)	Epithelial noduls
		Oral squamous cell papillomas	Papillomatous vegetation
		Common warts verrucae vulgaris)	Epithelial noduls
		Condyloma acuminata	Epithelial noduls
RNA-Viruses			
Retroviridae	HIV	AIDS	Candidosis
		Fungal infections	Recurrent herpetic ging
		Viral infections	Kaposi's sarcoma
		Tumors	Non-Hodgkin's lymph
		Auto-immune disease	Petechiae
		Bacterial infection	Necrotizing gingivitis
Picornaviridae	Enterovirus species Coxsackivirus	Herpangina	Ulcerous stomatitis
		Hand-foot-and-mouth disease	Stomatite

was often further characterized by a discrepancy between the amount of plaque and the intensity of the inflammation. A possible etiological role of candidal infection has been investigated (Winkler *et al*, 1988). The prevalence of LGE in HIV infected population varies from 0 to 49% (Porter *et al*, 1989; Klein *et al*, 1991; Slots and Rams, 1991; Lamster *et al*, 1997; Patton *et al*, 1998; Schuman *et al*, 1998; Ranganathan *et al*, 2000). This considerable variation can be due to the lack of clear diagnostic standardization and the heterogeneity of the populations studies. The existence of LGE as a periodontal disease entity that is strongly associated with HIV has been questioned by some (van Der Waal, 1997; Robinson, 2002) and others suggest that LGE is mostly misdiagnosed as gingivitis (Grbic *et al*, 1995; Robinson *et al*, 1996).

Necrotizing gingivitis, NP and necrotizing stomatitis (NS) may be different stages of the same disease. NG results in the destruction of one or more interdental papillae and remains confined to the marginal gingiva. NP extends beyond the marginal gingiva, involves the periodontal ligament and the alveolar bone, leading to a loss of attachment. NS extends past the mucogingival line into the mucosa and osseous tissue. Most studies show a higher prevalence of NG and NP in HIV infected patients than in non-HIV infected patients (Porter *et al*, 1989; Laskaris *et al*, 1992; Holmstrup and Westergaard, 1994; Schuman *et al*, 1998; Patton *et al*, 2002). NP may be used as a marker for immune deterioration with a 95% predictive value that $CD4^+$ cell counts have decreased below 200 cells μl^{-1}. If untreated the cumulative probability of death within 24 months is 72.9% (Glick *et al*, 1994).

Human immunodeficiency virus-associated NG, NP and NS were initially considered specific disease conditions. It is now thought that they do not differ from those found in immunocompetent patients (Reichart *et al*, 2003). Microbiologically there is no major difference between HIV-positive and HIV-negative subjects (Lamster *et al*, 1998; Cobb *et al*, 2003; Goncalves Lde *et al*, 2004). The prevalence of chronic periodontitis in HIV-positive subjects varies considerably from 5 to 69% (Holmstrup and Westergaard, 1998). When compared with HIV-negative counterparts, HIV-positive patients with chronic periodontitis suffer from a greater loss of attachment over time (Yeung *et al*, 1993; Ryder, 2002).

Conclusion

Several studies show a clear association between HIV infection and some distinct forms of periodontal infection, i.e. necrotizing lesions. Furthermore, reports of increased prevalence and severity of chronic periodontitis in HIV-positive subjects suggests that HIV infection predisposes to chronic periodontitis.

Herpesviruses

Herpesviruses are usually contracted in childhood from infected secretions such as saliva, may cause oral mucosal lesions during the primary infection, are capable of indefinite latency, and may be reactivated under various conditions. The main cause of reactivation is immune loss, and these infections may cause severe diseases in HIV infected and other immunocompromised patients (Roizman, 1996).

Eight human herpesviruses are discriminated. Subdivision of the family into three subfamilies is made upon the pathogenicity, the type of cells they infect and the properties of their growing. Alpha-herpes viruses include herpes simplex virus type 1 (HSV-1) and type 2 (HSV-2) and varicella zoster virus (VZV). This subfamily has a fast growing pattern, lyse-infected cells and remain latent in sensory nerve ganglia. Most often, they induce vesiculous lesions in skin or mucosa. Beta-herpes viruses include human cytomegalovirus (HCMV), human herpes virus 6 (HHV-6) and human herpes virus 7 (HHV-7). Their replication is slow and produces large, often multinucleated cells (cytomegalia). The viral genome remains latent in lymphoreticular tissue, secretory gland (i.e. salivary glands), kidneys and other tissues. HCMV can induce severe general diseases in immunocompromised patients in particular pneumonia, and primary infections during pregnancy can lead to severe congenital abnormalities. HHV- 6 and HHV-7's primary infection is often asymptomatic but can give rise to exanthema subitum (De Araujo *et al*, 2002). Gamma-herpes viruses include Epstein–Barr virus (EBV) and human herpes virus 8 (HHV-8). They infect lymphoid cells (lymphocytes B and T) but they can also be cytocidal for epithelial cells and fibroblasts. Latency of this subfamily frequently occurs in lymphoid tissues. EBV is responsible for infectious mononucleosis and HHV-8 may be implicated in Kaposi's sarcoma (Roizmann *et al*, 1992).

Herpes simplex viruses

Infections HSV-1 and HSV-2 are worldwide spread and usually affect skin and mucosa. Other herpetic diseases of clinical importance occur through ophthalmic, neurologic and, more rarely, organ system infection (Whitley and Roizman, 2001). Herpes simplex viruses are transmitted by close person-to-person contact by mucosal secretion or lesions. HSV-1, principally shed in the saliva, is transmitted directly (kissing) or indirectly (infected utensils or hands) and is mainly involved in oral-facial infections and encephalitis. HSV-2 is usually transmitted sexually and causes genital infection. However, HSV-1 and HSV-2 can both be found in oral-facial and genital infection (Rabenau *et al*, 2002; Buxbaum *et al*, 2003). Other ways of transmission include autoinoculation by fingers to the eyes or genital tract and transmission from infected mothers to neonates. Although herpes simplex virus infections are endemic in all human populations, the epidemiology varies between countries and type of population. Acquisition of herpesviruses, especially HSV-2, is largely dependent on the socio-economic status, sex, age and ethnic origin (Fleming *et al*, 1997). The prevalence in a representative population in the United States aged more than 12 years shows for example a seroprevalence of 67.6% for HSV-1 and 21.9% for HSV-2 (Xu *et al*, 2002).

Primary infection occurs in childhood from infected saliva or herpetic lesions. At the site of epithelial infection viral antigens induce a cell-mediated immunity response which is the key of recovery (lyse of infected cells) and latency (suppression of the full HSV DNA expression). Following replication in epithelial cells, some viral nucleocapsid ascend the local sensory neurons by retrograde axonal transport and establish lifelong latency in the corresponding spinal or cerebral ganglion (e.g. trigeminal ganglion following oral infection). Productive infection leads to the destruction of the neurons but most of them survive (Rozenberg, 2002).

Reactivation can occur at any time and may be triggered by immunosuppression, stress, trauma, ultraviolet irradiation, or fever. The specific mechanisms of reactivation remain unclear. Replication of the virus is induced in some latently infected neurons. Virus is then transported down the axon to the region of original infection. Recurrences are generally less severe than the primary infection and severity and frequency tend to diminish with time (Whitley and Roizman, 2001).

The primary herpetic infection is the most common gingival disease of viral origin (Kuzushima *et al*, 1991; McMillan *et al*, 1993). It may run an asymptomatic course in early childhood but can give rise to more severe manifestation with increasing of age infection. The clinical manifestations are: gingivitis, vesicles that leave ulcerations, cervical lymphadenopathy and fever (Amir *et al*, 1999). Healing takes 10–14 days. Pain can compromise food intake. The treatment includes maintenance of fluid and food intake, antipyretic and analgesic treatment and topical antiseptics to prevent bacterial superinfections. Acyclovir treatment seems to shorten the duration of all clinical manifestations (Amir, 2001). Acyclovir, a synthetic acyclic purine nucleoside analog, is a substrate with high specificity for herpes simplex and varicella-zoster-specified thymidine kinase. These kinases transform acyclovir to its monophosphate. Cellular enzymes further transform it to acyclovir triphosphate which is an inhibitor of, and a substrate for, herpes virus DNA polymerase.

Reactivation of herpes simplex virus can occur subclinically but about one-third of individuals become prone to clinical recurrences (Young *et al*, 1988; Lowhagen *et al*, 2002). The recurrences are usually found at the mucocutaneous junction of the lips (herpes labialis) but can also be intraoral typically on the palate or the gingiva (recurrent herpetic gingivostomatitis and chronic herpetic gingivostomatitis in immunocompromised patients) (Leigh, 1988; Nikkels and Pierard, 1999).

Varicella zoster virus

Varicella zoster virus is found in a worldwide distribution, with over 90% of seroprevalence among adults in the United States (Choo *et al*, 1995). Annual epidemics are more prevalent in temperate climates, occurring most often in late winter and spring. VZV is responsible for two universal human diseases: varicella (chickenpox) in childhood and herpes zoster in aged and immunocompromised persons. VZV enters by inhalation and replicates in the mucosa of the respiratory tract. Dissemination occurs via bloodstream and lymphatics and the virus multiplies in mononuclear leukocytes and capillary endothelial cells. The cutaneous rash results from the multiplication of the virus in epithelial cells, following which it ascends the axons of various sensory nerves and remains latent in their ganglions (Arvin, 1996).

Varicella is usually acquired during the first 5–10 years of life. As it affects almost all children, its annual incidence coincides with the birth rate. Varicella appears suddenly with or without prodormal fever and malaise. Vesicles and ulcers first appear in the mouth (usually on the palate and the tongue but also on the gingiva) followed by a cutaneous rash that spreads centrifugally from the head and the trunk. Lesions in the mouth are painful whereas lesions on the skin are painless but itchy, which may lead to secondary bacterial infection and permanents scars (Kolokotronis *et al*, 2001). Neurologic complications are uncommon but potentially serious (Hausler *et al*, 2002).

Herpes zoster results from reactivation of virus that remain latent in sensory ganglia. The epidemiology is affected by host factors and its incidence increases with advancing age to more than 10 cases per 1000 persons per year by age 75. Lesions are similar to those found in varicella but usually remain confined to a single dermatome and are thus unilateral. Apparition of lesions can be accompanied by severe pain and paresthesia. Intraoral lesions can be found if the third or second branch of the trigeminal ganglion is involved (Arvin, 1996), which may lead to alveolar bone necrosis (Smith *et al*, 1984; Arikawa *et al*, 2004).

Epstein–Barr virus

Epstein–Barr virus affects over 90% of humans (Cohen, 1997), and is usually transmitted by oral secretions or blood. The virus replicates in epithelial cells or B cells of the oropharynx. Nearly all seropositive persons actively shed virus in the saliva (Yao *et al*, 1985). Resting memory B cells are the main site of persistence of EBV in the body (Cohen, 1997). One to 50 circulating B cells per million are infected. The number of latently infected cells within a person remains stable over years (Babcock *et al*, 1998).

Whereas EBV infection in children is most often subclinic, EBV infection in adults results in infectious mononucleosis. Most of the symptoms are attributed to the proliferation and activation of T cells in response to infection. Most common symptoms of infectious mononucleosis are fever, lymphadenopathy and pharyngitis. Oral ulcers, palatal petechia and less commonly gingival ulcerations can be diagnosed (Rivera-Hidalgo and Stanford, 1999). EBV has also been associated to other diseases such as cancers and auto-immune diseases (Thorley-Lawson and Gross, 2004). Oral hairy leukoplakia (OHL) is the main lesion associated to EBV. Its presence signals most often a relatively advanced stage of immunosuppression, namely in HIV infection (Margiotta *et al*, 1999). OHL is a non-malignant hyperplasic lesion of epithelial cells which shows

evidences of non-cytolytic active EBV replication (Triantos *et al*, 1997). Clinically OHL appears as a raised, white, corrugated lesion that most often develops on the ventral-lateral aspect of the tongue and may be unilateral or bilateral (Schiodt *et al*, 1987a,b). The question whether OHL arises from the activation of EBV latent in the tongue or from superinfection by endogenous EBV shed via non-glossal sites or by exogenous EBV strains remains unresolved (Teo, 2002).

T cells of HIV infected patients suppress EBV-infected B cells less effectively than those in immunocompetent persons. Thus, patients with HIV have 10–20 times as many circulating EBV infected B cells than healthy persons (Birx *et al*, 1986). Patients with HIV also carry a higher load of EBV in their oropharyngeal secretions (Jenson *et al*, 1999).

Human cytomegalovirus

Human cytomegalovirus is the most common cause of congenital and perinatal infections. About 10% of infants are infected by the age of 6 months following transmission from their mother through the placenta, during delivery or by breast feeding (Pass, 1985; Stagno and Whitley, 1985). Even in industrialized countries HCMV affects 90% of the population by the age of 20 (Numazaki and Chiba, 1995). HCMV infects many different epithelial cells, endothelial cells, smooth muscle cells, mesenchymal cells, hepatocytes, granulocytes and monocyte-derived macrophages (Landolfo *et al*, 2003). HCMV is thus found in many body secretions including saliva, urine, semen and breast milk. HCMV infection, although generally subclinical, is responsible for cytomegalovirus inclusion disease and mononucleosis; under immunosuppressive conditions it can give rise to a wide variety of clinical manifestations (Landolfo *et al*, 2003). In HIV-infected patients, oral ulcers associated with HCMV have been reported (Scully and Porter, 1998) as well as gingival hyperplasia (Epstein and Scully, 1991).

Herpesviruses: an etiologic factor of periodontal diseases?

The involvement of herpesviruses in the etiology of periodontal diseases is suggested by their presence in gingival tissue, gingival cervicular fluid (GCF) and subgingival plaque, in the presence of periodontal disease. The hypothesis is challenged by several arguments regarding sampling, methods and interpretation. This chapter outlines the arguments pro and contra such a hypothesis.

Evidence for a potential role of herpesviruses in the etiology of periodontal disease

1. Virus detection in gingival tissue

Cultured epithelial cells and fibroblasts from clinically healthy human gingiva are susceptible to HSV infection (Zakay-Rones *et al*, 1982), suggesting that those cells could be a reservoir for the latent virus. Using an indirect immunofluorescence assay, HSV-1 antigens could be detected in four out of 14 gingival biopsies from periodontally diseased patients (Ehrlich *et al*, 1983). The presence of HSV antigens could also be shown in 26 out of 66 specimens of clinically healthy gingiva using a similar method (Amit *et al*, 1992). In addition, using a dot blot hybridization assay, HSV DNA was found in one specimen carrying intact gingival cells, suggesting that the virus may be present in the gingiva in the latent form. Other DNA viruses, not belonging to the herpes group have also been identified in gingival tissue. Human papilloma virus (HPV) for example was demonstrated in gingival specimens from periodontaly diseased subjects by southern blot hybridization (Madinier *et al*, 1992).

In periodontally diseased tissues a high proportion of inflammatory cells are infected by herpesvirus. Polymorphonuclear neutrophils (PMN), monocytes/macrophages, T and B lymphocytes were isolated from gingival biopsies of periodontitis lesions in 20 subjects using immunomagnetic cell sorting (Contreras *et al*, 1999b). The presence of HCMV, EBV-1, EBV-2, HHV-6 and HSV was then sought in them and in the whole tissue samples by means of a nested polymerase chain reaction (PCR). The cell fractions of 14 (70%), and the whole biopsies of 18 (90%) patients were positive for herpesviruses. Monocyte/macrophage cell fractions from 11 patients (55%) yielded DNA from HCMV, and one (5%) from HSV. T lymphocytes harbored HCMV DNA from four (20%) patients and HSV DNA from four (20%) patients. B lymphocytes carried EBV DNA in nine (45%) patients.

2. Higher frequency of virus detection in the gingival tissue of periodontitis sites than in healthy sites

In the 20 patients with periodontitis in the study of Contreras *et al* (1999b), HCMV DNA was detected in 13 biopsies, EBV DNA in 10, HSV DNA in seven and HHV-6 DNA in two. Out of three additional gingival biopsies from healthy gingiva, obtained from three additional healthy subjects, only one yielded positive result for HSV DNA; all other viruses were not detectable.

A later report from the same group (Contreras *et al*, 2000) showed that herpesviral DNA in gingival tissues could be detected in periodontally healthy or diseased subjects by nested PCR. Out of 14 samples from periodontally diseased tissues, HCMV DNA was detected in 12, and EBV-1 DNA in 11. Seven samples of healthy gingival tissues were obtained from periodontally healthy people, two from gingivitis and two from periodontitis. HCMV DNA was detected in only two and EBV-1 DNA in three.

3. Higher frequency of herpesvirus detection in GCF from periodontaly diseased sites than from gingivitis/healthy sites

Parra and Slots (1996) investigated the presence of HCMV, EBV, HSV, HPV and HIV in GCF samples from 30 patients with advanced periodontitis and 26 subjects with gingivitis. GCF samples were collected with sterile paper points from the two or three most diseased sites in each patient. Viral identification was

performed using nested PCR. Seventy-eight percent of advanced periodontitis patients were positive for at least one of the five tested viruses. HCMV was detected in 60% of the periodontitis patients, EBV in 30%, HSV in 20%, HPV in 17% and HIV in 7%. Only 31% of the gingivitis subjects were virus positive; and all were positive for HCMV only.

Contreras and Slots (1996) carried out a similar study to determine the frequency of HCMV, EBV-1, EBV-2, HSV and HIV in subgingival samples from 27 adults who each contributed sample from a periodontitis and a gingivitis site. Viral detection was again performed using a nested PCR. Eighty-nine percent of the patients yielded at least one of the five tested viral DNA from deep periodontal pockets, whereas only 56% yielded viral DNA from shallow periodontal sites. HCMV was found significantly more frequently in deep than in shallow pocket samples, with a presence in deep pockets of HCMV in 14, EBV-1 in 10, HSV in 7, EBV-2 in six patients and HIV in one patient.

The same group of researchers has published additional reports presenting data essentially confirming the results presented above (see Table 2). Viruses were always identified using nested PCR and the analyses were carried out in the same laboratory (Contreras and Slots, 1998; Contreras *et al*, 2000; Hanookai *et al*, 2000; Ting *et al*, 2000; Kamma *et al*, 2001).

4. Higher frequency of virus detection in subgingival plaque from periodontaly diseased than from healthy sites

Saygun *et al* (2002) examined the occurrence of HCMV, EBV-1 and HSV DNA in patients with chronic periodontitis and the relationship between these viruses and clinical parameters. These authors also used nested PCR for virus detection as described by Contreras and Slots (1996) and Parra and Slots (1996). Subgingival plaque samples were taken with paper points from 30 patients with chronic periodontitis and 21 randomly selected healthy controls. HCMV DNA was detected in 44.3% of chronic periodontitis patients and 14.3% of healthy persons. EBV-1 DNA was found in 16.7% of chronic periodontitis patients and 14.3% of healthy persons and HSV DNA in 6.7% of chronic periodontitis patients and not at all in healthy patients. Thus, the frequency of detection was lower compared with previous studies in which GCF samples were used.

In another study by the same group (Yapar *et al*, 2003), subgingival plaque samples, collected with sterile curette, were analysed from 17 patients with aggressive periodontitis and 16 healthy subjects. HCMV DNA was detected in 64.7% and EBV-1 DNA in 70.6% of

Table 2 Percentage of subjects on sites with the presence of selected herpes viruses in GCF

Authors	*Subjects*	*Periodontal pockets (%)*	*Gingivitis or shallow sites (%)*	*Sampling approach*	*Method of detection*
Parra and Slots (1996)	30 Patients with advanced periodontitis, 26 subjects with gingivitis	HCMV (60)[a] EBV (30)[a] HSV (20)[a] HPV (17)[a] HIV (2)[a]	HCMV (31)[a] EBV (0)[a] HSV (0)[a] HPV (0)[a] HIV (0)[a]	GCF sample with paperpoints	Nested PCR
Contreras and Slots (1996)	27 Adults with chronic periodontitis, periodontal and gingivitis sites in same patient	HCMV (59)[a] EBV-1 (37)[a] EBV-2 (22)[a] HSV (26)[a] HIV (4)[a]	HCMV (18)[a] EBV-1 (22)[a] EBV-2 (18)[a] HSV (7)[a] HIV (0)[a]	GCF sample with paperpoints	Nested PCR
Contreras and Slots (1998)	6 Chronic periodontitis patients, 3 localized	HCMV (89)[a]	HCMV (22)[a]	GCF sample with paperpoints	Nested PCR
Ting *et al* (2000)	11 Patients with localized juvenile periodontitis juvenile periodontitis patients	HCMV (72)[b] EBV-1 (63)[b] EBV-2 (9)[b] HSV (54)[b]	HCMV (18)[b] EBV-1 (18)[b] EBV-2 (0)[b] HSV (9)[b]	GCF sample with paperpoints	Nested PCR
Hanookai *et al* (2000)	19 Trisomy 21 patients with periodontitis lesions	HCMV (26)[b] EBV-1 (32)[b] HSV (16)[b]	HCMV (5)[b] EBV-1 (0)[b] HSV (0)[b]	Plaque samples with curette	Nested PCR
Saygun *et al* (2002)	30 Patients with chronic periodontitis, 21 healthy controls	HCMV (44.3)[a] EBV-1 (17.7)[a] HSV (6.7)[a]	HCMV (14.3)[a] EBV-1 (14.3)[a] HSV (0)[a]	Plaque samples by paperpoints	Nested PCR
Yapar *et al* (2003)	17 Patients with aggressive periodontitis, 16 healthy controls	HCMV (64.7)[a] EBV-1 (70.6)[a]	HCMV (0)[a] EBV-1 (6)[a]	Plaque sample with curette	Single PCR
Kubar *et al* (2004)	16 Patients with aggressive periodontitis, 15 healthy controls	HCMV (68.8)[b]	HCMV (0)[b]	Plaque sample with curette	Real-time PCR

HCMV, human cytomegalovirus; EBV, Epstein–Barr virus; HSV, herpes simplex virus; HPV, human papilloma virus; HIV, human immunodeficiency virus; GCF, gingival cervicular fluid.
[a]% of patients.
[b]% of sites.

aggressive periodontitis patients. In healthy subjects, HCMV DNA could not be detected and EBV-1 DNA in only one case. The results of this study revealed a high prevalence of HCMV and EBV-1 DNA in aggressive periodontitis sites. Such findings have been recently confirmed by (Kubar *et al*, 2004) by real-time PCR quantification (Table 2).

In another recent study (Saygun *et al*, 2004), 18 adults provided subgingival plaque samples from a periodontal abscess-affected site and a healthy control site. By using PCR they found a higher frequency of HCMV and EBV-1 DNA detection in abscess-sites than in healthy sites.

5. Detection of activated herpesvirus in the GCF of periodontal lesions

Contreras and Slots (1998) investigated the stage of activation of HCMV in six adult periodontitis patients and three juvenile periodontitis patients. They used the reversed transcription-PCR to examine mRNA transcription of subgingival HCMV. Specific primers for hybridization with the major capsid protein gene were applied to reveal active HCMV infection. HCMV major capsid protein transcripts were detected in deep periodontal pockets of two patients with adult periodontitis and two patients with localized juvenile periodontitis but not in any shallow periodontal sites of any patients. These findings suggested that active HCMV replication could occur in periodontal sites. It remained unclear if HCMV reactivation was related to the initiation or the progression of destructive periodontal disease.

A similar study was performed in 11 young adults with clinical and radiographic signs of localized juvenile periodontitis (Ting *et al*, 2000). Six patients were 10–14 years old. Five of them were HCMV positive; HCMV activation was found in deep pockets of all of these five positive patients. Five patients were older than 14 years; only three of them were HCMV positive and only one of them showed HCMV activation. No shallow site tested positive for virus activation. Herpesviruses could not be detected in every periodontal site in HCMV-positive individuals, and only in a fraction of positive sites was HCMV activation shown. The authors hypothesized that active HCMV infection could be associated with the initiation and the progression of localised juvenile periodontitis (LJP).

Herpesvirus reactivation may occur spontaneously or as a result of concurrent infections, fever, drugs, tissue trauma, emotional stress, exposure to ultraviolet light and other factors reducing the host immune defence. It is interesting to note that several recognized risk factors for periodontal disease, including HIV infection, emotional and physical stress, pregnancy and hormonal changes (Salvi *et al*, 1997) also have the potential to reactivate herpesviruses (Croen, 1991).This suggest that the underlying mechanism of several risk factors of periodontal disease may be the activation of the latent herpesvirus in periodontal tissues.

6. Interaction of herpesviruses with periodontal pathogens

Several studies examined a possible association between potential periodontopathic bacteria and herpesviruses in subgingival plaque. The subgingival detection of EBV-1, HCMV and other viruses could be associated with an increased presence of periodontal pathogens and periodontitis (Contreras *et al*, 1999a; Hanookai *et al*, 2000). In patients with localized juvenile periodontitis, the presence of *Actinobacillus actinomycetemcomitans* was associated with active HCMV infection (Ting *et al*, 2000). *Actinobacillus actinomycetemcomitans* tended to be more prevalent in samples showing active than latent HCMV infection. Similar data were generated in follow-up studies (Kamma *et al*, 2001; Slots *et al*, 2002; Kamma and Slots, 2003), examining the relationship in the occurrence of human herpesviruses and suspected periodontopathic bacteria in patients with early-onset periodontitis and subjects showing evidence of recurrent disease during the maintenance phase. Subgingival plaque samples were collected from two deteriorating and two stable periodontal sites in 16 subjects. HCMV, EBV-1 and HSVA were detected using a nested PCR method. *Porphyromonas gingivalis*, *Dialister pneumosintes*, *Bacteroides forsythus*, and *A. actinomycetemcomitans* were identified by 16S rRNA PCR. All herpesviruses were detected more frequently in active than in stable periodontal sites; the same was true for *P. gingivalis* and *D. pneumosintes*. The authors concluded that HCMV, EBV-1, HSV as well as *P. gingivalis* and *D. pneumosintes* were associated with active periodontitis and that immunosuppressive properties of herpesviruses could set the stage for overgrowth of subgingival periodontopathic bacteria. Statistically significant associations were established between the detection of herpesviruses, periodontopathic bacteria and several clinical variables. HCMV, as well as HSV was a significant predictor of the presence of subgingival *P. gingivalis*. In turn, *P. gingivalis* was positively associated with active periodontal disease, probing attachment level, probing pocket depth, bleeding upon probing, and patient age. Those data implicated HCMV, HSV and *P. gingivalis* as possible cofactors in the etiology of aggressive periodontitis, or as triggers of active tissue destruction.

Finally, a case report described the periodontal clinical and microbiological status of an 11-year-old boy having Fanconi's anemia (Nowzari *et al*, 2001). Samples of periodontal sites showed active HCMV infection *A. actinomycetemcomitans* could be cultured from these sites.

Challenges for the hypothesis of herpesviruses being involved in the etiology of periodontal diseases

In reviewing the evidence produced to implicate herpesviruses in periodontal destruction the following factors should be taken into consideration:

1. Investigators

Most clinical association studies have been carried out by the same group of investigators (Contreras and Slots, 1996, 1998; Parra and Slots, 1996; Contreras *et al*, 1999a,b, 2000, 2001; Hanookai *et al*, 2000; Ting *et al*, 2000; Kamma *et al*, 2001; Slots *et al*, 2002; Kamma and Slots, 2003; Kubar 2004; Saygun *et al*, 2004) or were performed in collaboration with them, and almost all

samples were analysed in the same laboratory. Confirmation by other, independent researchers is lacking.

2. Method
Gene amplification by PCR allows the detection of very small quantities of microorganisms or viruses through selective manipulation of DNA fragments. The so-called 'nested' PCR works with two primer pairs and two different amplification tests, one after the other, for one single target. Only the final amplification product, available after the second PCR reaction, is analysed. In addition to an enhanced sensitivity, it is believed that such a nested PCR test would also display higher specificity. However, just the opposite may be the case because this technology is very susceptible to contamination, and can produce false positive results (Burkardt, 2000).

Quantitative real time PCR might be a more appropriate method, allowing not only the detection, but also the quantification of a virus. In a recent study, this newer technology has been used to quantify HCMV in patients with aggressive periodontitis and in healthy subjects (Kubar 2004). Contrary to previous results, the virus DNA could not be detected in any healthy sites in this study.

3. Sample population
A higher frequency of co-infection and occurrence of EBV-2 and HHV-6 has been noted in HIV-positive than in HIV-negative periodontitis patients (Contreras *et al*, 2001). HIV was sought in two of the cited studies (Contreras and Slots, 1996; Parra and Slots, 1996); both of them in fact included some HIV positive subjects. In the other studies listed in Table 2, the HIV status was not investigated. As the serostatus was never determined, there remains some doubt whether the subjects included in several studies constitute a representative sample of the periodontitis affected population. A wide range of age and the unknown origin and socioeconomic status of study population constitutes a limitation in studies comparing different populations.

4. Inferences of causality
Considering the high frequency of virus detection in periodontal sites, the etiological role of viruses is difficult to establish. One difficulty directly relates to the sampling procedure: in diseased sites, with increased probing depths and bleeding on probing, higher volumes of GCF can be collected, and samples may contain blood cells more easily. In addition, such samples may contain increased quantities of subgingival biofilm. Thus, samples from diseased sites are more likely to contain viruses present in blood, independent of a specific association with the local disease process.

Active HCMV replication has been demonstrated in periodontal sites, and thus HCMV re-activation could have a triggering effect in the initiation or progression of periodontal disease. Here again, the association of two concurrent phenomena is not a proof of a cause and effect relationship. Herpesviruses are usually contracted in childhood, are capable of indefinite latency and may be reactivated under various conditions. Instead of the reactivation being the cause of periodontal disease activity, just the opposite may be the case: periodontal disease activity caused by bacterial infection may trigger virus reactivation.

Conclusions

Several studies, most of them from the same research group, have demonstrated an association of herpesviruses with periodontal disease. Viral DNA has been detected in gingival tissue, GCF and subgingival plaque from periodontaly diseased sites. In addition markers of herpesviral activation have been demonstrated in the GCF from periodontal lesions. Active HCMV replication in periodontal sites may suggest that HCMV reactivation triggers periodontal disease activity. Concerns regarding sampling, methods and interpretation cast doubts on the role of viruses as causes of periodontal disease.

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