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COMMENTARY

Periostin-deficient mice, a relevant animal model to investigate periodontitis or not?

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Periodontitis

Growing evidence indicates that periodontitis is highly prevalent in adult populations, according to a recent US survey underlining that 47% of the adult population is affected by periodontitis, 8.5% in its severe form. This rate increased to 64% in adults older than 65 years.[1] Clearly, periodontitis is a major health challenge, particularly affecting the ageing population, where the disease is the primary cause of tooth loss. Moreover, numerous epidemiological studies have found a high degree of association between diabetes mellitus, or coronary artery disease, and periodontitis as well as strong links between rheumatoid arthritis and periodontitis.[2]

Periodontal diseases refer to common inflammatory diseases caused by bacteria that affect the tissues surrounding and supporting the tooth, including inflammation of the gingiva, that is, gingivitis, the periodontal ligament (PDL), and the alveolar bone (AB), all of which lead to periodontitis. The role of genetic factors in the pathogenesis of periodontitis is emerging as few evidences support the contribution of some major genes to determine susceptibility of the host to this inflammatory process such a specific gene polymorphism for Il-1 upregulating this pro-inflammatory mediator.[3]

Gingivitis limited to the superficial gingival tissue is completely reversible by eliminating the bacterial stimulus using mechanical procedures such as tooth brushing or dental scaling. If not, the disease progresses to deeper tissues, and the so-called hallmark of the periodontitis is an AB loss resulting ultimaltely in extended tooth loss and oral disability.[4]

Anaerobic bacteria, mainly *Porphyromonas gingivalis (Pg)*, have traditionally been considered as causative agents of periodontitis, based on their virulence properties and strong association with diseased sites. However, *Pg*-induced periodontitis required the presence of commensal microbiota to cause periodontitis in germ-free mice.[5] In a newly proposed definition, periodontitis may result not from individual pathogens, but rather from polymicrobial synergy and dysbiosis, a condition characterized by an imbalance in the relative abundance or influence of species within a microbial community associated with a disease, which perturb the ecologically balanced biofilm associated with periodontal tissue homeostasis.[2]

It is now well acknowledged that the presence of bacterial species is necessary but not sufficient for the onset and progression of periodontitis. The recognition of microbial components as 'danger signals' by host immune cells and the subsequent production of inflammatory mediators is an essential step in periodontitis pathogenesis.[6] Indeed, production of pro-inflammatory cytokines (including interleukin-1β (IL-1β), IL-6 and tumor necrosis factor-α) by resident and recruited inflammatory cells acting synergistically seemed to be of particular importance in this process because of their ability to increase the recruitment and activity of osteoclasts through enhanced production of RANKL, mainly by T and B lymphocytes[7] (**Figure 1**).

Animal Models of Periodontitis

The first animal model of periodontal disease, reported in 1956 by Gupta and Shaw,[8] was the rice rat, showing a particular susceptibility to periodontal disease as early as 16 days of age. As with humans, the first step was a marginal gingivitis, predominantly in the region of the first mandibular molar. Edema, gingival pocket formation and accumulation of bacterial dental plaque occurred within 90–100 days. Beneath the gingival tissue, AB resorption took place and, as a result, the teeth drifted apart and were eventually exfoliated. The AB loss was evaluated by measuring the distance between the cemento–enamel junction and the crest of adjacent AB.[9] This is always the main criterion to evaluate AB loss in current animal models of periodontitis.

Today, two rodent models of periodontitis are useful, both inducing periodontal tissue inflammation secondary to a local infection.[10,11] Oral infection is achieved either by oral gavage or by local application using silk ligatures retentive of bacterial plaque around a first maxillary or mandibular molar, both models were then subjected to a wash-out period of 10 days with antibiotic treatment. Typically, gavage of a bacterial strain is orally administered in a 2% carboxymethylcellulose soft diet

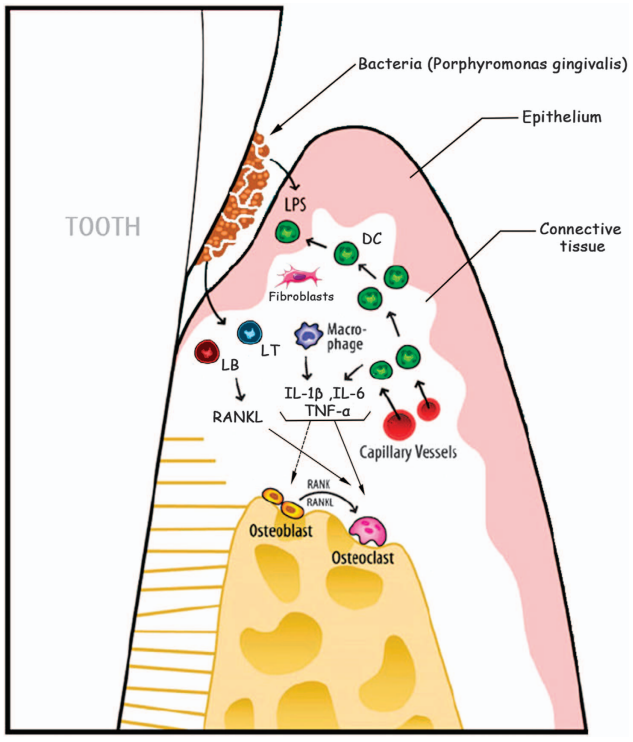

Figure 1 Main cellular and molecular mechanisms involved in the pathogenesis of the periodontitis (adapted from Giannobile WV:[16]). Resident cells of the gingiva, including epithelial cells, fibroblasts, dendritic cells (DC) and macrophages mediate the innate immunity, responding to the bacterial stimulation (via TLRs) by producing pro-inflammatory cytokines, mainly IL-1β, IL-6 and TNF-α. These cytokines are directly (→) or indirectly (----->), throughout osteoblasts, involved in the osteoclastogenesis leading to the alveolar bone loss. Macrophages and dendritic cells act as antigen-presenting cells for B and T cells which in turn produce the crucial osteoclastogenic factor RANKL.

containing bacteria three times per week for at least 4 weeks. Silk ligatures, impregnated or not with a bacterial strain, are placed and replaced if necessary every 3 days for at least 1 week. In the oral gavage model, substantial AB loss requires more than 4 weeks, whereas a 2- to 4-week period in the ligature model is sufficient. The oral gavage model is useful for addressing a wide variety of hypotheses related to periodontal pathogenesis, ranging from the role of host response, to virulence traits of pathogens, to the interconnections of those factors with systemic parameters. The silk ligature model is useful in investigating mechanistic and inflammatory aspects of the host immune response to the bacterial stimulus and in identifying potent therapeutic targets of particular interest in this inflammatory cascade. It must be remembered that these models demonstrate the inflammatory pathogenesis of periodontitis after bacterial stimulation. Although a single bacterial infection model cannot fully mimic human periodontitis, they share several features such as bacterial accumulation, formation of periodontal pockets, inflammatory infiltration of the deep periodontal tissues and destruction of these tissues.

Periostin-deficient Mice Model

Periostin-deficient (PKO) mice exhibit an early onset periodontal disease-like phenotype based on radiographic signs of AB destruction, external root resorption and widening of the PDL.[12] Inflammatory infiltrate, mainly neutrophils with fewer lymphocytes and plasma cells, is also discernible, but only in PDL and close AB concerning by the anchorage of collagen fibers from the PDL not in deeper AB, as is the case in humans. As opposed to aging humans, the severity of the periodontal defects in PKO mice can be moderated with growing age and a soft diet, indicating that the trigger of the inflammatory infiltrate in PDL is traumatic mastication. These data gave rise to a so-called 'genetic model of periodontitis'.[13]

In mice aged 13 months, Bonnet *et al.*[14] reported AB loss in the mandibular molar region (bone volume/trabecular volume (BV/TV)) decrease; $P<0.01$ vs wild type (WT)), a higher number and activity of osteoclasts ($P<0.01$ vs WT) and a decrease in the bone formation rate ($P<0.01$ vs WT). Basal bone, which does not belong to the periodontal tissues, was also affected in the same ranges. However, no sign of active inflammation was recorded in AB. There was only fibrous tissue in the bone medullar areas, suggesting that an early inflammatory episode had previously occurred in the mandible. Moreover, no periodontal pockets were recorded. These data indicate that PKO mice fed with a soft diet present alveolar osteopenia without overt periodontitis.

Discussion

In their study, Ren *et al.*, showed the primary importance of the *SOST* gene, a potent inhibitor of Wnt signaling pathway, and its product, sclerostin, in the prevention and recovery of periodontal defects in PKO mice used as a periodontitis model. They confirmed a sharp increase in SOST-positive osteocytes of the jawbone ($P<0.01$ vs WT), originally reported by others.[15] They evidenced osteogenic progenitors of PDL origin and osteocytes as key pathologic factors responsible for AB and PDL damages. The authors depicted an unpredicted change in the osteocytes of jawbones, from spindle- to round-shaped. Using pre-labeled fluorochrome specimens, the predominance of bone formation from progenitor cells of the PDL was shown, as compared with the bone formation from periosteum and endosteum ($P<0.05$). Removing SOST from PKO mice in a double KO model prevented the AB loss in 1-month-old animals and reversed completely the decrease of BV/TV recorded in PKO mice aged 5 months ($P<0.05$ vs WT). A treatment with a sclerostin antibody for 8 weeks, starting at the age of 1 month, evidenced a prevention of AB loss and a reversal of this bone loss, in volume and height, in 5-month-old PKO mice (BV/TV: $P<0.01$ and cemento–enamel junction: $P<0.01$ vs PKO controls). This recovery was correlated to the improvement of osteocyte morphology, including a decrease of surface area, dendrite length and dendrite number ($P<0.01$ in PKO mice vs WT).

These PKO mice experiments are the first to highlight a crucial role for PDL in AB formation and to underline the importance of osteocyte morphology in this process. They are also the first to ascertain a predominant role for SOST in the periodontal defects evidenced in PKO mice and to document the importance of the sclerostin/periostin balance in the physiopathology of AB.

Nevertheless, this study did not evidence at any moment an inflammation inside the AB. Therefore, whether or not an

inflammation in PDL is able to drive an AB loss is questionable. The authors speculated a direct interaction between collagen fibers of the PDL and osteocytes, which would be altered during periodontitis. This point remains to be investigated. Moreover, whether these PKO mice developed lesions compatible with periodontitis, that is, inflammatory driven AB loss consequential to bacterial stimulation, remains debatable. Periodontitis is characterized by inflammation in all the periodontal tissues (gingival tissue, AB and PDL), caused by pathogenic microflora and consequently, loss of connective tissue and crestal AB, but not basal bone. Undoubtedly, PKO mice exhibit AB and PDL disturbances; however, it is without a permanent inflammatory process and without bacterial stimulation. In this way, PKO mice do not mimic periodontitis. Therefore, whether PKO mice models are valuable models of periodontitis remains highly questionable. PKO mice could be useful to investigate the potent role of periostin in the bone resorption associated to periodontitis only if subjected to an experimental model of periodontitis (oral gavage or local application using silk ligatures) stimulated with a periopathogen commonly involved in its pathogenesis such as porphyromonas gingivalis and compared to wild type animals subjected to the same experimental model of periodontitis.

The relevance of sclerostin and periostin involvement in periodontitis and anti-sclerostin treatment has to be investigated in a relevant model before any conclusions can be made. Another way to observe effects of sclerostin in human periodontitis would be to investigate whether or not an anti-sclerostin treatment would ameliorate this periodontal disease in women having been treated for osteoporosis.

To date, periostin and sclerostin should be more considered as involved in the AB resorption rather than in the inflammatory process driving this bone resorption related to periodontitis.

Conflict of Interest

The authors declare no conflict of interest.

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