EDITORIAL

PERIODONTAL DISEASE AND BONE PATHOGENESIS: THE CROSSTALK BETWEEN CYTOKINES AND *PORPHYROMONAS GINGIVALIS*

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Periodontal disease is the most frequent cause of tooth loss among adults. It is defined as a plaque-induced inflammation of the periodontal tissues that results in a loss of support of the affected teeth. This process is characterized by destruction of the periodontal attachment apparatus, increased bone resorption with loss of crestal alveolar bone, apical migration of the epithelial attachment, and formation of periodontal pockets. Although the presence of periodontal pathogens such as *Porphyromonas gingivalis* is a prerequisite, the progression of periodontal disease is dependent on the host response to pathogenic bacteria that colonize the tooth surface. Nowadays, a growing body of literature has accumulated to investigate the association between bone diseases, periodontal pathogens and periodontal diseases. The integration of pathogen-associated molecular patterns from microorganisms with their surface receptors in the immune cells, induces the production of several cytokines and chemokines that present either a pro- and/or anti-inflammatory role and the activation of mechanisms of controlling this and the related disease, such as osteoporosis and rheumatoid arthritis. This review focuses on the evidence and significance of bone host cell invasion by *Porphyromonas gingivalis* in the pathogenesis of bone disorders, as well as the different lines of evidence supporting the role of cytokines in bone diseases.

Periodontal disease (PD) is a chronic bacterial infection that affects the gingiva and bone surrounding the teeth; the pathogenesis of human periodontitis was placed on a rational footing for the first time by Page and Schroeder in 1976 (1).

The connective tissue of the periodontium is separated from the oral cavity by a thin permeable junctional epithelium that has remarkable cell and fluid dynamics that allow the flow of bacterial toxins and inflammatory mediators (2), so the accumulation of bacteria in the gingival crevice might trigger an inflammatory process, which, if left untreated,

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destroys the periodontium and, eventually, results in tooth loss (3).

Spread of inflammation to the adjacent connective tissue drives the destruction of connective tissue and alveolar bone, that is, the cardinal sign of PD (4-5). Currently, an increasing body of literature has collected to investigate the cross-link between bone diseases and PD, that emerges either with the chronic or the aggressive form, and affects a significant portion of the population (2-5).

In 1998, Page (6) proposed that periodontitis may affect the host's susceptibility to systemic disease in three ways: by shared risk factors, by subgingival biofilms acting as reservoirs of gram-negative bacteria and through the periodontium acting as a reservoir of inflammatory mediators. The present editorial was conducted to focus on scientific published studies that have added to the basic knowledge and broader understanding of clinical and biological links between bone host cell invasion by a specific periodontal pathogen in the pathogenesis of the bone, with references to major historical studies and topical reviews.

Periodontal etiopathogenesis: the role of Porphyromonas gingivalis

The presence of periodontopathogens in the gingival sulcus such as *Porphyromonas gingivalis (P. gingivalis)* (7), in addition to genetic and environmental aspects seem to increase the susceptibility of some individuals to develop PD (8). This pathogen utilizes a multitude of virulence factors to evade host defenses as it establishes itself as one of the predominant pathogens in periodontal pockets (8). The pathogenic factors of *P. gingivalis* including fimbriae, hemagglutinin, capsule, lipopolysaccharide (LPS), outer membrane vesicles, organic metabolites such as butyric acid, and various enzymes such as Arg- and Lys-gingipains, collagenase, gelatinase and hyaluronidase, could contribute to the induction of chronic periodontitis in diverse ways (9). In fact, *P. gingivalis* could colonize gingival crevices by the fimbriae-mediated adherence to gingival epithelial cells, the proteases may have the ability to destroy periodontal tissues directly or indirectly, and the LPS could elicit a wide variety of inflammatory responses of periodontal tissues and alveolar bone loss (8-9).

In PD, gingipains degrade macrophage CD14, thus inhibiting activation of the leukocytes through the LPS receptor, thereby facilitating sustained colonization of *P. gingivalis* (9). Moreover, LPS can induce *in vitro* the osteoclast formation directly, and also indirectly by the cytokine production from gingival fibroblasts (10).

Although chronic exposure to bacteria and their products is a prerequisite for gingival inflammation and periodontal tissue destruction to occur, the major causative factor of soft- and hard- tissue breakdown associated with periodontitis is currently attributed to the host's immune-inflammatory response to bacterial challenge.

Effects of periodontal infection on systemic bone loss

While PDs have historically been deemed to be the result of an infectious process, other authors have proposed that PD may be an early manifestation of osteoporosis (11). The cross-link between these two diseases may be the bone-resorptive process: in fact, the diseases have a common denominator, loss of bone alveolar in PD and generalized in osteoporosis (12-15). Increased local production of cytokines associated with PD could rush systemic bone resorption by modulating the host response (Fig. 1).

Pleiotropic cytokine IL-6, produced by osteoblasts, may play a key role in this possible mechanism, because in normal bone homeostasis, IL-6 production stimulates osteoclastic activity subsequent in bone resorption, and certain lifestyle factors, such as smoking and low calcium intake, may impact the risk of developing osteoporosis and PD (16).

Role of RANK-L and related molecules

Nowadays, studies of crevicular fluid (GCF) from healthy individuals and patients with chronic periodontal disease demonstrated that increases in receptor activator of nuclear factor-kappa B ligand (RANK-L) mRNA and protein levels in periodontal tissues stimulate the differentiation of monocyte-macrophage precursor cells into osteoclasts and the maturation and survival of the osteoclasts, leading to alveolar bone loss (17-19). The severity of periodontal disease correlates slightly with RANK-L and Osteoprogerin (OPG) overexpression and more strongly with the RANK-L/OPG ratio (17).

Studies on humans have demonstrated that

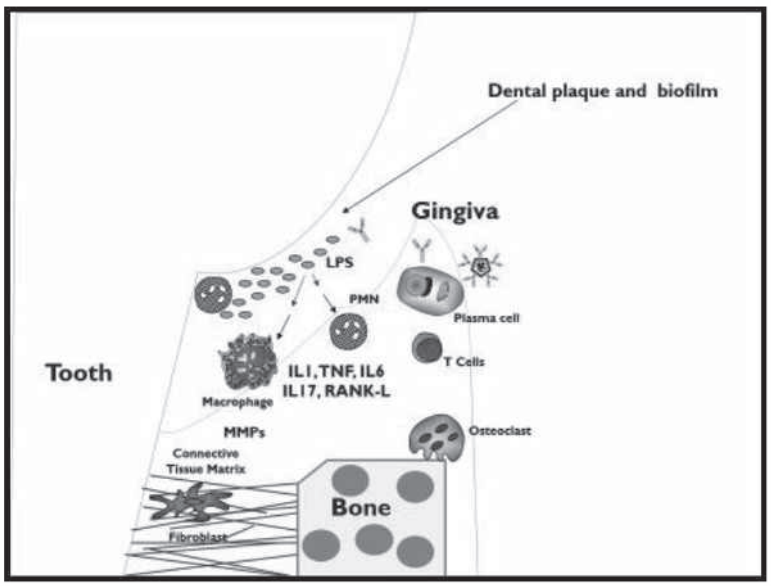

Fig. 1. *The host response. The pathogenetic events in periodontal disease are initiated, conducted and regulated by mediators of the acute and chronic inflammatory process produced by gingival resident cells, activated by infiltrating lymphocytes, junctional epithelium cells, vascular endothelium, mast cells, bone cells, the complement cascade and cytokines system.*

RANK-L levels in GCF were lower in healthy tissues or gingivitis, but increased in periodontitis (18-19) and, on the other hand, OPG levels were higher in healthy tissues than periodontitis, or gingivitis groups (17). Thus, GCF levels of RANK-L and OPG were oppositely regulated in periodontitis, but not in gingivitis, resulting in an enhanced RANK-L/OPG ratio.

Among the components of the oral flora, some organisms such as *P. gingivalis* may be strong inducers of RANK-L expression (18, 19), furthermore, *P. gingivalis* produces a unique class of cysteine proteinases termed gingipains that comprises Arg-gingipain and Lys-gingipain (19).

An amino acid sequence similar to the Arg-gingipain cysteine proteases may be involved in RANK-L production, as *P. gingivalis* strains carrying Lys-gingipain mutations fail to increase RANK-L levels (19). In addition, developments in the field of osteoimmunology, which examine the crosstalk of immune cells and bone, have uncovered a novel role for the RANK-L/RANK/OPG system in other processes such as in controlling autoimmunity or immune responses in the skin (20). The critical balance between osteoblast-mediated bone formation and osteoclast-mediated bone resorption has been described as "coupling" of bone formation to bone resorption (21).

Periodontium as a cytokine reservoir

Cytokines are regulatory low-weight proteins acting as mediators into genesis and control of immune and inflammatory response. During inflammatory response characteristic of periodontitis, proinflammatory cytokines, such as interleukin (IL)-1 α and β, IL-6, IL-17, tumor necrosis factor (TNF)-α, and interferon (INF)-γ can stimulate periodontal osteoblasts to express membrane-bound RANK-L (22-24). In addition to osteoblasts, RANK-L is expressed by a number of other cell types, mainly CD4+ T lymphocytes (25), playing a crucial role in T cell-mediated bone resorption. Through the RANK-L/RANK system, IL-17 may have a role in rheumatoid arthritis (RA), PD, and loosening of joint prostheses (26, 27).

IL-1 is a proinflammatory cytokine that plays an important role in immune regulation and inflammatory processes by inducing expression of many effector proteins, e.g. cytokines/chemokines, nitric oxide synthetase (NOS) and matrix metalloproteinases

(MMPs) (26-28). IL-1 includes two different but functionally similar molecules: IL-1α and IL-1β (28), genes which are all located on the long arm of chromosome 2 (region 2q13–14). IL-1α is mainly produced by keratinocytes of junctional epithelium or pocket epithelium, is a mediator of local inflammation and regulates intracellular event, instead IL-1β is primarily produced by activated macrophages and fibroblasts that release this extracellular protein (29). IL-1 upregulates complement system, Fc-receptor on polymorphonuclear neutrophils and monocytes, the expression of adhesion molecules upon fibroblast and leukocytes and induces the expression of homing receptors into the extracellular matrix for leukocytes and bone resorption by stimulating the proliferation and differentiation of osteoclast progenitors and activating formed osteoclast indirectly (28, 29).

Excessive and/or dysregulated activity of these mediators is associated with tissue destruction and therefore the synthesis, secretion and biological activity of IL-1 cytokines have been identified as therapeutic targets for common inflammatory disorders such as RA, diabetes and PD (30). Single nucleotide polymorphisms (SNPs) in the IL-1 promoter region could modulate this cytokine function that acts a fundamental role in the pathogenesis of PD, by affecting the susceptibility and severity of the affection (31).

The composite IL-1 genotype, known as periodontitis-associated genotype (PAG), obtained by the combination of the two rare alleles at the above-mentioned SNPs, has been extensively investigated in respect to the form of PD (chronic *vs* aggressive), smoke, ethnic group, and oral microbial pathogens present in the plaque biofilm (*P. gingivalis*) and other periodontal clinical parameters related to inflammatory status or to the progression of the disease (31, 32).

In a recent study, osteoblasts obtained from alveolar bone of PD patients underwent apoptosis in the presence of TNF-related apoptosis-inducing ligand (TRAIL), by interacting with its death receptors, (DR4, DR5) (33). However, its activity can be modulated by two decoy receptors, DcR1 and DcR2, as a result that, the sensitiveness of TRAIL-induced apoptosis is determined by the ratio of death and decoy receptor (33). Based on studies such as these, it can be projected that TNF superfamily,

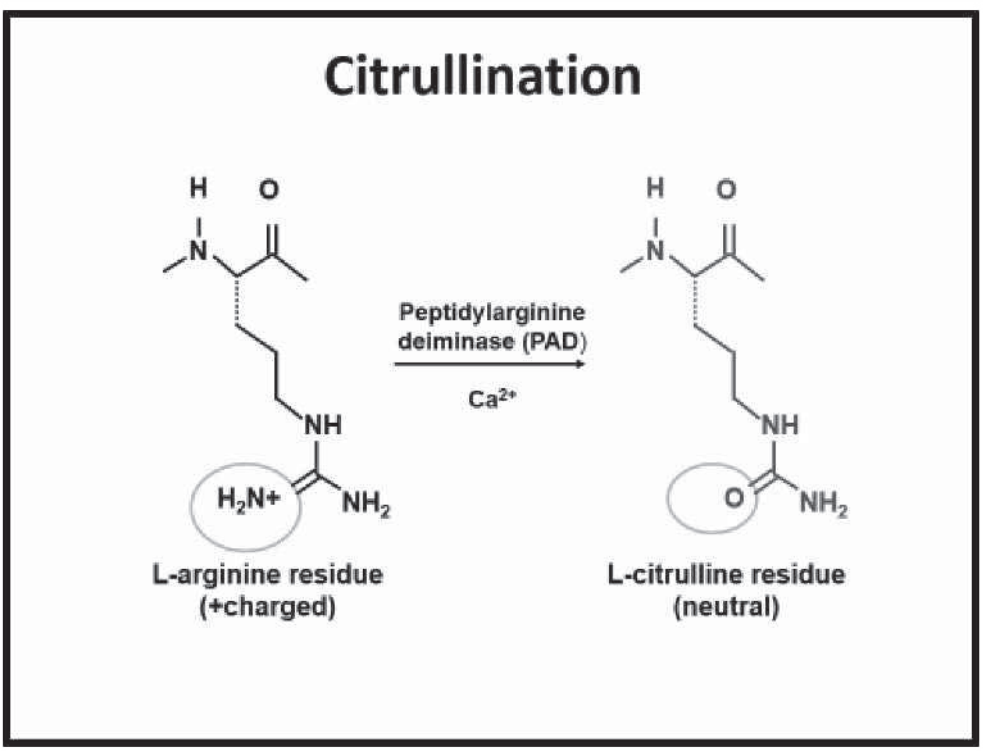

Fig. 2. *Citrullination: changes in secondary and tertiary conformation of proteins, neoepitopes formed.*

and in particular TRAIL, plays a role in controlling the expression of the cytokines that stimulate bone resorption during PD.

Inflammatory cytokines, IL-6 and INF-γ were considered as mediators of periodontal destruction because mononuclear cells and T cells extracted from periodontitis patients expressed more INF-γ and IL-6 compared to their peripheral blood counterparts from healthy controls (31-33).

Rheumatoid arthritis and citrullination : a link with Porphyromonas gingivalis

As reported to date, inflammation is clearly a central component of many chronic diseases.

Post-translational modifications such as phosphorylation, glycosylation and citrullination are very common processes that can increase the morphological and the functional diversity of the proteome, modifying a specific part of an amico acid after its synthesis.

Citrulline formation depends on the conversion of arginine catalyzed by peptidylarginine deiminase (PAD) enzymes (Fig. 2) (32). However, citrulline can be formed even as a side product when nitric oxide (NO) is generated from arginine by NO-synthetase (NOS) (32, 34).

In the oral cavity, nitrate is reduced to nitrite by nitrate-reducing bacteria (71) and nitrite is reduced to NO by nitrite-reducing bacteria (34). Additionally, in the oral cavity, two peroxidases are present; one is salivary peroxidase that is derived from saliva and the other is myeloperoxidase that is derived from leukocytes migrated into the oral cavity, so in this way, the formation of nitrite and NO by oral bacteria results in the production of reactive nitrogen oxide species (RNOS) by various reactions (34). Taking this mechanism into consideration, the decrease in pH in the oral cavity results in the injury of oral tissue cells.

There is a higher salivary arginase activity in periodontitis patients compared to healthy controls; in fact, the increased salivary arginase activity in periodontitis, perhaps causing a decrease in NO synthesis, also leads to a decrease in the antibacterial property of saliva and causes periodontal tissues to become more susceptible to existing pathogens

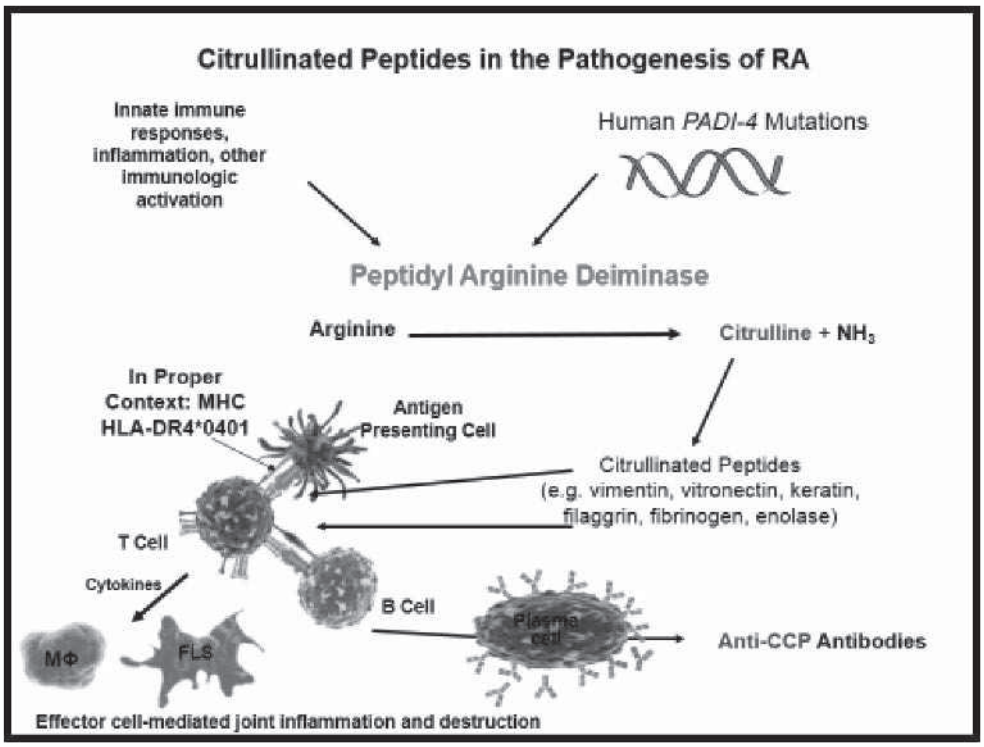

Fig. 3. *Crosslink between PAD and RA. Consistent associations between PAD and antibody responses against citrullinated peptides in the pathogenesis of RA. In RA patients, PD is a strong predictor of anti-CCP antibodies.*

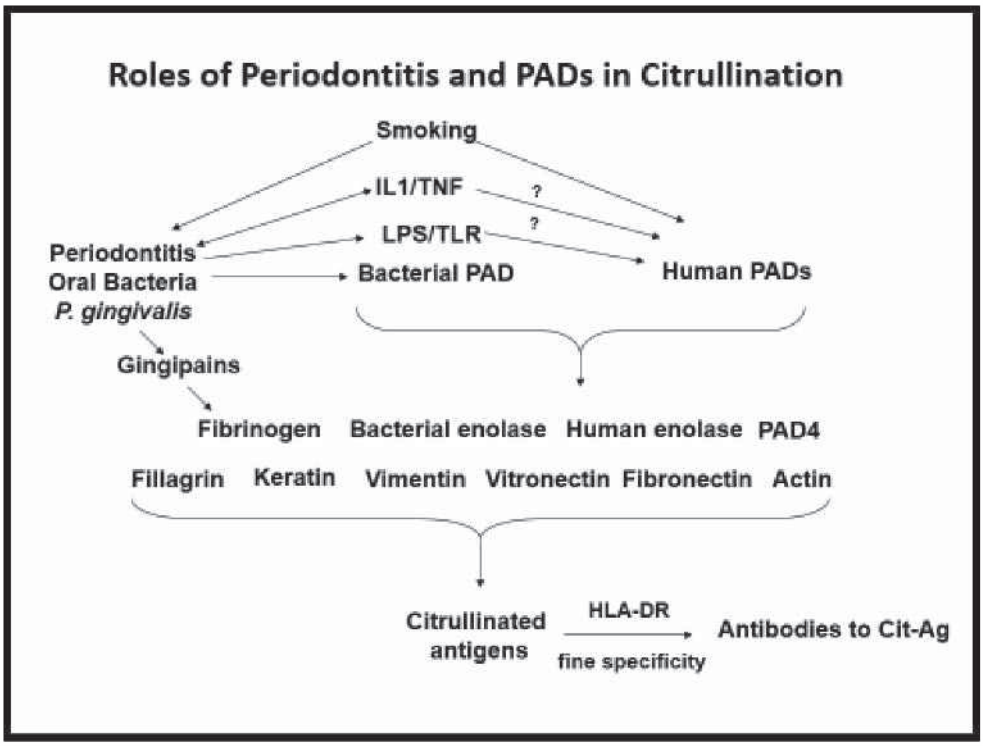

Fig. 4. *P. gingivalis has endogenous PAD. Hypothesized release of NH3+ leads to buffering of crevicular fluid and enhances organism evasion of host defense thus serving as virulence factor.*

(32, 34). Elevated NO production is a reflection of an immune-activated state in which inflammatory cytokines and other mediators have up-regulated inducible nitric oxide synthase (iNOS) (34).

The anti–Cyclic Citrullinated Peptide Antibodies (anti-CCP) are produced locally in the inflamed synovium of Rheumatoid arthritis (RA) patients (Fig. 3), suggesting that citrullinated proteins are located in the inflamed synovium (32). This has led to speculation that individuals predisposed to PD are exposed to citrullinated antigens that, in the proinflammatory context of PD associated to *P. gingivalis* PAD, become systemic immunogens (Fig. 4). In fact*, P. gingivalis* possesses that unique microbial enzyme, PAD, the human equivalent of which has been identified as a susceptibility factor for RA, and its proliferation depends on secretion of PAD (32). PAD catalyzes the deamination of peptidylarginine residues of various peptides to produce peptidylcitrulline and ammonia therefore, from this point of view, is possible to wonder about the presence of anti-CCP antibodies in PD.

DISCUSSION

There is a growing appreciation in international scientific literature of oral bacteria interactions, and their effects alone and in concert emphasizes the complex interplay among different species in amplifying local inflammatory and immunoregulatory pathways. Throughout the microbial world, there are strain differences within a species and these differences sometimes influence virulence.

The results of a recent study of bacterial species showed a potential synergistic partnership between *Aggregatibacter actinomycetemcomitans (A. actinomycetemcomitans)*, *Filifactor alocis (F. alocis)*, and *Streptococcus parasanguinis (S. parasanguinis)* which are key members of localized aggressive periodontitis (LAP), as this consortium

is strongly associated with bone loss (35). However, while it has been proposed that *S. parasanguinis* culd be found in the upper third of the pocket, both *A. actinomycetemcomitans* and *F. alocis* have been shown to be present in the deeper portions of the pocket, resting closer to the pocket epithelium, and these temporal and anatomical associations need additional *in vivo* confirmation.

In recent years, research on the topics of periodontal bacteria in bone diseases, such as *P. gingivalis,* has confirmed long-standing observed cross-talk between these diseases. Although the unique contribution to periodontal disease propagation by *P. gingivalis* as a single species may be considerable, the likelihood for considerable redundancies of function with other bacteria seems likely. In a recent study, conducted on a sample of 22 subjects with PD, the anti-CCP antibody was not found in sera of 21 (U/ml<10), while only in 1 subject with probing depth of 7.1 mm was identified positive for anti-CCP (22.2 U/ml) (32). The results, even if the study was conducted with some limits, i.e. limited sample size, absence of healthy controls not observed levels of *P. gingivalis* antibody response and lower systemic inflammation, do not support a role for anti-CCP in diagnoses of PD (32). We can speculate from these findings alone whether infection with *P. gingivalis* serves as a trigger for RA onset, or alternatively acts to modify immune responses in subjects following disease onset, or both, particularly considering that the levels of bacteria antibody response in this cohort seems to be one of the enrollment criteria including RA positivity. It is possible that these null findings result from its lower specificity and our inability to distinguish smaller differences in anti-CCP expression with a limited sample size. On the other hand, it is also possible that differences in antibody responses to *P. gingivalis* based on disease status may be due to other factors (e.g., gene-environment associations like pollution, tobacco etc.). In fact, gene-environment associations are important in RA susceptibility, with an association existing between smoking, HLA- DRB1 'shared epitope' alleles, PTPN22 and anti-CCP.

Cigarette smoking was much more frequent in subjects with RA and PD compared to healthy controls, which is important given separate associations of smoking with both PD and RA risk. However, these are both multifactorial, chronic diseases, and their complex etiologies and pathogenesis themselves remain incompletely understood.

According to the evidence-based dentistry (36), researchers and clinicians are directed to therapeutic approaches that actually represent the gold standard in PD treatment, such as laser applications (37), bone substitutes (38, 39), and the new emerging field of stem cells from oral tissues (40).

As is evident from the studies in international scientific literature that continue to emerge, a multidisciplinary collaboration is necessary to fully understand the relationships between the host response resulting from periodontal pathogens in the pathogenesis of the bone, as well as the different lines of evidence supporting the role of periodontal bacteria in bone diseases.

CONCLUSIONS

Nowadays, periodontics has evolved from a simplistic model to a more complex interplay between infection and host response, because genetic factors are a new addition to the list of risk factors for periodontal and related systemic diseases. The association between oral and systemic health has highlighted the importance of periodontal health and treatment, with the consequence that dental assessment and attention to oral hygiene assume an increasingly important part for the clinical management of patients with osteoporosis and rheumatoid arthritis.

Together with patient education, these factors improve the probability of patient treatment acceptance, which will improve oral and systemic health. Thus, although many studies have been conducted to understand this process, many questions still need to be clarified in order to utilize this knowledge in the comprehension of the development of PD.

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