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Human Viruses as Risk Indicators for Periodontal Disease

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Background

Periodontitis is one of the most complex infectious diseases of the human body. Infectious agents must be able to colonize periodontal sites, overcome local host defences, proliferate in periodontal sites and participate in the breakdown of periodontal tissues (Slots and Genco 1984). Interplay between the periodontal infectious agent and the host immune responses reflects the dynamic which develops in a multistep process periodontitis. The oral cavity supports more than 700 bacterial species and the periodontal pocket area harbors more than 400 bacterial species (Parra and Slots 1996). Periodontopathic bacteria, such as Porphyromonas gingivalis and Tannerella forsythia, possess virulence factors involved in colonizing periodontal sites, neutralizing local host defences and destroying periodontal tissues, and individual periodontal lesions may harbor millions of genomic copies of herpesviruses as well as papillomaviruses, human immunodeficiency virus, human T-lymphotropic virus type I, torquesenovirus, hepatitis B and C viruses, Epstein-Barr Virus and cytomegalovirus (Amaliy 2012, Holt and Ebersole 2005, Saygun et al 2008, Slots 2009, Slots and Genco 1984, Slots and Ting 1999). Herpesvirus-infected periodontal sites tend to exhibit more breakdown than herpesvirus-free sites, and herpesviral active infection is associated with an elevated risk of progressive periodontal disease (Slots et al 2005). Additionally, healthy periodontal sites of periodontitis patients may harbor more herpesviruses than healthy periodontal sites of individual with generally healthy periodontium (Dawson 2009). The host immune response attempts to control both pathogenic bacteria and viruses in periodontal sites. However, it is still unclear if various immune mediators, such as certain cytokines and chemokines, exert a primarily protective or destructive role in periodontal disease.

Sufficient levels of proinflammatory substances protect against bacterial periodontopathogens, however excessive level will destruct periodontal tissue (Rateichak 2009). Successful immune control of a periodontal infection depends on a highly coordinated series of host defences (Cutler and Teng 2007). The host identifies pathogens as foreign bodies by recognizing pathogen-associated molecular patterns (Mahanonda and Pichyangkul 2007). Pathogen recognition receptors and signaling pathways subsequently activate cells of the immune system. Cytokines mediate the interaction and regulation of immune cells. Optimally, the host executes immune responses sufficient to control the pathogens, but also ensures suppression of excessive immune reactions in order to limit the pathological consequences of inflammation. If uncoordinated, the host...
immune response by itself may cause pathosis.

Herpesviruses

The eight human members of the herpesvirus family infect parenchymal cells, connective tissue cells, epithelial cells, various hematopoietic cells and other cell types, and can cause a variety of illnesses by mechanisms that are direct, indirect or immunoregulatory (Fellin 2007, Slots 2005, Slots 2009). In some, it causes gingivostomatitis or a subclinical infection and can then lie dormant in the trigeminal ganglion. In response to external stimuli such as stress, cold weather or virus infection, the virus can be activated and cause herpes labialis (cold sores) (Wade 2009). Current evidence strongly implicates viruses, particularly cytomegalovirus and other herpesviruses, in the pathogenesis of periodontitis (Amali 2012, Slots 2007, Slots 2010). The majority of adults are carriers of Epstein-Barr virus and cytomegalovirus. Once infected, a person harbors the herpesvirus for life. Clinical and experimental evidence show that viruses can directly affect the hystostasis of microbial population as well as modulate the host response to bacterial pathogens. Active herpesvirus infection evokes strong innate and adaptive immune responses, which include both immune activation and immune suppression (Crough and Khanna 2009, Mocarski et al 2007, Rickinson and Starff 2007). Key effector cells of the innate immune system are dendritic cells, monocyte/macrophage and natural killer cells, whose function is to limit the viral burden until cells of the adaptive immunity become available to suppress the infection. The effector cells recognize viral proteins via toll-like receptors, natural killer cell receptor or other pattern recognition receptors. Herpesvirus DNA reacts with toll-like receptor 9, which is significantly up-regulated in periodontitis lesions compared with gingivitis lesions (Kajita et al 2007a, Kajita et al 2007b). Cells of innate immunity employ cytokine secretion and cell-mediated cytotoxicity as the primary anti-herpesvirus effector mechanisms. Macrophages and polymorphonuclear leukocytes can destroy antibody-coated virions or virus-infected cells via reactive oxygen species, nitric oxide and activated caspases. Natural killer cells are an important source of interferon-gamma and are able to kill herpesvirus-infected cells via virus-specific antibody-dependent cell-mediated cytotoxicity or via antibody-independent mechanisms. In addition, natural killer cells share similarities with cytotoxic T cells and may play a role in adaptive immunity (Sun et al 2009).

Herpesvirus infections in immunocompetent individuals also induce antibody production against herpesvirus proteins and patients with periodontitis exhibit an elevated level of antibodies against herpesviruses, however it does not ensure a favorable clinical outcome (Hochman et al 1998, Kajita et al 2007a, Kajita et al 2007b, Svaan et al 2006).

Bacteria

Bacteria are the predominant microorganism in the human mouth. They are present in large numbers, of around 100 million per milliliter of saliva, and are the primary constituent of dental plaque, where they are around 1 billion per milligram. All types of bacteria are found; Gram-positive and Gram-negative, obligate aerobes, facultative anaerobes, obligate anaerobes, saccharolytic and proteolytic (Wade 2009). Bacterial infections evoke functionalities of both the innate immune system and the adaptive immune system. Bacterial species attach to specific toll-like receptors to establish some degree of specificity in the innate immune system and subsequently in the adaptive immune system (Hirschfield 2001, Kinane 2007). Bacterial pathogens detected by toll-like receptors
on the surface of macrophages activate NF-κB mediated transcription of cytokines and chemokines (Nagasawa 2007). Macrophage-released cytokines and chemokines recruit neutrophils in the innate immune system and serve as antigen-presenting cells for lymphocytes in the adaptive immune system. Neutrophils phagocytize and kill ingested bacteria by means of reactive oxygen species, anti-microbial proteins, degradative enzymes and other microbial pathways (Dennison 1997).

Extracellular pathogenic bacteria activate Th2 cells, which release anti-inflammatory cytokines and commit B cells to antibody production. *P. gingivalis* seems to primarily evoke a Th2-type response in periodontitis patients (Houri-Haddad et al. 2007). Antibacterial antibodies of the IgG1 subclass play an important role in opsonization and complement activation (Dennison 1997). Lipopolysaccharide of *P. gingivalis* and of other periodontopathic bacteria can also activate complement via the alternative pathway and induce the release of pro-inflammatory cytokines (Dixon 2004). Complement assists antibodies by acting as an opsonin, by lysing bacterial cells and by attracting lymphocytes and neutrophils to the site of infection.

Intracellular bacteria trigger a Th1-mediated immune response, which includes the release of interferon-gamma, pro-inflammatory cytokines and the IgG2 antibody isotype (Elkins 2007, Tithal 2008). IgG2 serum antibody occurs at high levels in patients with localized aggressive periodontitis, and despite being a less efficient opsonin than IgG1 and IgG3, seems to protect against tissue destruction (Schenkein 2007). *P. gingivalis, A. actinomycetemcomitans* and other periodontal species have the ability to invade cells of the periodontium and thus may trigger a Th1, as well as a Th2, immune response (Christersson 2002, Colombo 2006, Lamont 2002, Li 2008, Rudney 2005, Tribbie et al. 2009, Vitkov 2005). *P. gingivalis*-specific T cells can produce both Th1 and Th2 cytokines irrespective of the type of antigen-presenting cells (Gemmell 2002).

**Herpesviral-bacterial model of periodontitis**

The finding of abundant herpesviruses in periodontitis lesions redefines the pathogenicity of the disease. The core of the development of periodontitis proceeds from bacteria to herpesvirus to bacteria (Slots 2000). Initially, bacteria in the dental biofilm induce gingivitis, which permits latent herpesviruses, embedded in the DNA of macrophages, T lymphocytes and B lymphocytes, to infiltrate the periodontium (Contreras 1999). Cytomegalovirus can replicate in gingival tissue, which may help to sustain the periodontal infection (Hai 2006). Re-activation of the latent herpesviruses may occur spontaneously or during periods of decreased host defence, as a result of drug-induced immunosuppression, concurrent infection, unusual and prolonged emotional stress, hormonal changes, physical trauma, etc. Not coincidentally, most herpesvirus-activating factors are also suspected risk factors/indicators for periodontitis (Reddy 2007). In response to the active herpesvirus infection, the host elicits a robust T-cell mediated immune response, comprised primarily of CD8+ T cells. To counteract the hostile host environment, herpesviruses in turn execute strategies to down-regulate antiviral host defences. Herpesviruses evade immune responses by disintegrating components of the MHC and interfering with antigen presentation, by silencing natural killer cells, by expressing a viral homolog of IL-10, by diverting potent cytokine responses and by inhibiting apoptosis (Slots 2005). The encounter between antiviral host defence and virally mediated anti-host
Figure 1. Herpesviral-bacterial model of periodontitis (Slots 2009).
responses results in a major release of pro-inflammatory cytokines that have the potential to activate osteoclasts and impair antibody-mediated host defences against exogenous-like bacterial species, such as \textit{P. gingivalis} and \textit{A. actinomycetemcomitans} (Botero et al. 2008, Han 2007, Saygun et al. 2008). The ensuing increase in pathogenic bacteria provides additional mechanisms of periodontal tissue destruction (Holt and Ebersole 2005). Both cytomegalovirus and other herpesviruses can exert an acute cytopathogenic effect on fibroblasts, epithelial cells, keratinocytes, endothelial cells, inflammatory cells and bone cells (Britt 1996). An active herpesviral infection in severely immunocompromised patients may directly destroy periodontal cells and tissue by cytotoxic mechanisms, as seen in a patient with necrotizing ulcerative gingivitis and anoma. Herpesvirus infections may participate in oral collagen degradation, as suggested by \textit{in vivo} and \textit{in vitro} studies, and potentially interfere with periodontal tissue turnover and healing (Botero et al. 2008, Saboia-Dantas 2008).

In the herpesviral-bacterial model of periodontitis, herpesvirus-related cytopathogenic effects, immune evasion, immunopathogenicity, latency, re-activation from latency and tissue/site tropism comprise important aspects of periodontal pathosis. It is likely that the early stage of periodontitis in immunologically naive hosts involves an active herpesviral infection that primarily causes cytopathogenic effects, whereas most clinical manifestations in immunocompetent individuals are secondary to cellular or humoral immune responses. The proposed model may help to clarify at least some of the clinical features of periodontitis (Saygun 2004, Slots 2005). The propensity for site tropism of herpesviruses may explain why periodontal tissue destruction can differ markedly from tooth-to-tooth in the same patient or from surface-to-surface in individual teeth. A vigorous anti-herpesvirus host defence may ensure a prolonged period of periodontal stability, even in the presence of virulent bacteria. Herpesvirus re-activation from a latent state may trigger a burst of periodontal tissue damage and progressive disease. However, most immunocompetent individuals experience episodes of oral herpesvirus re-activation lasting only a few hours or a few days, the duration of which is too short to initiate or aggravate clinical periodontal disease (Mark 2008).

Conventional periodontal therapy can reduce the periodontal load of herpesviruses. Mechanical debridement has suppressed subgingival Epstein-Barr virus and cytomegalovirus to undetectable levels in patients (Saygun 2005, Wu 2006). After repeated debridement, many patients with periodontitis yielded no cytomegalovirus, but were found to have Epstein-Barr virus and herpesvirus-7, suggesting that cytomegalovirus is particularly susceptible to the effects of periodontal therapy (Rotola 2008). In patients with Papillon-Lefèvre syndrome, mechanical debridement and systemic amoxicillin-metronidazole suppressed subgingival Epstein-Barr virus, cytomegalovirus and \textit{A. actinomycetemcomitans} to undetectable levels and prevented further loss of periodontal attachment (Pacheco 2002). The decrease in post-treatment herpesvirus counts is probably caused by a reduction in gingivitis and thus in the number of virally infected inflammatory cells. Similarly, low herpesvirus counts in healthy periodontal sites are probably the result of a virtual absence of infected inflammatory cells. Cytomegalovirus was also not detected in healthy peri-implant sites (Nowzari 2008). The treatment data suggest that diseased periodontal sites are an important source of salivary herpesviruses. The potential of periodontal therapy to decrease herpesvirus levels in saliva may reduce the risk of herpesvirus transmission.
and herpesvirus-related disease among close acquaintances.

The herpesviral-bacterial model of periodontitis provides a rationale for considering new approaches to disease prevention and treatment. A patient who exhibited refractory periodontitis and high Epstein-Barr virus subgingival copy count was treated with valacyclovir HCl at 500 mg twice a day for 10 days. The treatment suppressed subgingival Epstein-Barr virus to an undetectable level for at least one year and resulted in a dramatic clinical improvement. Vitamin C supplementation for 90 days may improve plasma vitamin C levels, reduce periodontopathic bacterial load and reduce viral load of Epstein-Barr virus but not cytomegalovirus (Amaliya 2012, Sunde 2008).

Major advances in periodontal treatment to determine when antiviral intervention is appropriate are dependent upon screening of periodontal viruses using diagnostic DNA microarrays that are able to simultaneously detect herpes simplex virus and Epstein-Barr virus.

Future management of periodontal diseases may benefit from anti-viral immunotherapy; either prophylactic vaccines which harness the immune system of healthy subjects to prevent infection with decrease-causing viruses, or therapeutic vaccines, which stimulate the immune system into combating existing viruses and disease.

Conclusion

The etiopathogenesis of periodontitis includes virulence factors of herpesviruses and bacteria, host immune responses against viral and bacterial infections, and manipulation of host-cell processes by the infectious agents. Herpesviruses may induce periodontitis by triggering specific tissue-destroying pathways of the immune system and by predisposing an individual to bacteria carriage or increased bacterial load. However, ongoing research is required to better understand the molecular contribution of herpesviruses versus bacteria to periodontal pathosis.

An active herpesvirus infection correlates with periodontitis disease activity and may be a major contributor to the periodontal immune response. Herpesviruses are potent inducers of proinflammatory cytokines that have the potential to activate osteoclasts and matrix metalloproteinases. An active herpesvirus infection can also impair antibacterial immune mechanisms and potentially cause an upgrowth of periodontopathic bacteria. Some periodontopathic bacteria may reactivate a latent herpesvirus infection. Synergism among herpesviruses and bacteria may play an important role in the onset and progression of periodontitis. The inflamed periodontium appears to be a major site for Epstein-Barr virus and cytomegalovirus accumulation and re-activation, especially in the progressive phase of periodontal disease. Immunosuppressive factors are potential triggers of herpesvirus re-activation and, perhaps for that reason, are also major risk factors for periodontitis.

Conventional periodontal therapy, systemic amoxicillin-metronidazole, anti-herpesvirus drugs or supplemental vitamin C can reduce the periodontal load of herpesviruses. Therapeutic treatment to control herpesviruses by vaccination will help develop fields for future investigation.

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