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EDITORIAL

HERPESVIRUSES AND PERIODONTAL DISEASE: A CAUTIONARY TALE

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Periodontitis is an inflammatory disease of bacterial origin, characterized by an inconstant progression of lesions affecting the tooth supporting tissues. In spite of more than half a century of research efforts, the clinician still lacks any specific molecular or microbial diagnostic tool to predict the progression of periodontal lesions. Recently, several reports have proposed a role for some herpesviruses in the etiology of destructive phases of periodontitis. This paper critically analyzes these data in the light of consolidated knowledge that was developed in the characterization of virus-bacteria cooperative interactions, and proposes new topics of investigation to clarify the role of herpesviral infections in periodontitis and their potential predictive role as markers of progression.

Periodontitis comprises a number of different infectious disorders characterized by inflammation and a generally inconstantly progressive breakdown of the tooth-supporting tissues. In patients at risk the clinical history of periodontitis can show shorter or abolished periods of remission leading to rapid severe tooth mobilization and loss. To date, no definite molecular or microbiological markers of disease progression have been identified that can be applied to routine diagnostics.

The etiology of periodontitis – general concepts

Although the bacterial origin of periodontal disease is not to be disputed, its etiopathogenesis is still largely obscure, as a consequence of several problems: i) the periodontal microbiota is a large and complex community (about 400 potential periodontal bacteria, organized in communities of 100–200 cultivable species in a single individual) (1); ii) molecular approaches have shown that about half of the hundreds of bacterial species colonizing subgingival sites have never been cultured (2), and we lack any information on their pathogenicity and virulence, though are aware that some of them positively correlate with periodontal disease (3); iii) pathogenic species have been shown to include strains characterized by significant genomic diversity in regions coding for determinants of pathogenicity and virulence (4); iv) the taxonomic collocation of several periodontal bacteria is still uncertain; v) periodontitis is a clinical complex and not just a single disease.

Few species are considered as true periodontal pathogens, including *Actinobacillus (Aggregatibacter) actinomycetemcomitans*, *Porphyromonas gingivalis*, *Tannerella forsythia*,

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and *Dialister pneumosintes*, while other species are thought to participate in tissue damage generation, though are unable to initiate infection, including *Prevotella intermedia*, *Prevotella nigrescens*, *Campylobacter rectus*, streptococci, staphylococci, enteric rods and *Pseudomonas aeruginosa* (5).

In recent years, several reports have suggested a possible role for different human herpesviruses as major pathogens in aggressive periodontitis (6). This hypothesis is interesting, since viruses are actually being considered as strong promoters of bacterial infections, and the biological behaviour of herpesviruses fits well into the clinical history of periodontitis.

Virus-bacteria mixed infections

The relevance of viral infections for the incidence and progression of bacterial infections was almost neglected until it became of dramatic actuality during the pandemic Spanish flu in 1918, when more than 40 million people died, prevalently from secondary bacterial pneumonia. In the following decades a number of reports showed that influenza viruses predispose to severe bacterial pneumonia. For more than half a century afterwards, researchers argued that viruses damage the mucous membranes and allow bacterial pathogens a foothold to start acute infections (7). According to a later hypothesis, viruses promote bacterial infections by altering local immunity effectors to cause leukopenia (8). This mechanism is difficult to accept, since leukocytosis is the most frequent feature in mixed infections (7), and bacterial cytotoxins, rather than viruses, account for most of the dysfunctional effects influencing leukocyte functions in these cases (9).

The results of recent research contributed to demonstrating that these cooperations are prevalently multi-factorial (10). However, viral multiplication within host tissues may induce significant damage to mucous membranes that can prime tissues for subsequent bacterial colonization and infection. These alterations must be rather specific since bacterial infections have been shown to be promoted by viruses, irrespective of their epithelial damaging potential and, more importantly, chemically-induced epithelial damage does not predispose to bacterial colonization and/or infection (11).

Modifications affecting epithelia

An increasing number of reports show that different virus-induced modifications at the surface of epithelia are central in favouring bacterial colonization. In particular, alteration of mucous secretions, by increasing their content in fibrin, serum proteins, and inflammatory mediators, creates an excellent nutritional environment for bacteria that may find optimal conditions to colonize host tissues and to express their virulence potential (12). Moreover, virus multiplication may modulate bacterial adhesion, colonization and/or internalization by inducing modifications of the presence and concentration of specific cell-membrane receptors recognized by bacterial adhesins. In fact, i) bacteria may recognize specific viral proteins exposed at the surface of infected cells, acting as specific receptors (13); ii) virus-dependent cellular responses may induce *de novo* synthesis and/or over-expression of surface proteins recognized by colonizing bacteria (14); iii) viral enzymes may expose cryptic receptors (15), and iv) fibrin, fibronectin, collagen and other matrix elements deposited during the regenerative process may provide attachment sites.

Effects of virus infection on immune responses

Using a variety of different mechanisms, viruses are able to subvert the innate and acquired immune responses, and this ability may be exploited by pathogenic bacteria to colonize and multiply within virus-infected tissues. Innate immunity, through the activity of pattern recognition receptors, plays a primary role in the host defence against pathogenic microorganisms by activating different signalling cascades that generate an inflammatory response. The activation of the innate immune response by both viruses and bacteria, contemporaneously colonizing the same host tissues, leads to the activation of signalling cascades that in turn generate humoral and cellular responses at a rate that is not merely additional (13, 16). This activation results in the production of proinflammatory cytokines and chemokines (IL-1, IL-6, TNF-α, MIP-1-α, and γ-interferon), as well as of the anti-inflammatory cytokine IL-10 (17). These conflicting stimuli can prove dysfunctional, depriving professional phagocytes of their potential to clear the pathogens (11).

Herpesviruses and their potential role in periodontitis

Research studies published in the last decade indicate that herpesviruses could play an important role as putative periodontal pathogens or as co-pathogens (6). Most reports deal with the role of the Epstein-Barr virus (EBV) and of type 1 human cytomegalovirus (HCMV) in aggressive periodontitis, but representatives of all the three sub-families of herpesviruses have been associated to periodontitis.

Herpesviruses developed persistence, as perhaps their most relevant feature, to be able to co-evolve with their hosts for long periods of time. While relatively harmless in immunocompetent hosts, they can cause diseases with significant morbidity and mortality in immunocompromised patients. Herpesviruses have a biphasic infection cycle consisting of latent and lytic phases, so that, following the primary infection, they usually show lifelong persistence. Eight herpesviruses infect humans: the alphaherpesviruses herpes simplex virus 1 and 2 (HSV-1 and -2), and varicella-zoster virus (VZV), the betaherpesviruses HCMV and human herpesviruses 6 and 7 (HHV-6 and -7), and the gammaherpesviruses EBV and Kaposi sarcoma-associated herpesvirus (KSHV). Of these, HCMV, EBV and, to a minor extent, HSV-1 have been repeatedly associated with periodontitis (18-21).

All three of these viruses are able to infect the gingival epithelia, and HCMV and EBV can affect granulocytes and monocyte-derived macrophages or B lymphocytes. Persistent infections caused by these three viruses are extremely diffused in the world population (22) and reactivations may frequently occur subclinically. Herpesviruses try to evade specific antiviral responses of the host by a complex network of actions, including interference with the activation of major histocompatibility complex class I and class II-restricted T lymphocytes and natural killer cells, modification of the function of cytokines and their receptors, interaction with complement factors, and modulation of signal transduction and transcription factor activity and other cellular functions.

Thus, the ability of herpesviruses to alter cellular surfaces and to subvert the immune response makes them likely candidates, probably in cooperation with opportunistic bacterial pathogens, to play a role in the development and/or in the progression of periodontal diseases.

Herpesviruses and their putative role in periodontitis

In spite of several years of intensive research in the field, a univocal and undisputed demonstration of the role of herpesviruses in the etiology of periodontitis has not yet been provided. Evidence of a role of herpesviruses in the etiology of periodontitis relies upon the following considerations: a) different herpesviruses have been detected in gingival tissues, both in the epithelial component and in fractions containing neutrophils, monocytes/macrophages, T and B lymphocytes (23-25); b) herpesviruses (mainly HCMV and EBV) have been detected with higher frequencies in the gingival tissues of periodontitis sites than in healthy sites (25); c) herpesviruses can be detected at higher frequencies in the gingival crevicular fluid from periodontally-diseased sites than from gingivitis and/or from healthy sites (26-30); d) herpesviruses (mainly HCMV and HSV-1) have been detected at higher frequencies in subgingival plaque samples from chronic periodontitis sites (31). Moreover, if aggressive periodontitis-affected sites or periodontal abscess sites alone are considered, EBV and HCMV correlate at highly significant rates with disease activity (32-33); e) HCMV-specific replicative mRNAs can be detected in the crevicular fluid of patients with active sites of adult and localized juvenile periodontitis (26, 29); f) several recognized risk factors for periodontal disease, including HIV infection, emotional and physical stress, pregnancy and hormonal changes, are well-known factors implicated in the reactivation of herpesviruses; g) herpesviruses can interact with periodontal pathogens (34). A positive correlation was found between active infection by HCMV and isolation of *A. actinomycetemcomitans,* both from a qualitative and a quantitative point of view (29, 35). Positive correlations have been found also for other periodontal pathogens such as *P. gingivalis*, and *D. pneumosintes* (35). In these cases the correlation was extended also to EBV and HSV-1, suggesting that the immunosuppressive effects of herpesviruses may play a relevant role in these cases.

Although interesting and fascinating, the above-

discussed data deserve some critical considerations that have been recently drawn (36). In synthesis, it was noted that: i) the absolute majority of studies that associate herpesviruses to periodontitis have been substantially carried out by a single group of investigators. Data from totally independent laboratories are expected to fully confirm these data; ii) most of these studies were performed with false positive prone techniques and were not confirmed, or were confirmed only in part when more reliable techniques were used (33); iii) only a limited number of patients were studied who were not characterized for the presence of predisposing conditions, such as positivity to HIV (27-28), and for other important epidemiologic parameters.

Infection by herpesviruses is mainly contracted in childhood and only a few subjects are spared. Consequently, the majority of adults carry one or more latent herpesviruses in their gingival tissues, as supported by the high positivity rates for herpevirus DNA in gingival tissues that have been reported.

Thus, in the absence of any other strong biological evidence, it is quite impossible to definitely assess a direct etiologic role for these viruses in periodontitis, especially considering that very often viral reactivation coincides with activation of periodontitis. These observations cannot exclude that herpesviruses may be reactivated within periodontal sites by the activity (cellular damage, etc.) of periodontal pathogens.

More data regarding precise pathogenetic relationships between herpesvirus, periodontal pathogens and periodontitis are certainly needed to draw any definite conclusion on the role of these viruses on periodontal diseases. Innovative *in vitro* and *in vivo* models together with evaluation of patients suffering of periodontitis, treated with combinations of anti-bacterial and anti-viral drugs, are needed to fully elucidate this point. In this context, recently Sunde and co-workers (18) observed that, with few exceptions, in the above-discussed studies, positive samples were always near to the detection limit of the adopted technique. If the impact of viral replication on the bacterial environment is real, then it should be expected that the bacterial profile differs between sites with or without virus, which was reported to be real only in few cases (25, 29).

An insight into pathogenetic mechanisms

Among the several pathogenetic mechanisms that may explain the potential role of viruses in a cooperative interaction with bacterial pathogens, an alteration of cellular receptors enhancing susceptibility to bacterial adherence or internalization in virus-infected cells should be investigated in detail as a potential mechanism. This hypothesis was first investigated by Sanford and colleagues (1978) (37) in a pioneer study that constitutes a basic experimental model still adopted in many laboratories. However, only one report is at present available that examines this issue in relation to periodontitis. Teughels and colleagues (2007) (20) explored the interaction between HCMV and *A. actinomycetemcomitans* in an *in vitro* epithelial adherence assay. They infected HeLa cells with HCMV prior to bacterial challenge, and demonstrated a significant increase of bacterial adherence to HCMV positive cells. This phenomenon has been observed for *Salmonella typhimurium* on semi-permissive HCMV-infected A549 epithelial cells (38). The relative enhancement of adherence in the case of *A. actinomycetemcomitans* was similar to that reported for other viral-bacterial interactions, and its magnitude was bacterial-strain-dependent.

Since it had been reported that inactivated viruses could enhance bacterial invasivity (14), infections with inactivated viruses were carried out (38), and a significant increase in adherence (though to a lesser extent to that induced by active viruses) was reported. Similar interactions were reported for Coxsackie virus (39) (possibly due to host cell membrane alterations by decreasing the *Zeta* potential or increasing the cellular cation concentration) and for Rhinovirus (14) (due to effects on cellular receptors). No experiments were addressed to this point by the authors, although experiments performed in different conditions suggest that HCMV-infected epithelial cells secrete factors that exert a paracrine effect on the adjacent epithelium, and thus augment bacterial colonization (10). The significance of these findings in the development of periodontitis remains unclear. Nevertheless, it is likely that synergistic interactions between microorganisms in the oral cavity have important implications in the onset and progression of periodontitis.

Concluding remarks

The hypothesis of a possible role for herpesviruses

as inducers of the genesis and/or progression of periodontal disease is certainly a fascinating one. To date, available data do not allow to draw any definite conclusion, the interpretation of these results not being univocal. Much work remains to be done, and precise molecular mechanisms must be identified together with their effective role in the onset and progression of periodontitis as demonstrated, both *in vitro* and *in vivo*, by multidisciplinary approaches.

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