Cytomegalovirus infection in symptomatic periapical pathosis

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Abstract

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Aim To compare the presence of human cytomegalovirus (HCMV) and Epstein–Barr virus (EBV) infections in samples from 25 symptomatic and 19 asymptomatic periapical lesions.

Methodology Periapical samples were collected by sterile curettes in conjunction with apicectomy. cDNA-based HCMV and EBV identification was performed on total mRNAs extracted from peripapical tissues, using primers for genes transcribed during the productive phase of the herpesvirus infection. Statistical analysis was performed using chi-squared test.

Results HCMV was detected in 100% of the symptomatic and in 37% of the asymptomatic study lesions. EBV was identified only in HCMV-infected periapical lesions. The difference in occurrence of HCMV and EBV between symptomatic and asymptomatic periapical lesions was statistically significant ($P < 0.0001$).

Conclusions The noteworthy finding of this study was the ubiquitous occurrence of HCMV active infection in symptomatic periapical pathosis. EBV may contribute to periapical pathogenesis in a subset of symptomatic lesions. HCMV and EBV infections may cause periapical pathosis by inducing cytokine and chemokine release from inflammatory or connective tissue cells, or by impairing local host defences resulting in heightened virulence of resident bacterial pathogens. Knowledge about the role of herpesviruses in periapical pathosis seems important to fully delineate the pathogenesis of endodontic infectious diseases. HCMV and probably EBV should be added to the list of putative pathogenic agents in symptomatic periapical disease.

Keywords: cDNA, Epstein–Barr virus, human cytomegalovirus, periapical pathosis, PCR.

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Introduction

Periapical lesions involve polymicrobial infections that can be acute or chronic and give rise to a variety of clinical and radiographic manifestations. The conditions that are causally involved in the initiation of periapical pathosis remain obscure. It is generally agreed that pulpal necrosis is a necessary antecedent but not the cause of periapical pathosis. Sundqvist (1976) showed that non-infected necrotic pulps did not result in periapical pathosis, whereas pulpal necrosis associated with pathogenic bacteria led to periapical breakdown. Black-pigmented Gram-negative anaerobic asaccharolytic rods seem to assume a major role in the development of many cases of symptomatic periapical disease (Sundqvist 1976, Sundqvist *et al.* 1989, Siqueira *et al.* 2001). Bacteria other than black-pigmented anaerobes have also been related to the flare-up of periapical inflammation and acute symptoms (Haapasalo 1986, Lewis *et al.* 1986, Hashioka *et al.* 1992, Gomes *et al.* 1996, Sabeti & Slots 2004). Nonetheless, most major endodontopathic bacteria seem to belong to the Gram-negative anaerobic group of microorganisms (Dahlén 2002). Acute periapical infection eventually turns into a chronic state of inflammation predominated by macrophages, lymphocytes and plasma cells (Nair 1997, Márton & Kiss 2000). However, many chronic periapical lesions develop without an antecedent acute phase. Although bacteria and host-related inflammatory responses are unquestionably involved in periapical pathosis, the pathophysiological events that

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trigger an acute exacerbation of endodontic disease remain uncertain.

Various herpesviruses, especially human cytomegalovirus (HCMV) and Epstein–Barr virus (EBV), have recently emerged as putative pathogens of marginal periodontitis (Slots 2002) and of periodontal abscesses (Saygun *et al.* 2004). Membership in the family Herpesviridae is based on the structure of the virion (Roizman & Pellett 2001). The prototypical structure of herpesviruses consists of a double-stranded DNA genome encased within an isosahedral capsid and an amorphous proteinaceous tegument, which is surrounded by a lipid bilayer envelope derived from the host cell membrane. Herpesviral replication takes place in the nucleus of the host cell and involves the expression of immediate-early, early and late classes of genes. Late (structural) genes are expressed during the productive (lytic) phase of herpesviral infections. After primary exposure, herpesviruses establish latency in various host cell reservoirs, from which they may reactivate periodically (Sissons *et al.* 2002). The main effector cells of the immune control of herpesviruses are CD8(+) cytotoxic T-lymphocytes, whose T-cell receptor complex recognizes major histocompatability complex (MHC) class I molecules of viral peptides on infected and professional antigen-presenting cells (Tortorella *et al.* 2000).

Given the similarity in pathological features between marginal and apical periodontitis, it is reasonable to assume that herpesviruses also play roles in periapical pathosis. As an extension of previous findings of a high rate of occurrence of HCMV and EBV in periapical lesions (Sabeti *et al.* 2003a,b, Sabeti & Slots 2004), the aim of this study was to delineate the relative importance of HCMV and EBV active infections in symptomatic periapical lesions. A model is proposed to clarify how herpesviruses may contribute to the pathophysiology of some types of aggressive periapical disease.

Materials and methods

A total of 25 symptomatic and 19 asymptomatic teeth with periapical lesions were studied. Symptomatic teeth exhibited swelling, pain, discomfort on biting, or sensitivity by percussion or palpation. Asymptomatic teeth revealed no signs or symptoms of acute periapical disease at the time of study. Periapical study lesions ranged in radiographic size from 2 × 2 to 15 × 16 mm. None of the study teeth demonstrated moderate or severe types of marginal periodontitis.

Periapical samples were collected in conjunction with apicectomy, which was being performed due to radiographic evidence of incomplete periapical healing following conventional root canal treatment. Prior to administrating local anaesthetics, the teeth, gingiva and mucosa of the sample area were washed with 0.12% chlorhexidine and patients rinsed with 0.12% chlorhexidine mouthwash for 30 s. Using a sterile No. 15 blade, an intra-sulcular incision was extended 1–2 teeth mesially and distally from of the study tooth, followed by a vertical release incision mesially. A full-thickness mucoperiosteal flap was then reflected exposing the periapical lesion area. Access through the cortical bony plate was obtained using a sterile explorer and/or No. 4 or 6 sterile high-speed surgical round burs with sterile water coolant in the area of osteotomy. Using a sterile curette, a periapical specimen for virological identification was placed in an empty plastic vial and immediately frozen.

cDNA methodology was used to identify transcription of herpesviral genes indicative of herpesvirus active infection (Sabeti *et al.* 2003b). PCR primers for HCMV were 5′-CCCGTCGTCGACGTCGTGATT-3′ and 5′-GGAAACACGAACGCTGACGT-3′ that amplify the virion tegument pp65 gene, which is transcribed late during the infectious cycle (Pande *et al.* 1991, Solache *et al.* 1999). Primers for EBV were 5′-TCCACCACACCCAGGCAC-3′ and 5′-TCTTACGAGCTGTAAGAGGG-3′ that amplify the EBV-encoded nuclear EBNA-2 transcriptional activating factor (Lin *et al.* 1993). Details of the standardization, sensitivity and validation of the cDNA methodology are described elsewhere (Tal-Singer *et al.* 1997). Positive and negative controls for HCMV and EBV included infected and non-infected leucocytes from human peripheral blood. The sensitivity of the PCR assay was 400 herpesvirus genomic copies, including the dilution factor of the samples. No cross-reactivity was observed with a variety of oral bacterial strains. Amplicons were identified in agarose electrophoretic gel, and visualized for size using a UV lamp at 320-nm wavelength.

Results

HCMV and EBV occurred more frequently in symptomatic than in asymptomatic periapical lesions ($P < 0.0001$; chi-squared test). As seen in Table 1, all symptomatic lesions showed presence of HCMV transcript whereas only 37% of asymptomatic lesions revealed the virus. HCMV and EBV coinfection was detected in 76% of symptomatic and in 26% of

Table 1 Human cytomegalovirus and Epstein–Barr virus transcripts in symptomatic and asymptomatic periapical lesions

Herpesviruses	Symptomatic lesions ($n = 25$)	Asymptomatic lesions ($n = 19$)
Cytomegalovirus (no Epstein–Barr virus)	6 (24)	2 (11)
Epstein–Barr virus (no cytomegalovirus)	0	0
Cytomegalovirus + Epstein–Barr virus	19 (76)	5 (26)
Neither cytomegalovirus nor Epstein–Barr virus	0	12 (63)

Values are given as *n* (%). $P < 0.0001$ (chi-squared test).

asymptomatic periapical lesions. HCMV monoinfection was identified in 24% of symptomatic lesions and in 11% of asymptomatic lesions, whereas EBV monoinfection was not observed in any of the 44 lesions studied.

Discussion

The present study provides compelling evidence that herpesviruses participate in the pathogenesis of symptomatic periapical pathosis. A remarkable 100% of the symptomatic periapical lesions showed HCMV transcript. That 18% of periapical lesions yielded HCMV as the sole virus studied, whereas no lesion revealed EBV moninfection, is suggestive of HCMV being the more important endodontopathogen of the two viruses. A previous study described only a single periapical lesion exhibiting EBV monoinfection (Sabeti & Slots 2004). However, similar to marginal periodontitis and various nonoral infections (Slots 2002), HCMV and EBV may serve as copathogens in severe cases of endodontic disease. The detection of HCMV–EBV coinfection in 76% of symptomatic periapical lesions points to the importance of herpesvirus interactions in endodontic infections. HCMV reactivation has potential to transactivate EBV, which may constitute a mechanism of increased pathogenicity (Aalto *et al.* 1998). As this study identified herpesvirus active infections, it is not known if the PCR-negative periapical sites harboured the study viruses in a latent stage.

Herpesvirus activation may induce significant immunosuppressive and immunomodulatory effects in periapical sites. Herpesviruses can trigger an array of host responses that include dysregulation of macrophages and lymphocytes and have as purpose to downregulate the antiviral host immune response (Boeckh & Nichols 2003). Host impairment includes silencing of natural killer cells, inhibition of apoptosis, and destruction of components of MHC class I pathway within macrophages, markedly impairing their principal role in antigen presentation (Michelson 1999). In addition, HCMV encodes a unique homologue of interleukin (IL)-10, a T helper (Th) cells type 2 cytokine that antagonizes Th1 responses, and its immunosuppressive properties may help HCMV circumvent detection and destruction by the host defence system (Kotenko *et al.* 2000). HCMV has also the ability to inhibit the expression of macrophage surface receptors for lipopolysaccharide, which impairs responsiveness to Gram-negative bacterial infections (Hopkins *et al.* 1996). HCMV can cause severe illness and death in people exhibiting a compromised immune system, including organ and bone marrow transplant recipients, HIV-infected people, individuals on immunosuppressive drugs and newborns infected during pregnancy (Pass 2001).

Herpesvirus infections also affect cytokine networks (Mogensen & Paludan 2001). Cytokines and chemokines play important roles in the first line of defence against human herpesvirus infections and also contribute significantly to regulation of acquired immune responses. However, herpesviruses are able to interfere with cytokine production or divert potent antiviral cytokine responses by a diverse array of strategies, which allow the viruses to survive throughout the lifetime of the host (Alcami & Koszinowski 2000, Tortorella *et al.* 2000). HCMV infection stimulates a Th1-dominance with a proinflammatory cytokine production of IL-1β, IL-6, IL-12, tumour necrosis factor (TNF)-α, interferon (IFN)-α/β, and IFN-γ (Mogensen & Paludan 2001) and prostaglandin E_2 (PGE_2) (Mocarski 2002). 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) (Mogensen & Paludan 2001). INF-γ, TNF-α and IL-6 exert particularly high antiviral activity. Even if proinflammatory activities basically serve a positive biological goal by aiming to overcome infection or

tissue invasion by infectious agents, they can also give rise to detrimental effects when a challenge becomes overwhelming or with a chronic pathophysiological stimulus. In an effort to counteract ongoing inflammation, the proinflammatory response triggers the release of anti-inflammatory mediators, such as transforming growth factor-β and IL-10 (Haveman *et al.* 1999). In addition, herpesviruses display great inventiveness when it comes to diverting potent antiviral cytokine and chemokine responses to their benefit (Tortorella *et al.* 2000). PGE_2, which is a key mediator of the periapical inflammatory response (Márton & Kiss 2000), increases rapidly in response to exposure of cells to HCMV, bacterial lipopolysaccharide and the cytokines IL-1β and TNF-α (Vane *et al.* 1998), and PGE_2 may under certain circumstances support HCMV replication (Mocarski 2002, Zhu *et al.* 2002). Undoubtedly, a periapical HCMV infection can induce a multiplicity of interconnected immunomodulatory reactions, and various stages of the infection may display different levels of specific inflammatory cells and mediators, underscoring the complexity of HCMV–host interactions in periapical disease.

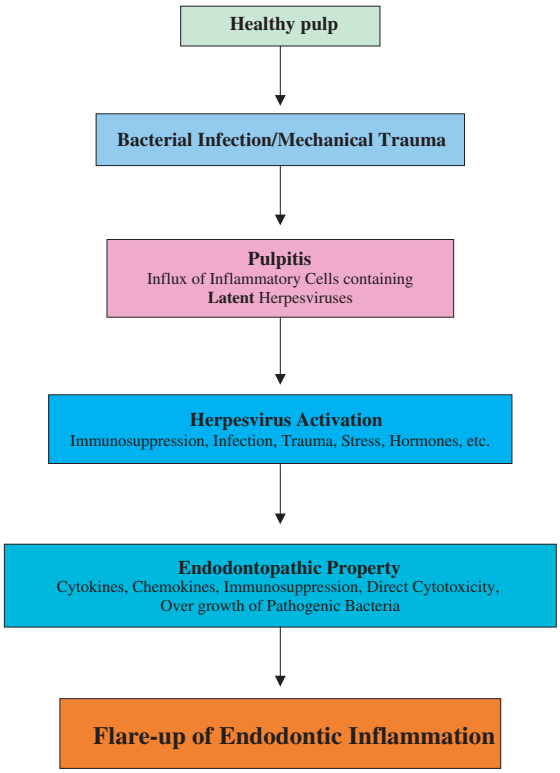

Figure 1 Herpesviruses in symptomatic endodontic pathosis.

Figure 1 suggests an infectious disease model for the development of periapical pathosis based on the concept of herpesvirus–bacteria–host interactive responses. Herpesvirus infection of periapical sites may be important in a multistage pathogenesis by altering local host defences. Initially, bacterial infection or mechanical trauma of the pulp cause inflammatory cells to enter pulpal and periapical tissues. In infected individuals, latent HCMV reside in periodontal macrophages and T-lymphocytes and latent EBV in periodontal B-lymphocytes (Contreras *et al.* 1999). Reactivation of herpesviruses from latency may occur spontaneously or during periods of impaired host defence as a result of immunosuppression, infection, physical trauma, hormonal changes, etc. Maybe not coincidentally, various herpesvirus-activating factors are also known risk factors for periapical flare-ups (Walton & Fouad 1992, Imura & Zuolo 1995). Herpesviral activation leads to increased inflammatory mediator responses in macrophages and probably also in resident connective tissue cells within the periapical lesion. After reaching a critical viral load, activated macrophages and lymphocytes may trigger a cytokine/chemokine 'storm' of IL-1β, TNF-α, IL-6, prostaglandins, interferons and other multifunctional mediators, which in an enclosed area have the potential to propagate states of pain (Vane *et al.* 1998, Rittner *et al.* 2002, Rutkowski & DeLeo 2002, De Jongh *et al.* 2003) and bone resorption (Stashenko *et al.* 1987, Wang *et al.* 1997, Lader & Flanagan 1998, Kawashima & Stashenko 1999). Several of the herpesvirus-associated cytokines and chemokines are prominent in periapical lesions (McNicholas *et al.* 1991, Lim *et al.* 1994, Takayama *et al.* 1996, Nair 1997, Wang *et al.* 1997, Kawashima & Stashenko 1999, Márton & Kiss 2000, Radics *et al.* 2003). Herpesvirus-induced immune impairment may also cause an upgrowth of resident Gram-negative anaerobic bacteria (Slots 2002, Sabeti & Slots 2004) whose lipopolysaccharide can induce cytokine and chemokine release from various mammalian cells and may act synergistically with HCMV in stimulating IL-1β gene transcription (Wara-aswapati *et al.* 2003). Moreover, in a vicious circle, triggering of cytokine responses may activate latent herpesviruses and in so doing may further aggravate periapical disease. However, it should be remembered that available data on herpesviruses in periapical pathosis are relating only statistical associations. To determine if herpesvirus activation is the cause or a consequence of symptomatic periapical disease, the proposed model for infectious periapical disease needs to be validated in animal

experiments and in prospective human studies. Definitive evidence of the importance of herpesviruses in periapical pathosis will probably require a selective suppression of the viruses in randomized trials.

In conclusion, endodontic inflammation can be initiated by a variety of infectious agents and is mediated by both cellular components, such as macrophages and leucocytes, and molecular components, including cytokines and chemokines, many of which possess pro- or anti-inflammatory properties, with harmful or beneficial effects. In this study, a strong statistical link was identified between HCMV and EBV productive infections and symptomatic periapical lesions. It can be hypothesized that reactivation of latent herpesviruses is involved in driving the pathological process of some types of symptomatic periapical disease. An important research question for the future is to determine the extent to which periapical herpesviruses influence the release of tissue-destructive cytokines and the progressive course of various types of endodontic disease. Vaccines that are currently being developed against herpesviruses warrant testing for their ability to induce a protective immune response against apical periodontitis. The detection of herpesvirus DNA in periapical lesions has brought a new dimension to our knowledge of periapical infections and calls for the inclusion of herpesviruses in studies on the pathogenesis of periapical pathosis. The present herpesviral findings may have future therapeutic relevance as well.

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