Literature Review

The role of immune system in the development of periodontal disease: a brief review

O papel do sistema imune no desenvolvimento da doença periodontal: uma breve revisão

Abstract

Periodontitis is a highly complex and multi-factorial disease. This review summarizes some immunological factors involved in the development and control of this oral disease, such as: the participation of inflammatory cells in local inflammation, the synthesis of chemotaxis proteins with activation of the complement system and a range of antimicrobial peptides, such as defensins, cathelicidin and saposins. The integration of pathogen-associated molecular patterns (PAMPs) from microorganisms with their surface receptors in the immune cells, induces the production of several cytokines and chemokines that presents either a pro- and/or anti-inflammatory role by stimulating the secretion of a great variety of antibody subtypes and the activation of mechanisms of controlling the disease, such as the regulatory T cells. Although several studies have tried to clarify some of the immune mechanisms involved in periodontal disease, more studies must be conducted to understand its development and progression and consequently to discover new alternatives for the prevention and treatment of this severe inflammatory disease.

Key words: Immunology; periodontitis; cytokines; chemotaxis; antibodies

Resumo

A periodontite é uma doença altamente complexa e multifatorial. Esta breve revisão reúne alguns fatores imunológicos envolvidos no desenvolvimento e controle desta doença oral, tais como: a participação de células inflamatórias no local da inflamação, a síntese de proteínas quimiotáticas através da ativação do sistema complemento e a presença de alguns dos peptídeos antimicrobianos, como defensinas, catelicidinas e saposinas. A interação de padrões moleculares associados à patógenos (PAMPs) de microrganismos com seus receptores de superfície, em células imunológicas, induz a produção de várias citocinas e quimiocinas que apresentam função pró- e/ou anti-inflamatória estimulando a secreção de uma grande variedade de subtipos de anticorpos e a ativação de mecanismos de controle da doença, como as células T reguladoras. Embora vários trabalhos tentem esclarecer alguns dos mecanismos imunológicos envolvidos na doença periodontal, estudos adicionais são necessários para ampliar conhecimentos sobre o desenvolvimento, a progressão e, consequentemente, para se descobrir novas alternativas de prevenção e tratamento desta grave doença inflamatória.

Palavras-chave: Imunologia; periodontite; citocinas; quimiotaxis; anticorpos

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Introduction

Periodontal disease is a chronic bacterial infection that affects the gingiva and bone that supports the teeth. This chronic inflammatory disease results from the response to bacteria in dental biofilm and may remain confined to the gingival tissues with minimal tissue alterations or this disease may progress to extreme periodontal destruction with the loss of attachment and alveolar bone. In addition to the presence of periodontopathogens; such as *Porphyromonas gingivalis, Aggregatibacter actinomycetemcomitans* and *Tannerella forsythia*; genetic and environmental factors seem to increase the susceptibility of some individuals in developing this severe inflammatory disease (1) (Fig. 1). Therefore, there is general support for this concept of periodontal disease. It is also well recognized that the presence of only pathogenic bacteria is insufficient to cause periodontitis. Progression of this disease occurs due to a combination of factors, including the presence of periodontopathic bacteria, high levels of proinflammatory cytokines, matrix metalloproteinases (MMPs), prostaglandin E2 (PGE2), low levels of anti-inflammatory cytokines including inter-leukin-10 (IL-10), transforming growth factor (TGF-β) and tissue inhibitors of MMPs (TIMPs) (2,3). However, the immune response initiated by periodontal disease seems to be much broader. Therefore, this review summarizes some immune mechanisms involved in periodontal disease.

Innate immune response in periodontal disease

Histological examination of gingivitis or periodontitis lesions reveals that the polymorphonuclear leukocyte (PMN) appears to play a key role in the maintenance of the periodontal health (3). These cells are present in the junctional epithelium in large numbers and appear to wall off the underlying tissues from the bacterial biofilm. The presence of these PMNs is the result of the existence or generation of chemotactic factors in the gingival sulcus and underlying tissues (4). Extra-oral infections and early severe forms of periodontitis frequently affect patients with diseases such as leukocyte adhesion deficiency and chronic or cyclic neutropenia. Molecular defects in PMNs, with a variety of functional consequences, result in accelerated periodontitis. The failure of PMNs to transmigrate into the endothelium results in an increase on the inflammatory response and reduces the protective response against periodontal pathogens (5).

Periodontal epithelium provides a physical barrier to infection and has an active role in the innate host defense, because the epithelial cells are in constant contact with bacterial products (6). In the presence of active disease, the epithelial migration causes a deep periodontal pocket resulting in bacterial invasion, inflammation and destruction of the connective tissue, with subsequent bone loss and possible tooth loss. The epithelium can participate in the infection by signaling further innate and acquired immune responses (Fig. 2). Epithelial cells may also respond to bacteria by increasing their proliferation, by altering their

cell signaling events, and by changing the cell differentiation and cell death and altering tissue homeostasis. Langerhans cells and dendritic cells of bone marrow origin that are located within the epithelium are a connecting link with acquired immunity. Langerhans cells in the epidermis and oral mucosa are responsible for communication with the immune system (7).
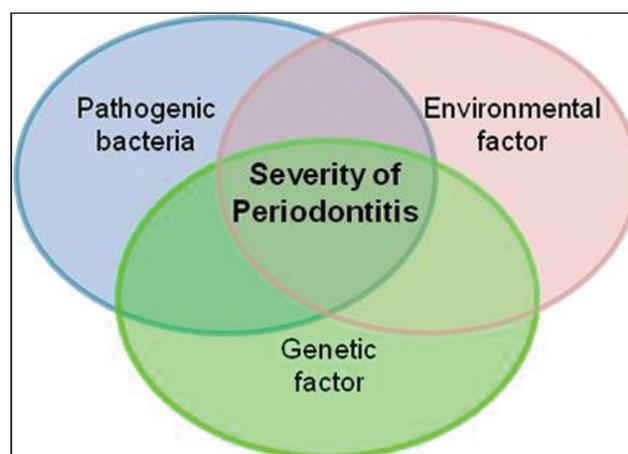

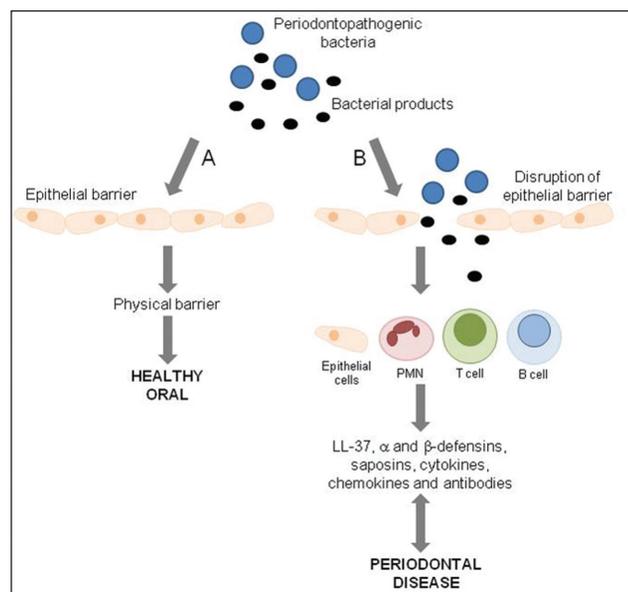**Fig. 1**. Factors involved in the severity of periodontitis.

Fig. 2. Some mechanisms involved in periodontal disease. (A) The intact epithelial barriers may delay the entry of periodontopathogenic bacteria and their products in oral tissues, maintaining the health of periodontum (B) Disruption of epithelial barriers allows the entry of periodontopathogenic bacteria and their products in the tissues host. In response, cells of the innate and adaptive immunity are activated and produce LL-37, defensins, saposins, cytokines and chemokines against invasors agents. PMN, polimorphonuclear cells.

It is now recognized that epithelia throughout the body produces a diverse range of antimicrobial peptides in at least four families (α-defensins, β-defensins, cathelicidins, saposins) that have been found in humans (8). These peptides have been associated with saliva or are present in the dentogingival junction region. The oral sulcular pocket and junctional epithelia of the gingival are all associated with the expression of defensin, more specifically β-defensins hBD-1, hBD-2 and hBD-3 (6). The integrity of the epithelial barrier is specifically disrupted by different microbial pathogens that attack cell-cell junctions and thereby dissociate cells from each other (9). Families of natural antibiotic peptides or proteins are expressed in epithelia and by neutrophils. These proteins have an activity against Gram-positive and Gram-negative bacteria, as well as against yeast and some viruses. These antimicrobial peptides function by associating with the anionic microbial surface, then aggregating to form pores or disrupt the microbial membranes, although new evidence potentially suggests additional cytoplasmic targets (10). These peptides complement the antimicrobial factors of saliva, such as the histatins, lyzozyme, and salivary immunoglobulins, having a specific role in the innate host defense in response to an infection (4).

The α-defensins and cathelicidin LL37 are proteolytically active peptides, present in high levels in neutrophils that migrate through the junctional epithelium to the gingival sulcus (8). Some investigators believe that the primary role of β-defensins may be to signal other innate and acquired immune responses while LL37 and α-defensins may be most important for their antimicrobial properties in the gingival sulcus (11). These peptides are capable of activating the classical complement pathway and appear to upregulate IL-8 production by epithelial cells, which may enhance neutrophil recruitment to the site of infection (12).

Innate immunity has a considerable ability to recognize bacteria as non-self agents because these microorganisms present PAMPs in the bacterial wall, which are recognized by pattern recognition receptors (PRRs) on immune cell surfaces. PAMPs are invariant and represent conserved molecular patterns that are essential for microbial survival. They are found in bacterial lipopeptides, peptidioglycan, flagellin and DNA, with others that are specific either for Gram-negative (lipopolysaccharide (LPS)) or Gram-positive (lipoteichoic acid) bacteria (4,13). The toll-like receptor (TLR) family is the best characterized class of PRRs and detects multiple PAMPs (14,15). Mammalian TLRs comprise a large family that consists of at least 11 members. TLRs 1-9 are conserved between humans and mice, and each of these recognizes a unique molecular pattern associated with different classes of pathogens. TLR4 recognizes LPS, which is a major cell wall component of Gram-negative bacteria (16). Reports have suggested that TLR2 may recognize several atypical types of LPS from *Leptospira interrogans* and *P. gingivalis*. TLR2 and TLR4 recognize bacterial components that are mainly present in the bacterial cell membrane. TLR5 recognizes flagellin, which is a protein component of the flagella

extending out from the outer membrane of Gram-negative bacteria. TLR9 recognizes unmethylated CpG motifs that are found in the bacterial genomic DNA and also in viral DNA (15,17).

TLRs are expressed on a variety of cells, including both lymphoid and nonlymphoid cells, and on various epithelial surfaces, including dendritic cells. TLR2, TLR3, TLR4 and TLR5 are differentially expressed on oral, bronchial and gastrointestinal epithelia. Pathogen recognition by TLRs expressed from epithelial cells leads to the production of cytokines, chemokines and antimicrobial peptides that induce the recruitment of more inflammatory cells to the infected sites (4,18). The interactions of TLRs with commensal microorganisms are also required to maintain epithelial homeostasis (19).

Bacterial LPS can subsequently interact with macrophage or dendritic cell receptors, including CD14 and TLRs, to stimulate the production of inflammatory cytokines and other mediators (4). Some authors have indicated that the model of tissue destruction, which focuses on the production of IL-1 as a key mediator of periodontal tissue destruction, includes the stimulation of collagenolytic and bone-destructive agents, such as MMPs and PGE_2 (4,20). It is important to note that cells other than macrophages are present in periodontal lesions (such as fibroblasts) which also produce inflammatory cytokines, lipid mediators, and MMPs and are likely to participate in the accumulation of these molecules (15,21,22).

Adaptive immune response in periodontal disease

The adaptative immune response is activated when the epithelial barrier, with its antimicrobial peptides and other components of innate systems, are breached (Fig. 2). Cytokines or interleukins are integral with this response and represent intercellular messengers (23,24). In agreement with Gemmell et al. (2), the immune response to infection is regulated by the balance between T helper (Th) 1 and Th2 cytokines. The differentiation of Th1 and Th2 T cell subsets is determined by a number of factors, including the antigen itself, antigen dose, route of administration, nature of the antigen-presenting cell and co-stimulatory molecules. IL-18, as a cofactor with IL-12, is recognized as a cytokine that is able to enhance the maturation of naïve T cells to Th1 cells (25). However, there is not a consensus about the Th1/Th2 immune response in periodontal disease. Some studies have shown a decrease in Th1 responses in periodontitis, while others have shown increased Th2 responses. However, other studies have indicated a dominance of the Th1 response over the Th2 response, with other studies showing a predominance of Th0 cells in periodontitis (25,26). This variable knowledge confuses the current understanding of the development and control of periodontitis.

By the end of the 19th century, studies recognized that diapedesis was a fundamental mechanism of the host defense, as this mechanism involved the process of leukocyte emigration. Leukocyte invasion of tissues can be induced by several substances, including IL-1, tumor

necrosis factor-α (TNF-α), and bacterial LPS when injected *in vivo* (27). However, in 1996, other chemokines (from chemotactic cytokines) have raised considerable interest for their selective recruitment and activation of leucocytes (27). Chemokines are a large family of small proteins that are structurally similar to heparin-binding proteins, which are classified into 4 subfamilies according to the configuration of cysteine residues near the N-terminus, depending on whether the first 2 cysteines are separated (CXC, CX3C) or not (CC, C) by an intervening amino acid (28). Chemokine receptors are named according to the family of their ligands, with the two major subfamilies designated CCR and CXCR. These are synthesized by several cell types, including endothelial, epithelial, and stromal cells, such as fibroblasts, mast and bone cells, as well as leukocytes (27). Interestingly, in addition to the crucial role of chemokines in cell trafficking, chemokine messengers initiate signal transduction events leading to other biological processes, such as angiogenesis, cell proliferation, apoptosis, tumor metastasis, and host defense (28).

On the other hand, related to periodontal disease, Boyle et al. (29) suggested that the integrity of bone tissues depends on the maintenance of an equilibrium between bone resorption by osteoclasts and bone deposition by osteoblasts. The major regulatory mechanism of osteoclast activity seems to be performed by members of the TNF family of receptors, RANK (receptor activator of nuclear factor-β), osteoprotegerin (OPG), and the RANK ligand (RANKL). RANK is expressed on osteoclastic precursors and on mature osteoclasts, while RANKL, a transmembrane protein, is expressed particularly on osteoblasts under homeostatic conditions. Interactions between RANK and RANKL are required for the differentiation and activation of osteoclasts, an event regulated by OPG, which strongly inhibits bone resorption by preventing RANK-RANKL engagement (29). Interestingly, RANKL also induces the production of some substances, such as MCP-1/CCL2, which could contribute to bone resorption (30). Osteoblasts are found to express several chemokines receptors during synthesis, which can modulate their function through the binding of chemokines. Additionally, an osteoclast can produce important chemokines which are involved in the recruitment of neutrophils and different lymphocyte subsets, suggesting an interesting role for osteoblasts in the development of the inflammatory immune reaction (31). Furthermore, the production of chemokines, with the consequent chemoattraction of inflammatory cells in the bone environment, may contribute to the disruption of bone homeostasis, resulting in tissue destruction (32). Therefore, the subsets of leucocytes the form the inflammatory infiltrate, Th1 or Th2 cells, can determine the level of bone destruction/deposition because these population cells differ in their migratory properties (33).

Both T and B cells are present in periodontal disease tissues. The infiltrate in the periodontal lesion consists of lymphocytes, macrophages, neutrophils and mast cells that migrate to the tissue, guided by the different concentrations of chemokines and cytokines. INF-γ (Th1 response) produced by T cells would enhance the phagocytic activity of macrophages and neutrophils and contain the infection. However, if there is IL-4 production (Th2 response), B cells are activated and start antibody production (34). Studies have verified the strong antibody responses against specific bacterial antigens, such as the LPS or leukotoxin of *A. actinomycetemcomitans* and the LPS or hemaglutinin of *P. gingivalis*, which were associated with less severe disease in patients with aggressive or chronic periodontitis (4,35,36). The protective role of local antibody production, as detected by elevated concentrations of gingival crevice fluid antibodies, is unclear (37). Some researchers have suggested that, in the chronic phase of the disease, the antibody response is generally protective, facilitating bacterial clearance and arresting the progression of the disease (38).

Regulatory T cells

Beyond Th1 and Th2 subpopulations of T cells, which determine the response to infection based on the cytokine pattern induced, a distinct subset of CD4+ T cells, called regulatory T cells, has received intense focus since last decade, due to their role in the regulatory network that controls immune responses. Three distinct regulatory T (Treg) cells have been described. Naturally occurring Treg cells, CD4+and CD25+ T cells, originate directly from the thymus during the early stages of fetal and neonatal T cell development. These cells make up approximately 5-10% of the peripheral T cell pool and constituently express several suppressing mediators, such as CD25 (α chain IL-2 receptor), glucocorticoid induced TNF-R (GITR), cytotoxic T lymphocyte Ag-4 (CTLA-4), CD103 and the transcription factor Foxp3 (39).

In contrast to the intrathymic natural Treg cells, inducible Treg cells are generated in the periphery, after a pathogen is eliminated to prevent secondary autoimmunity. Their generation is dependent on peripheral factors, such as the maturation and type of the stimulating antigen-presenting cells (APC) (40), availability of cytokines such as TGF-β (41) and the presence of low-dose antigens (42). Finally, inducible Treg cells corresponds to Tr1 and Th3 regulatory T cells. Tr1 cells are characterized by a pattern of cytokine production, producing high levels of IL-10 and TGF-β and IL-5, low amounts of IFN-γ and IL-2, and no IL-4 (43), and have been shown to prevent the development of Th1-mediated autoimmune disease and suppress the immune response to pathogens, tumors and alloantigens (44). The Th3 subset of regulatory T cells is described by primarily secreting TGF-β and IL-1 inhibitor, both of which have an important role in immune regulation (39,45). Actually, the presence of regulatory T cells has been related to several immune responses, such as allergy, asthma, atopic dermatitis, rhinitis (46), inflammatory dermatoses, i.e. spongiotic dermatitis, psoriasis, lichen planus and leishmaniasis (47) and recently has been described in participating with Treg cells in periodontal disease (48-50).

As mentioned previously, inflammatory periodontitis lesions are caused by a group of periodontopathic bacteria and are characterized by large numbers of B cells and plasma cells together with a significant number of T cells and different concentrations of Th1 and Th2 cytokines (23,24). Among the subsets of T cells, the participation of regulatory T cells in periodontal disease has been largely studied. Some search showed that identifying CD4^{+}, CD25^{+}, CTLA-4^{+}, mRNA as a transcription factor Foxp3 and several cytokines and chemokines (such as IL-10, IL-4, IL-17, TGF-β and RANKL markers of Treg cells in periodontal lesions (46). Additionally, the increased frequency of Treg cells were evidenced by Cardoso et al in inflammatory infiltrate of gingival tissues from patients with chronic periodontal disease (50) strengthening the idea to involvement of cells type in pathogenesis of periodontal disease. However, the accurate function of Treg cells in the development of periodontal disease remains unknown and the understanding of the role of population cells will impact the current understanding and treatment of periodontal disease.

Conclusions

In this review, a brief introduction of periodontal disease, focusing on the important participation of periodonto-pathogenic bacteria associated with proinflammatory, environmental and genetic factors was made. It was noted that some individuals that are more susceptible to this oral pathology present with periodontal disease. The complex immune mechanism, including innate and adaptive systems, which may interfere in the development and progression of the periodontal disease, was described. However, there was no consensus regarding the pattern of the immune response in controlling this oral disease, even if the response generated may have a role in controlling or exacerbating the clinical situation observed in patients affected by periodontitis. Thus, although many studies have been conducted to understand this process, many gaps still need to be clarified in order to utilize this knowledge in the comprehension of the development of periodontal disease.

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