Current understanding of periodontal disease pathogenesis and targets for host-modulation therapy

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1 | INTRODUCTION

Chronic periodontitis is clinically characterized by loss of gingival tissue attachment to the tooth, deepening of the gingival crevice (designated "periodontal pocket" in periodontitis), degradation of the periodontal ligament, and loss of alveolar bone. This destructive process is associated with the presence of subgingival microbial communities and a dense immuno-inflammatory infiltrate in the periodontium that may lead to tooth loss if not appropriately treated. In gingivitis, a reversible form of periodontal disease that does not result in bone loss, the inflammatory process is restricted to the gingival epithelium and the connective tissue without affecting the deeper compartments of the periodontium. However, it should be noted that gingivitis is a major risk factor and a necessary prerequisite for periodontitis and can result in an increase in the serum levels of inflammatory biomarkers such as C-reactive protein.

The first systematic model to describe the temporal development of host response events leading to the development of periodontitis dates back to the 1970s when Roy Page and Hubert Schroeder defined 4 types of histopathologic lesions: "initial," "early," and "established," exemplifying distinct and sequential stages of gingivitis, and "advanced," featuring bone loss and clinically manifested as periodontitis. The development of periodontitis correlated with an increase in complexity of the cellular infiltrate, from one dominated by neutrophils (ie, the initial lesion), to one containing elevated numbers of macrophages and T cells (ie, the early lesion), and then to one containing B and plasma cells that predominate in established and advanced lesions. Subsequent discoveries, including the characterization of specialized subsets of innate and adaptive leukocytes and the dissection of their crosstalk interactions, has offered a more nuanced and mechanistic understanding of periodontal disease pathogenesis with profound implications for treatment.

The host immune response to the subgingival tooth-associated biofilm can be potentially protective, thus maintaining balanced host-microbe interactions and a healthy periodontium ("tissue homeostasis"). Besides the microbiota, additional challenges or potential insults that may contribute to the induction of local immunity include ongoing damage from mastication and perhaps also from exposure to dietary and airborne allergens/particles. In this regard, a predominantly T-cell-rich infiltrate and a network of antigen-presenting cells (macrophages, dendritic cells), as well as neutrophils, constantly and proactively patrol the periodontium. By contrast, a clinically healthy periodontium contains minimal numbers of B cells and plasma cells, and the numbers of neutrophils in healthy periodontium are lower than those present in gingivitis or periodontitis. Small populations of gammadelta T cells and innate lymphoid cells may also be seen in healthy gingiva and are thought to contribute to protective immunity and maintenance of tissue homeostasis.

However, in individuals with susceptibility to periodontitis, the host response is ineffective, dysregulated, and destructive.

Although bacteria are required for disease pathogenesis, it is predominantly the host inflammatory response to this microbial challenge that can ultimately inflict damage upon the periodontal tissues. Resistance or susceptibility to periodontitis appears to be determined by multiple factors, including genetic, epigenetic, and environmental (such as smoking, stress, and diet) factors, aging, and systemic diseases (such as diabetes) that may modify the host response in either a protective or a destructive direction (Figure 1). Observations that gingivitis in certain individuals may remain stable indefinitely (ie, without necessarily progressing to periodontitis) is...
consistent with the notion that periodontitis requires a susceptible host.

Recent microbiome and mechanistic studies have offered an improved understanding of the nature of host-microbe interactions in periodontitis. Specifically, such studies in humans and animal models have revealed that: (a) the periodontitis-associated microbiota is considerably more diverse and complex than previously thought; and (b) the bacteria involved act in disease through polymicrobial synergy and dysbiosis. In other words, periodontitis is not caused by a single or a select few bacterial species (“periodontopathogens”) and does not appear to qualify as an infection in the classical sense of the term. Rather, periodontitis is associated with dysbiosis (i.e., an alteration in the abundance or influence of individual species within the polymicrobial community, relative to their abundance or influence in health). Whereas dysbiosis can lead to destructive inflammation, the reverse is also true. In this regard, inflammatory tissue breakdown products (e.g., degraded collagen and heme-containing compounds, which are sources of amino acids and iron, respectively) are released into the gingival crevicular fluid. In the gingival crevice/pocket, these inflammatory spoils can be used as nutrients to fuel the selective expansion of a subset of bacterial species (e.g., proteolytic and asaccharolytic pathobionts), thereby exacerbating the imbalance of the microbiota (dysbiosis). In this regard, the addition of serum, hemoglobin, or hemin to an in vitro-generated oral multispecies community selectively induces the outgrowth of pathobionts, which interestingly upregulate genes that encode proteases, hemolysins, and molecules involved in hemin acquisition. This conversion of the original “homeostatic” community into a “dysbiotic” one can
also increase the community’s proinflammatory potential. Thus, an initial inflammatory response to subgingival biofilm development (for instance, because of the absence of adequate oral hygiene) may select for “inflammodilic” pathobiotic bacteria (incipient dysbiosis), which, upon further growth in a nutritionally favorable inflammatory environment, can further exacerbate inflammation. This positive-feedback loop of dysbiosis and inflammation can ultimately cause overt periodontitis in susceptible individuals (Figure 1).

If left untreated, periodontitis may not only lead to tooth loss but might also affect mastication, esthetics, and quality of life.34-36 Almost 50% of adults are affected by some form of periodontal disease (ranging from mild to severe), and nearly 10% are afflicted by severe periodontitis.37-39 Although highly variable between different communities, the prevalence of gingivitis is quite high (reaching >80%) in some.40-42 Current standard-of-care therapy for both gingivitis and periodontitis aims to remove the pathogenic microbial biofilm through mechanical debridement (scaling and root planing). However, scaling and root planing is only partially effective for the majority of patients with periodontitis, and some individuals (“refractory periodontitis patients”) do not respond favorably to this procedure.43 Therefore, periodontitis represents a significant health and economic burden.35,44,45 As tissue damage in periodontitis is mediated primarily by the host inflammatory response, which is also exploited by the dysbiotic microbial community for growth and persistence, it can be reasoned that host-response modulation approaches may be promising adjunctive treatments to conventional periodontal therapy. The main objective of this review is to summarize and discuss host-modulation therapies in periodontitis, some of which have already been tested in humans and many more of which are currently being pursued at a preclinical level. Emphasis will be placed on their mechanisms of action and on their perceived safety and potential for clinical application. First of all, however, a brief outline of the mechanisms of inflammation induction and resolution is given, to provide a better understanding of the relevance and rationale of the therapeutic interventions discussed.

2 | INFLAMMATION AND ITS RESOLUTION

Inflammation constitutes an important component of the overall biological response, whereby host tissues attempt to cope with various threats, such as invading pathogens, damaged cells, and irritants. Through adaptive changes of the local vasculature and release of various soluble mediators that interact with neutrophils and other cell types, the main functions of inflammation are: (a) elimination of the initial cause of infection or cell injury; (b) clearance of apoptotic and necrotic cells and debris; and (c) initiation of tissue repair.

In response to tissue infection, injury, or inflammation, neutrophils are the first cells to be recruited from the circulation to the afflicted site. The process of neutrophil extravasation involves a complex cascade of low- and high-affinity adhesive interactions of neutrophils with the endothelium.46-48 Briefly, circulating neutrophils initially engage in transient rolling interactions with the vascular endothelium, mediated by the binding of glycoprotein ligands on neutrophils to their endothelial cell-surface receptors (P- or E-selectin). This rolling-dependent deceleration of neutrophils enables them to interact with chemokines deposited on the luminal surface of endothelial cells.49 Chemokine- and selectin-induced signaling in neutrophils cooperatively induces their beta-2 integrins to adopt an extended and high-affinity conformation that can effectively bind their counter-receptors on endothelial cells, namely the intercellular adhesion molecules-1 and -2.50-51 The beta-2 integrins (heterodimeric molecules, each containing a distinct CD11 subunit and a common CD18 subunit) are required for firm adhesion of neutrophils onto the endothelium and their subsequent crawling on the endothelial surface, which enables neutrophils to find appropriate sites for extravasation. Firm adhesion and intraluminal crawling are primarily mediated by the beta-2 integrins lymphocyte function-associated antigen 1 (CD11a [integrin alpha L]+CD18) and macrophage-1 antigen (CD11b [integrin alpha M]+CD18), respectively.46-48 and genetic deficiency in beta-2 integrins (owing to CD18 mutations; leukocyte adhesion deficiency) results in few or no neutrophils in peripheral tissues such as the gingiva.52 The function of beta-2 integrins, and hence neutrophil extravasation, can be physiologically regulated. A major such mechanism is mediated by a protein, the developmental endothelial locus-1, which is secreted by endothelial and other cells. The developmental endothelial locus-1 protein binds lymphocyte function-associated antigen 1 and interferes with its adhesive function, thereby downregulating neutrophil recruitment to the periodontium and other peripheral tissues.53,54

Besides chemokines, other important inflammatory mediators that interact with and activate neutrophils include arachidonic acid derivatives, such as prostaglandins and leukotrienes55 and complement activation products, such as the anaphylatoxins C3a and C5a.54 The complement system contains some 50, fluid-phase, cell-surface-associated or intracellular proteins that trigger and regulate signaling pathways, thus mediating immune surveillance and homeostasis.56,57 Complement activation is triggered through distinct initiation pathways (classical, lectin, or alternative), all of which converge at the third component of complement (C3), leading to enzymatic generation of effector molecules that facilitate the action of antibodies and phagocytes to clear microbial pathogens (via opsonization by C3b), promote recruitment and activation of inflammatory cells (via the C3a and C5a anaphylatoxins), and lyse susceptible pathogens (via the C5b-9 membrane attack complex). However, when complement is dysregulated or overactivated, it can drive or exacerbate the pathogenesis of a number of inflammatory diseases, including periodontitis.58

Although traditionally associated with acute inflammation, neutrophils are now increasingly appreciated as major players in chronic inflammatory conditions, including atherosclerosis, psoriasis, rheumatoid arthritis, and periodontitis.59-61 In fact, neutrophils are functionally versatile and mediate previously unanticipated functions, including regulation of adaptive immune leukocytes.48,59,62-64 For instance, by releasing the chemokines C-C motif ligand 2 and C-C...
motif ligand 20, neutrophils can recruit T-helper 17 cells. T-helper 17 cells are CD4+ T-helper cells that release interleukin-17 at sites of inflammation and, in periodontitis, represent an osteoclastogenic subset that link T-cell activation to inflammatory bone loss. Moreover, neutrophils secrete B-lymphocyte stimulator and tumor necrosis factor ligand superfamily member 13 (also known as a proliferation-inducing ligand or APRIL), 2 key cytokines that promote the survival, proliferation, and maturation of B lymphocytes into plasma cells.

The histopathology of periodontitis involves elements of both innate and adaptive immunity. Neutrophils, antigen-presenting cells, and T and B lymphocytes form a sophisticated network of interactions between themselves and with humoral systems, such as complement, to stage immune and inflammatory responses in the periodontium. It is now well established that complement has functions above and beyond its traditional role of tagging and eliminating microbes. For instance, complement can amplify immune and inflammatory responses by synergizing with toll-like receptors on innate leukocytes and is able to regulate the activation and differentiation of B cells and T-cell subsets. In periodontitis, these complex interactions lead to inflammation-induced bone loss, which is largely mediated by a triad of proteins consisting of tumor necrosis factor ligand superfamily member 11 (also known as RANKL), its functional receptor (tumor necrosis factor receptor superfamily member 11A, also known as RANK), and its decoy receptor (tumor necrosis factor receptor superfamily member 11B, also known as osteoprotegerin). Tumor necrosis factor ligand superfamily member 11 is produced by activated T and B lymphocytes as well as by osteoblasts in the inflamed periodontium. The binding of cell-surface or soluble tumor necrosis factor ligand superfamily member 11 to tumor necrosis factor receptor superfamily member 11A on osteoclast precursors triggers osteoclast maturation and activation, although this tumor necrosis factor ligand superfamily member 11/tumor necrosis factor receptor superfamily member 11A-driven process is antagonized by the decoy receptor tumor necrosis factor receptor superfamily member 11B.

The ideal outcome of an inflammatory response is its timely termination so that it does not become chronic with potentially adverse effects. Indeed, nonresolving inflammation underlies the pathogenesis of many chronic conditions, including periodontitis. The successful resolution of inflammation is an active and well-coordinated process that involves a series of steps. These include downregulation of proinflammatory mediators and upregulation of regulatory or pro-resolution mediators, termination of neutrophil recruitment, clearance of apoptotic neutrophils by tissue phagocytes (efferocytosis), and initiation of tissue repair.

A “lipid-mediator class switching” process takes place during the resolving phase of inflammation. This temporal switch signifies a transition from an environment rich in proinflammatory prostaglandins and leukotrienes to one with high levels of proresolving mediators, which include arachidonic acid-derived lipoxins and omega-3 polyunsaturated fatty acid-derived resolvins and protectins. Resolvins are derived from docosahexaenoic acid (D series resolvins) or eicosapentaenoic acid (E series resolvins). When released within the vascular lumen, lipoxins and resolvins can suppress neutrophil transmigration to tissues through several mechanisms, such as modulation of adhesive molecules in both neutrophils and the endothelium. For example, lipoxin A4 blocks leukotriene- or peptide-leukotriene-dependent neutrophil adhesion by downregulating the expression of P-selectin on endothelial cells and of macrophage-1 antigen (CD11b/CD18) on neutrophils. Additionally, lipoxins and resolvins can inhibit neutrophil recruitment by downregulating expression of beta-2 integrin and intercellular adhesion molecule 1 and upregulating production of nitric oxide (an inhibitor of leukocyte adhesion to vascular endothelium) in endothelial cells. In addition to restraining neutrophil infiltration, proresolving lipid mediators promote neutrophil apoptosis and their phagocytosis by macrophages at the inflamed site.

The phagocytic uptake of apoptotic cells is designated efferocytosis and is mediated by a number of endocytic (efferocytic) receptors, including the T-cell immunoglobulin- and mucin-domain-containing molecules -1 and -4, the scavenger receptor CD36, the receptor tyrosine-protein kinase Mer, and the integrins alpha-V beta-3, alpha-V beta-5, and CD11b/CD18 (also known as macrophage-1 antigen and complement receptor-3). These receptors do not necessarily work independently of each other but actually appear to engage in cooperative interactions. It should also be noted that not all of these receptors may be expressed by a given phagocyte, and the associated clearance mechanisms may operate in a tissue-specific manner.

Efferocytosis is also facilitated by phosphatidylserine exposed on the outer membrane surface of apoptotic cells. This is because phosphatidylserine functions as a major “eat-me” signal and interacts, either directly or indirectly and with the help of “bridging molecules”, with different types of efferocytic receptors on macrophages. An example of the former mechanism involves the efferocytic receptor T-cell immunoglobulin- and mucin-domain-containing molecule-4 which can directly bind phosphatidylserine. An example of indirect interaction involves the bridging molecules developmental endothelial locus-1 and the related milk fat globule-epidermal growth factor-8 (also designated lactadherin), which promote efferocytosis by each binding to integrin alpha-V beta-3 (via an RGD motif on the N-terminal part of the molecules) and to phosphatidylserine (via discoidin-1 like domains in the C-terminal part of the molecules). Moreover, tyrosine-protein kinase Mer can bind phosphatidylserine-bearing apoptotic cells via the opsonin known as growth arrest specific factor-6. Not all efferocytic receptors require phosphatidylserine to function. For instance, macrophage-1 antigen (CD11b/CD18), also designated complement receptor-3, on macrophages can mediate the efferocytosis of iC3b-coated apoptotic cells, in other words, this receptor depends on the opsonin iC3b generated as a result of complement activation. Besides their role in apoptotic cell recognition, integrins actively crosstalk with other efferocytic receptor pathways in the context of inflammation resolution. For instance, the T-cell immunoglobulin- and mucin-domain-containing molecule-4 can induce stimulation of Src-family kinases and focal adhesion kinase and integrin activation. In this way, T-cell
immunoglobulin- and mucin-domain-containing molecule-4 engages integrins as coreceptors to activate signals required for the engulfment of apoptotic cells. Efferocytosis serves more functions than simply waste disposal, that is, clearing apoptotic cells and preventing secondary necrosis and inflammation. Efferocytosis also reprograms the transcriptional profile of the efferocytic macrophage, switching its phenotype to a "resolving" macrophage. Specifically, upon efferocytosis, macrophages are reprogrammed to reduce the expression of proinflammatory cytokines (eg, interleukin-6 and interleukin-23) and increase the expression of regulatory cytokines, such as transforming growth factor-beta and interleukin-10. Liver X receptors and peroxisome proliferator-activated receptors are ligand-activated transcription factors belonging to the nuclear receptor superfamily and play important roles in efferocytosis and the resolution of inflammation. Liver X receptors comprise 2 isoforms (liver X receptor alpha and liver X receptor beta) and are activated by endogenous oxysterols (ie, oxidized derivatives of cholesterol). Endogenous ligands of peroxisome proliferator-activated receptors (existing in the distinct isoforms alpha, beta/delta, and gamma) include fatty acids and other lipids. Engagement of apoptotic neutrophils triggers liver X receptor signaling in efferocytic macrophages in response to the sterol lipids of the apoptotic plasma membrane. Liver X receptor signaling, in turn, upregulates the expression of tyrosine-protein kinase Mer, thereby enhancing further efferocytosis, and furthermore inhibits proinflammatory gene expression. Peroxisome proliferator-activated receptor beta/delta signaling is also induced during apoptotic cell uptake, apparently by polyunsaturated fatty acid ligands derived from the apoptotic cell plasma membrane, and induces the expression of C1q (a complement component that binds phosphatidylserine) and milk fat globule-epidermal growth factor 8, both of which can promote the phagocytosis of apoptotic cells by macrophages. Consistent with this, macrophages deficient in peroxisome proliferator-activated receptor beta/delta have impaired capacity to perform efferocytosis and to switch to an anti-inflammatory and pro-resolving phenotype. Overall, macrophages exhibit

**FIGURE 2** Targets for host-modulation interventions in periodontitis. Periodontitis arises from the disruption of host-microbe homeostasis in susceptible individuals, leading to dysbiosis and destructive inflammation that not only activates osteoclastogenesis and bone loss but also provides nutrients (tissue breakdown products) that enable the dysbiotic microbiota to grow and persist. Shown are important therapeutic targets and potential interventions, most of which are currently at an experimental stage (see the text for details). C, complement; Del-1, development endothelial locus-1; IL, interleukin; MMPs, matrix metalloproteinases; NSAIDs, nonsteroidal anti-inflammatory drugs; OPG-Fc, osteoprotegerin (tumor necrosis factor receptor superfamily member 11B) fused to the Fc part of IgG; PGE2, prostaglandin E2; RANKL, receptor activator of nuclear factor-kappaB ligand (tumor necrosis factor ligand superfamily member 11); SPM, specialized pro-resolving mediators; TLR, toll-like receptor; TNF, tumor necrosis factor; Treg, regulatory T-cell
remarkable plasticity, which is crucial for successful resolution of inflammation, and hence are an important target for host modulation in inflammatory diseases.

3 | HOST MODULATION

Host-modulation therapy involves a treatment concept that aims to alter the status or function of the host to treat a disease. In periodontitis, host modulation predominantly refers to efforts to manipulate the immune response in ways that prevent or ameliorate tissue damage. The purpose of some of these approaches is to break a self-sustained vicious cycle that links microbial dysbiosis and destructive inflammation and underlies the chronicity of periodontitis (Figure 1). Conceivably, by controlling the host inflammatory response, it becomes possible to limit the food supply to a microbiota that sustains dysbiosis, thereby creating an environment that can reverse dysbiosis to recover a microbial flora that is compatible with periodontal health. Ideally, effective host-modulation therapy can restore the balance between proinflammatory and anti-inflammatory mediators, arrest disease development, and promote an environment that is conducive to inflammation resolution and periodontal tissue repair. It would not be feasible to discuss all proposed host-response modulation approaches, most of which are at an experimental stage and have not yet been tested in clinical trials. Priority has been given to those for which the mechanisms of action are adequately understood (Figure 2).

4 | NONSTEROIDAL ANTI-INFLAMMATORY DRUGS

Nonsteroidal anti-inflammatory drugs block the cyclooxygenase pathway of arachidonic acid metabolism and have been considered for the treatment of periodontitis. Nonsteroidal anti-inflammatory drugs can be nonselective or selective depending on their ability to block the activity either of both isoforms of cyclooxygenase (ie, cyclooxygenase-1 and cyclooxygenase-2) or only of cyclooxygenase-2, respectively. Cyclooxygenase-1 is constitutively expressed and cyclooxygenase-2 is an inducible isoform expressed in response to inflammation. Cyclooxygenase inhibition blocks the production of prostaglandins, including prostaglandin E2. Prostaglandin E2 is produced by various resident (eg, fibroblasts) and recruited (eg, macrophages and neutrophils) cell types in the periodontium in response to lipopolysaccharide or proinflammatory cytokines. Prostaglandin E2 functions as a vasodilator and promotes vascular permeability as well as bone resorption in periodontitis. Animal model-based studies and clinical trials have shown that nonsteroidal anti-inflammatory drugs can inhibit alveolar bone resorption. Although these drugs could improve the clinical outcome of mechanical periodontal treatment, the results decline rapidly after drug withdrawal. Moreover, current formulations have serious adverse effects that preclude their prolonged use for periodontal therapy. Nonselective nonsteroidal anti-inflammatory drugs are associated with gastrointestinal mucosal damage and renal toxicity. The use of selective cyclooxygenase-2 inhibitors is also problematic as they can induce prothrombotic side-effects, which may be attributed to reversal of cyclooxygenase-2-dependent attenuation of expression of tissue factor, a molecule that activates blood clotting.

5 | ANTI-CYTOKINE THERAPY

Anti-cytokine treatments involve the use of neutralizing monoclonal antibodies or receptor antagonists to block the action of proinflammatory cytokines. Such approaches to inhibit the effects of tumor necrosis factor, interleukin-1, or interleukin-17 resulted in inhibition of periodontitis in preclinical models, thereby also confirming the involvement of these cytokines in inflammatory bone loss. Periodontitis and rheumatoid arthritis share common inflammatory pathways. In this context, a systematic review analyzed the effects of several anti-rheumatic drugs on periodontal inflammation and related biomarkers in rheumatoid patients with periodontitis. Such drugs include infliximab (monoclonal antibody to tumor necrosis factor), etanercept (a soluble form of tumor necrosis factor receptor), and anakinra (an interleukin-1 receptor antagonist). The authors’ conclusion was that there is currently limited evidence to suggest that existing anti-cytokine therapies can reduce periodontal inflammation, at least in patients with both periodontitis and rheumatoid arthritis. The insufficient evidence was probably a result of the small number of studies performed, which moreover were not specifically designed to address efficacy in periodontitis. Anti-cytokine therapy has potentially adverse effects on immunity, which may be less serious when the drugs are administered locally than when administered systemically. Another potential concern is that specific blockade of a single cytokine may not be very effective if destructive inflammation is driven by a redundant cytokine network. This may not be an issue, however, when a specific cytokine pathway is heavily implicated in immune pathology, as is the case with an aggressive form of periodontitis that is associated with leukocyte adhesion deficiency and is driven specifically by the interleukin-23/interleukin-17 axis. Indeed, anti-interleukin-23 therapy (through ustekinumab, a monoclonal antibody that blocks the common interleukin-12/interleukin-23 p40 subunit) in a patient with leukocyte adhesion deficiency type 1 inhibited gingival expression of interleukin-17 and resolved inflammatory lesions without adverse reactions after 1 year of systemic treatment.

6 | SPECIALIZED PRO-RESOLUTION MEDIATORS

As discussed in more detail above, specialized proresolving lipid mediators, including arachidonic acid-derived lipoxins and omega-3 polyunsaturated fatty acid-derived resolvins and protectins, can mediate inflammation resolution via a variety of mechanisms. A newly dissected proresolving mechanism by which resolvins can terminate further neutrophil recruitment involves their ability to...
upregulate endogenous inhibitors of neutrophil transmigration. Specifically, resolvin D1 was shown to counteract interleukin-17-induced downregulation of the integrin beta-2 antagonist, developmental endothelial locus-1, thus potentially contributing to the resurgent expression of developmental endothelial locus-1 during the resolution of inflammation.\(^{91,115}\) A number of studies have established the ability of specialized proresolving lipid mediators to protect against experimental periodontitis in different animal models, such as rats, rabbits, and pigs.\(^{119-123}\)

Clinical studies have indicated that dietary supplementation with omega-3 polyunsaturated fatty acids (precursors of resolvins and protectins) may provide a benefit to the treatment of the disease, especially when combined with aspirin.\(^{108,124,125}\) In this regard, aspirin acetylates and changes the enzymatic function of cyclooxygenase-2, triggering the production of E-resolvins from eicosapentaenoic acid and D-resolvins and protectins from docosahexaenoic acid.\(^{126}\) To date, these intervention studies have involved small sample sizes. Larger-scale clinical trials are required to substantiate the initial promising results.

### 7 | PROBIOTICS

Probiotic microorganisms (probiotics) are being considered as a therapeutic strategy to prevent or treat human inflammatory diseases. The mechanisms of action of probiotics are incompletely understood but they appear to modulate both the microbiota and the host response.\(^{127,128}\) Several probiotic preparations have been shown to alter the periodontal microbial ecology in a manner that attains a balanced composition compatible with health, although this effect is transient and ceases after the end of the treatment.\(^{129}\) This might be caused by the low persistence in the oral cavity of the probiotic strains used, most of which are not oral organisms. Regarding host modulation, probiotics were shown to promote barrier function and the activity of T-regulatory cells, and to inhibit proinflammatory responses. Several probiotic strains were shown to mitigate experimental periodontitis in animal models. For instance, gastric intubation of Lactobacillus gasseri SBT2055 in mice inhibited Porphyromonas gingivalis-induced gingival inflammation and alveolar bone loss.\(^{130}\) In mice, topical application of Lactobacillus brevis CD2 was shown to inhibit ligation-induced gingival inflammation and bone loss and reduce the counts of gram-negative bacteria.\(^{131}\) A major mechanism by which L. brevis CD2 exerts anti-inflammatory action is through suppression of nitric oxide synthesis.

In a subsequent human study, lozenges containing L. brevis CD2 or placebo were applied topically in the mouth (3 times daily for 14 days) and allowed to dissolve slowly. The probiotic had a modest effect in reducing signs of experimental gingivitis in L. brevis CD2-treated volunteers compared with the placebo-treated group.\(^{132}\) A number of clinical studies have used Lactobacillus reuteri; overall, the results from these investigations indicate that this probiotic treatment can be an effective adjunctive treatment to conventional periodontal therapy as it leads to significant improvement in clinical indicators of periodontal disease (reviewed in refs.\(^\text{127,129}\)). Although the combination of probiotic treatment and scaling and root planing is generally more effective than scaling and root planing alone in reducing clinical indices of periodontal inflammation, human studies performed so far involve small sample sizes and are quite heterogeneous (because of the use of different probiotic strains, different doses, and different modes of administration). Therefore, strong conclusions cannot be readily drawn. Indeed, a recent meta-analysis concluded that more clinical studies, especially long-term studies, are required to strengthen the notion that L. reuteri-based probiotics have clinical efficacy as an adjunct to scaling and root planing.\(^{133}\) Thus, long-term efficacy, as well as safety, of probiotics need to be established before reliable clinical recommendations can be made.\(^{129}\)

### 8 | COMPLEMENT

Early clinical studies have shown considerably higher levels of complement activation products in the gingival tissue and the gingival crevicular fluid of patients with periodontitis than in healthy control individuals.\(^{134-140}\) Consistent with these observations, induction of experimental gingivitis in human volunteers caused progressive complement activation correlating with increased clinical inflammation.\(^{141}\) Conversely, successful periodontal treatment which resolved clinical inflammation inhibited complement activation in the gingival crevicular fluid of the treated patients.\(^{142}\) A cause-and-effect relationship between complement and periodontitis was established in preclinical mouse models using strains deficient in key complement components (C3 or C5aR1): mice deficient in C3 or C5aR1 were found to be protected against experimental periodontitis compared with wild-type controls.\(^{143,144}\)

The functional significance of C3 in experimental mouse periodontitis and the central location of this molecule in the complement cascade have prompted investigations into whether inhibition of C3 could provide a therapeutic benefit for patients with periodontitis. The inhibitor used was Cp40, an improved analog of the compstatin family of C3 inhibitors.\(^{145-147}\) The original compstatin was discovered by screening a phage-displayed peptide library and was identified as a 13-residue cyclic peptide ([CCVQDWHHRC]T-NH2).\(^{148}\) All members of the compstatin family block the activation of C3 exclusively in humans and nonhuman primates. Mechanistically, they bind C3 and inhibit its binding to, and cleavage by, the C3 convertase, thus blocking the generation of downstream effectors regardless of the initiation pathway of complement activation.\(^{145,146}\) Compared with the original version of compstatin, Cp40 has several improved features, including stronger inhibitory action, higher affinity for the C3 target, and better pharmacokinetic parameters.\(^{146}\) Specifically, Cp40 has subnanomolar affinity for C3 (K_D = 0.5 nM/L, where K_D is the specific equilibrium dissociation constant for formation of the enzyme-substrate complex) and a plasma half-life (~40 hours) that exceeds expectations for most peptidic drugs.\(^{146,147,149}\)

When administered locally by intragingival injection in a preventive setting, Cp40 inhibited inflammation and bone loss in young adult nonhuman primates subjected to ligation-induced
immune cells, such as T-helper 17 cells.\textsuperscript{152-157} Although complement is a potent proinflammatory and pro-osteoclastogenic cytokine (Figure 3). In this respect, strong suppressive effect on interleukin-17, a potent proinflammatory, or immunological parameters in their blood or tissues relative to those of control animals, despite complete inhibition of C3 in the plasma. In addition, wounds inflicted in the skin of the AMY-101-treated monkeys did not show signs of infection, but rather showed a trend toward faster wound healing compared with controls.\textsuperscript{152} Moreover, AMY-101 was shown to be free of local irritation when injected intragingly into nonhuman primates.\textsuperscript{151} More recently, AMY-101 was evaluated in a first-in-human clinical trial in healthy volunteers, in which it was shown to be safe and well tolerated.\textsuperscript{161,163} Overall, the safety and efficacy features of locally administered AMY-101\textsuperscript{143,150,151} suggest that this is a promising approach meritig investigation as a host-modulation therapy in human periodontitis.

9 | HOMEOSTATIC PROTEINS COMPRISING EPIDERMAL GROWTH FACTOR-LIKE AND DISCOIDIN-LIKE DOMAINS

The secreted homologous proteins developmental endothelial locus-1 and milk fat globule-epidermal growth factor-8, which have been mentioned briefly above in the context of neutrophil recruitment and efferocytosis, have an overall identity of about 50% at the amino-acid sequence level and are structurally quite similar. At the N-terminus, both molecules have epidermal growth factor-like repeats (developmental endothelial locus-1 has 3 such repeats and milk fat globule-epidermal growth factor-8 has 2), the second of which contains an integrin-binding RGD motif. At the C-terminus, both developmental endothelial locus-1 and milk fat globule-epidermal growth factor-8 have 2 discoidin I-like domains which can bind phospholipids and glycosaminoglycans.\textsuperscript{90,92,164-166} Both proteins were shown to have important homeostatic functions and to inhibit periodontitis in mouse and nonhuman primate models.\textsuperscript{53,167,169}

As mentioned earlier, developmental endothelial locus-1 can regulate integrin beta-2-dependent neutrophil functions, including firm adhesion to the endothelium, thereby suppressing neutrophil recruitment to sites of inflammation.\textsuperscript{53,54,170} (Figure 3). As a consequence, developmental endothelial locus-1-deficient mice display clessive neutrophil infiltration in the gingiva and develop spontaneous periodontal inflammation.\textsuperscript{53} Moreover, developmental endothelial locus-1 is expressed by osteoclasts, in which it regulates tumor necrosis factor ligand superfamily member 11-induced differentiation as well as restraining their resorptive function.\textsuperscript{167} More recently, developmental endothelial locus-1 was shown to contribute to the resolution of periodontal inflammation.\textsuperscript{91} In both human and murine periodontitis, clearance of inflammation correlated with restoration of the expression levels of this protein after its initial downregulation during initiation of inflammation. Resolution of experimental periodontitis in mice failed in developmental endothelial locus-1 deficient mice, and developmental endothelial locus-1 was functionally connected with resolvins. Indeed, developmental endothelial locus-1 was shown to act as a nonredundant downstream effector of inflammation resolution mediated by resolvin D1 and was required for optimal production of resolvin D1 and resolvin E1.\textsuperscript{91} Therefore, developmental endothelial locus-1 is not only a crucial effector of periodontal inflammation resolution but is also positively cross-regulated with resolvins,\textsuperscript{91,118} probably leading to the generation of a positive-feedback loop that fortifies resolution of inflammation. Developmental endothelial locus-1 was also shown to promote the resolution of acute monosodium urate crystal-induced peritoneal inflammation through its ability to promote effective clearance of apoptotic neutrophils (efferocytosis) through the integrin alpha-V beta-3-dependent mechanism discussed above.\textsuperscript{91} Consistent with its regulatory actions, recombinant developmentand endothelial locus-1 fused to the Fc part of IgG was shown to inhibit periodontal tissue inflammation and bone loss in both mice and nonhuman primates subjected to ligature-induced periodontitis.\textsuperscript{167} Apparently, developmental endothelial locus-1 can block periodontitis by suppressing upstream activities for inflammatory cell recruitment and downstream processes pertaining to osteoclastogenesis, as well as promoting the resolution of inflammation. These findings may potentially translate to human periodontitis because the immune system and periodontal tissue anatomy of monkeys is similar to that of humans, and monkey periodontitis shares important clinical, microbiological, and immunohistological features with human periodontitis.\textsuperscript{171}
Milk fat globule-epidermal growth factor-8 expressed by macrophages was shown to promote efferocytosis and hence the resolution of inflammation. As noted above, milk fat globule-epidermal growth factor-8 is structurally similar to developmental endothelial locus-1 in that it contains critical features (an RGD motif in the N terminus and discoidin-like domains in the C terminus) which enhance apoptotic cell phagocytosis. Moreover, milk fat globule-epidermal growth factor-8 has direct anti-inflammatory activity in macrophages and may promote the degradation of both connective tissue and the underlying bone by inducing the production of matrix metalloproteinases and RANKL from stromal cell types. Del-1, development endothelial locus-1; ICAM-1, intercellular adhesion molecule 1; G-CSF, granulocyte colony-stimulating factor; IL, interleukin; LFA-1, lymphocyte function-associated antigen 1; MMPs, matrix metalloproteinases; PGE2, prostaglandin E2; RANKL, receptor activator of nuclear factor-kappaB ligand (tumor necrosis factor ligand superfamily member 11); ROS, reactive oxygen species; Th17, T-helper 17 cell; TNF, tumor necrosis factor.

**FIGURE 3** Proinflammatory functions of interleukin-17 with potential for periodontal tissue destruction. Synergistic complement and toll-like receptor signaling in antigen-presenting cells enhance interleukin-17 production by adaptive immune cells (eg, T-helper cell 17). Interleukin-17, in turn, acts predominantly on innate immune and stromal cells to promote inflammatory responses. By upregulating granulocyte colony-stimulating factor, interleukin-17 can orchestrate the production of neutrophils in the bone marrow and their mobilization to the circulation. By inducing CXC chemokines, interleukin-17 can induce the chemotactic recruitment of neutrophils to the periodontium. Additionally, interleukin-17 facilitates neutrophil recruitment by inhibiting negative regulators of the leukocyte adhesion cascade. Specifically, interleukin-17 can inhibit endothelial cell production of development endothelial locus-1, a homeostatic protein that suppresses neutrophil adhesion and extravasation by blocking the interaction between the lymphocyte function-associated antigen 1 integrin on neutrophils and the intercellular adhesion molecule-1 on endothelial cells. Moreover, interleukin-17 activates macrophages and may promote the degradation of both connective tissue and the underlying bone by inducing the production of matrix metalloproteinases and RANKL from stromal cell types. Del-1, development endothelial locus-1; ICAM-1, intercellular adhesion molecule 1; G-CSF, granulocyte colony-stimulating factor; IL, interleukin; LFA-1, lymphocyte function-associated antigen 1; MMPs, matrix metalloproteinases; PGE2, prostaglandin E2; RANKL, receptor activator of nuclear factor-kappaB ligand (tumor necrosis factor ligand superfamily member 11); ROS, reactive oxygen species; Th17, T-helper 17 cell; TNF, tumor necrosis factor.
transcription factors involved in the expression of genes that regulate cell growth and differentiation, and metabolic homeostasis. Liver X receptors and peroxisome proliferator-activated receptors are constitutively found in the nucleus where they form heterodimers with retinoid X receptors that bind to specific response elements in target genes, even in the absence of ligand binding. However, when not occupied by ligands, liver X receptor/retinoid X receptor and peroxisome proliferator-activated receptor/retinoid X receptor heterodimers are transcriptionally inactive as they are complexed with corepressor proteins. By contrast, ligand occupancy of the heterodimers induces conformational changes that release the corepressor proteins and recruit coactivator proteins to the transcriptional complexes, leading to target gene expression.

More recently, liver X receptors and peroxisome proliferator-activated receptors were linked to the regulation of inflammation, thus placing them at the intersection of metabolism and immunity. As alluded to earlier, liver X receptors and peroxisome proliferator-activated receptors play important roles in the clearance of apoptotic neutrophils (effe... 


cytosis) by tissue phagocytes. Liver X receptors are also critical for the ability of developmental endothelial locus-1 to induce a proresolving macrophage phenotype in the context of effe... 


cytosis.

Accumulating evidence suggests that peroxisome proliferator-activated receptor activation may have protective effects in periodontitis. The activation of peroxisome proliferator-activated receptor delta (also known as peroxisome proliferator-activated receptor beta, and hence often designated peroxisome proliferator-activated receptor beta/delta) was shown to suppress the ability of *P. gingivalis* lipopolysaccharide to activate matrix metalloproteinase-2 in human gingival fibroblasts. Mechanistically, peroxisome proliferator-activated receptor beta/delta signaling – triggered by the selective agonist GW501516 – inhibited transcription of mRNA and protein expression of NADPH oxidase 4, thereby attenuating the production of reactive oxygen species that would, in turn, induce matrix metalloproteinase-2 activation. Consequently, GW501516-induced activation of peroxisome proliferator-activated receptor beta/delta activation in human gingival fibroblasts blocked *P. gingivalis* lipopolysaccharide-induced degradation of collagen types I and III. Given the important role of matrix metalloproteinases in periodontitis pathogenesis, these findings may provide mechanistic support for an in vivo rat study showing that systemic administration of a selective peroxisome proliferator-activated receptor beta/delta agonist (GW0742) attenuated ligature-induced periodontal inflammation and tissue damage. Protective results in ligature-induced periodontitis in rats were obtained also by administering synthetic agonists of peroxisome proliferator-activated receptor alpha or peroxisome proliferator-activated receptor gamma.

11 | TARGETING ADAPTIVE IMMUNE CELLS

B lymphocytes and plasma cells are abundant in the inflammatory infiltrate of advanced chronic periodontitis in humans. The survival, proliferation, and maturation of B lymphocytes depends on 2 cytokines of the tumor necrosis factor ligand superfamily, namely tumor necrosis factor ligand superfamily member 13 (also known as a proliferating-inducing ligand) and B-lymphocyte stimulator, which are both upregulated in human periodontitis. Antibody-mediated neutralization of tumor necrosis factor ligand superfamily member 13 or B-lymphocyte stimulator in mice was shown to diminish the B-cell numbers in the gingival tissue and inhibit periodontal bone loss. Therapeutic approaches targeting tumor necrosis factor ligand superfamily member 13 and/or B-lymphocyte stimulator are under clinical development for other inflammatory (or autoimmune) diseases, and a neutralizing monoclonal antibody to B-lymphocyte stimulator (belimumab) has been approved by the US Food and Drug Administration for the treatment of systemic lupus erythematosus. Interestingly, anti-B-cell therapy using rituximab (a chimeric monoclonal antibody to CD20) in patients with rheumatoid arthritis resulted in significant reduction of indices that measure clinical periodontal inflammation and tissue destruction. Thus, although further studies are required for reliable conclusions, limited evidence suggests the potential of B cell-targeted therapies for the treatment of periodontitis.

CD4+ Forkhead box protein p3-positive regulatory T cells down-regulate the induction and proliferation of effector T cells and can be protective in inflammatory and autoimmune conditions. In experimental periodontitis in mice, regulatory T cells appear in high numbers after the peak appearance of tumor necrosis factor ligand superfamily member 11-expressing CD4+ T cells, and selective regulatory T-cell depletion exacerbates inflammation and bone loss. Regulatory T cells therefore mitigate inflammatory tissue damage. However, their protective potential may be compromised in inflamed tissues. For instance, interleukin-23-activated gammadelta T cells restrain regulatory T cells and shift the balance in favor of effector T helper cells and, moreover, render effector T cells refractory to suppression by regulatory T cells. However, local treatment of mice or dogs with a chemoattractant formulation for regulatory T cells, consisting of C-C motif chemokine ligand 22 encapsulated in degradable polymer, enhanced the recruitment of regulatory T cells and inhibited experimental periodontitis in these models.

A recent study has implicated T-helper 17 cells in the inflammatory destruction of tissue in experimental periodontitis. Specific genetic inhibition of T-helper 17 cell differentiation (by knocking out the critical transcription factors signal transducer and activator of transcription 3 or retinoic acid-related orphan receptor gamma t) not only led to the depletion of T-helper 17 cells in gingival tissues but also significantly blocked inflammatory bone loss in a murine model of ligature-induced periodontitis. Human relevance for the importance of T-helper 17 cells in periodontal disease pathogenesis was established by showing that patients with genetically impaired development of T-helper 17 cells (caused by signal transducer and activator of transcription 3 loss-of-function mutations; autosomal-dominant hyper IgE syndrome) exhibited significantly decreased periodontal inflammation and bone loss compared with age- and gender-matched healthy controls and patients with periodontitis. From a translational perspective, pharmacological inhibition of retinoic acid-related orphan receptor gamma t using the
small-molecule inhibitor GSK805, which inhibits development and function of T-helper 17 cells,\(^{193}\) resulted in significantly decreased periodontal inflammation and bone loss in mice.\(^{195}\) GSK805 preferentially targeted the expansion of T-helper 17 cells without affecting other interleukin-17-secreting cellular sources, such as gammadelta T cells or innate lymphoid cells that do not appear to contribute to periodontal disease pathogenesis. This study therefore has shown that GSK805 is a selective inhibitor of T-helper 17 cells in periodontitis and can provide protection against this inflammatory disease.

### 12. APPROACHES FOR DIRECT INHIBITION OF PERIODONTAL TISSUE DESTRUCTION

A persisting, nonresolving inflammatory response in the periodontal tissue can exert damaging effects through several mechanisms. These include the induction of matrix metalloproteinases, which cause connective tissue degradation,\(^{177}\) and of tumor necrosis factor ligand superfamily member 11, which stimulates the differentiation and activation of osteoclasts that resorb bone.\(^{72,75,194}\) The destruction of connective and bone tissue can be blocked directly by targeting the effector mechanisms.

Tetracyclines attracted considerable interest when it was discovered that subantimicrobial doses of these antibiotics could block the activity of matrix metalloproteinases, the levels of which are elevated in inflamed gingiva and can cause collagen breakdown.\(^{195}\) Treatment of periodontitis patients with systemically delivered doxycycline (a potent tetracycline for inhibiting collagenolytic activity) has produced variable results but was generally beneficial as an adjunct to scaling and root planing.\(^{196,197}\) In this respect, treatment with a combination of doxycycline plus scaling and root planing could promote clinical attachment gain, reduction in probing depths, and suppression of gingival crevicular fluid levels of certain matrix metalloproteinases, relative to the combination of a placebo plus scaling and root planing. Subantimicrobial doxycycline (20 mg, taken twice daily) has received US Food and Drug Administration approval and has been marketed as Periostat for the treatment of human periodontitis.\(^{196,197}\)

Bisphosphonates are antiresorptive drugs that bind the mineral component of bone (hydroxyapatite crystals) and interfere with the action of osteoclasts. One possible mechanism of action of bisphosphonates is promotion of osteoclast apoptosis,\(^{198}\) and these drugs have been used for the prevention and treatment of osteoporosis.\(^{199}\) Experiments in animal models of periodontitis showed that systemically administered bisphosphonates could protect against bone loss without affecting inflammation. Similarly, decreased alveolar bone loss and improved mineral density was demonstrated in patients with periodontitis administered bisphosphonates (risedronate or alendronate) as an adjunct to scaling and root planing; however, a significant improvement of clinical inflammatory parameters was not consistently observed.\(^{200-202}\) For instance, alendronate did not significantly reduce the gingival index but displayed a trend for improved attachment level. From a safety perspective, osteonecrosis of the jaw is a serious adverse effect observed in a subset of patients receiving bisphosphonates.\(^{200-202}\)

Another approach to directly inhibit bone loss is to prevent the differentiation of osteoclasts by blocking the interaction of tumor necrosis factor ligand superfamily member 11 with its receptor tumor necrosis factor receptor superfamily member 11A on the surface of osteoclast precursors. Proof-of-concept was obtained by showing that tumor necrosis factor receptor superfamily member 11B, the natural inhibitor of tumor necrosis factor ligand superfamily member 11 (administered as a fusion protein with the Fc portion of IgG), and antibody to tumor necrosis factor ligand superfamily member 11 both inhibit periodontal bone loss in rats.\(^{203,204}\) Denosumab is a humanized monoclonal antibody that binds tumor necrosis factor ligand superfamily member 11 and prevents its interaction with tumor necrosis factor receptor superfamily member 11A, and has been used for treating osteoporosis.\(^{205,206}\) The efficacy of denosumab has not been specifically tested in patients with periodontitis, although it should be noted that osteonecrosis of the jaw has also been reported in patients treated with denosumab.\(^{207}\)

Osteoclasts can also be regulated, directly or indirectly, by secreted frizzled-related proteins.\(^{208,209}\) For instance, secreted frizzled-related protein 1 was shown to bind tumor necrosis factor ligand superfamily member 11, thereby preventing its interaction with tumor necrosis factor receptor superfamily member 11A and hence inhibiting osteoclastogenesis.\(^{209}\) The main targets of secreted frizzled-related proteins are, nevertheless, the Wnt family of proteins. This is because secreted frizzled-related proteins are structurally related to the frizzled receptors of Wnt proteins and can act as decoy receptors that antagonize interactions between Wnt protein and frizzled receptor.\(^{210}\) In this context, protein Wnt 5a and secreted frizzled-related protein 5 constitute a typical ligand/antagonist pair.\(^{210}\) The binding of osteoblast-derived protein Wnt 5a to a coreceptor complex involving receptor tyrosine kinase-like orphan receptor-2 and frizzled on osteoclast precursors induces noncanonical Wnt signaling that upregulates tumor necrosis factor receptor superfamily member 11A expression. This, in turn, sensitizes the precursors to the action of tumor necrosis factor ligand superfamily member 11, thereby promoting osteoclast differentiation.\(^{208}\) Importantly in this regard, local intrangular injection of secreted frizzled-related protein 5 in mice subjected to ligature-induced periodontitis resulted in inhibition of alveolar bone loss, which correlated with decreased numbers of osteoclasts in tissue sections.\(^{211}\) However, given the tight connection between inflammation and osteoclastogenesis, the anti-osteoclastogenic effect of secreted frizzled-related protein 5 could, additionally or alternatively, be attributed to its anti-inflammatory properties.\(^{211,212}\) Interestingly, there is a reciprocal relationship between secreted frizzled-related protein 5 and protein Wnt 5a expression in human periodontal health and disease, with protein Wnt 5a dominating in periodontally diseased tissue and secreted frizzled-related protein 5 dominating in periodontally healthy tissue.\(^{211}\) In principle, secreted frizzled-related protein 5 might potentially be both a biomarker and a therapeutic agent in periodontitis.
The first essential condition for immunization against any microbiially induced disease is to define the causative agents(s). Thus, the concept of vaccination against periodontitis started to emerge only after specific microorganisms, such as *P. gingivalis*, Tannerella forsythia, Treponema denticola, and Aggregatibacter actinomycetemcomitans were implicated as putative etiologic agents in periodontal diseases in the late 1980s. A challenge in vaccine development for periodontitis is that the disease primarily results from collagen tissue damage of the host immune response rather than from direct bacterial action. Therefore, vital to success in developing a periodontitis vaccine is a good understanding of the mechanisms that induce inflammatory tissue damage while generally failing to control subgingival microbial communities. In other words, a vaccine-induced antimicrobial response should not activate a destructive inflammatory response. In this regard, it should be considered that vaccine adjuvants have off-target effects related to induction of trained innate immunity; such effects might confer non-specific heterologous protection against subsequent infections or lead to immune responses that exacerbate inflammatory diseases.

Another challenge for vaccine development is that periodontitis is initiated by synergistic and dysbiotic microbial communities rather than by a few select “periodontopathogens.” Despite these considerations, there may be a sufficient rationale for vaccination targeting *P. gingivalis*. In a mouse model of periodontitis, *P. gingivalis* acts as a keystone pathogen (ie, exerts community-wide effects that promote the pathogenicity of the entire biofilm). In this context, *P. gingivalis* subverts the host response in a manner that uncouples inflammation (which is enhanced) from bactericidal activity (which is impaired), thereby leading to the emergence of a dysbiotic microbiota in which inflammophilic pathobionts aggravate the inflammatory response and cause tissue destruction. If *P. gingivalis* exerts similar effects in human periodontitis, at least in a subset of patients (discussed below in more detail), then it may be a promising therapeutic target.

The first attempts for vaccination against *P. gingivalis* were performed in rats and utilized whole bacterial cells or broken-cell preparations. However, because of the undesirable reactogenicity of these types of vaccine, subsequent attempts in animal models have focused on defined microbial proteins or genetically engineered subunits thereof. Such approaches using subunit vaccines have so far focused primarily on *P. gingivalis* virulence factors, particularly its cysteine proteinases (RgpA, RgpB, and Kgp gingipains), hemagglutinin B, as well as its fimbriae. Immunization with defined subunit immunogens necessitates the use of proper adjuvants more than immunization with whole bacterial cells, as the latter contain intrinsic adjuvant substances (eg, lipopolysaccharide). Some studies have utilized Freund’s complete or incomplete adjuvant or cholera toxin, while others made use of adjuvants which have been approved for use in humans, such as aluminum hydroxide (alum) and monophosphoryl lipid A, either alone or supplemented with trehalose dicorynomycolate.

Subcutaneous immunization of rats with the hemoglobin-binding domain of *P. gingivalis* gingipain could induce specific IgG and moderate protection against alveolar bone loss. The same immunogen, when given in Freund’s complete adjuvant, was able to potentiate the antibody responses but failed to confer protection against bone loss. This finding may have been a result of inflammatory responses induced or primed by the adjuvant, thus interfering with the potential of the immunogen to protect against bone resorption. This study therefore highlights the significance of selecting appropriate adjuvants that can promote specific protective immunity without immunopathological side-effects. In another study, rats were immunized with a mixture of gingipains (RgpA and Kgp) in Freund’s incomplete adjuvant, resulting in specific high-titer serum IgG2a responses and protection against *P. gingivalis*-induced periodontal bone loss. Although the control rats were positive for *P. gingivalis*, this bacterium was undetectable by DNA probe analysis of subgingival dental plaque samples taken from the RgpA/Kgp-immunized animals. Thus, besides possible neutralization of gingipains by antibody, it is possible that *P. gingivalis* may have been cleared by IgG2a-mediated opsonization and subsequent FcγRII-dependent phagocytosis and killing. Alternatively, specific IgG2a could inhibit colonization by *P. gingivalis*. More recently, a chimera vaccine combining key gingipain sequences formulated in alum induced an antigen-specific IgG1 and T-helper cell 2-biased response that protected mice from *P. gingivalis*-induced periodontitis. Although the role of the T-helper 2 cells in periodontitis is incompletely understood, this subset does not seem to be involved in periodontitis pathogenesis, in contrast to the T-helper 17 cell subset. Another approach to immunization against *P. gingivalis*-induced periodontal disease has exploited Streptococcus gordonii vectors that express cloned segments of the major fimbriae (FimA) of *P. gingivalis*. Oral immunization of rats with these recombinant bacteria elicited specific salivary IgA and serum IgG responses and protection against subsequent *P. gingivalis*-induced periodontal bone loss.

Vaccination studies in macaque monkeys are particularly valuable as periodontitis in these animals more closely resembles the human disease than the more convenient and relatively inexpensive rodent models and, importantly, these monkeys naturally harbor *P. gingivalis*. However, monkey studies to investigate immunity to periodontal disease have so far had limited success. A few examples are given here. Subcutaneous immunization of monkeys using, as immunogen, purified *P. gingivalis* cysteine proteinase in Freund’s incomplete adjuvant, elicited specific antibody responses but did not suppress *P. gingivalis*. Moreover, there were no significant differences between immunized and control animals with regard to periodontal bone loss. However, in another monkey study, subcutaneous immunization with cysteine proteinase in Syntex Adjuvant Formulation-M (an oil-in-water emulsion stabilized by Tween 80 and pluronic polyoxyethlene/polyoxypropylene block copolymer L121) led to considerably decreased levels of prostaglandin E2 in the gingival crevicular fluid and correlated with significantly reduced bone loss as compared with nonimmunized controls. This study suggests that suppression of inflammatory mediators by immunization, possibly because of reduced pathogenic challenge as a consequence of antibody-dependent inhibition of colonization or killing, is a potentially protective mechanism against periodontal disease. In
a similar gingipain-based vaccination study conducted by the same research group, protection against bone loss was associated with decreased counts of *P. gingivalis* as well as decreased total subgingival bacterial load.\textsuperscript{231} These findings are consistent with the concept that the presence of *P. gingivalis* benefits the entire microbial community, as anticipated by the keystone-pathogen hypothesis.\textsuperscript{47}

Additional studies are needed to establish the vaccination strategies and the immune response mechanisms that have optimal efficacy in controlling periodontal disease in primates. Specifically, much more has to be done to define the most favorable adjuvant formulations and immunization routes (mucosal routes are considered to be safer than systemic ones), in order to develop vaccine candidates that can be effective, not only in inducing immune responses to the bacteria but also in suppressing the disease. Whether a gingipain-based vaccine can have a significant protective impact on periodontal disease in humans remains to be determined. Although *P. gingivalis* is only one of a plethora of community bacteria implicated in periodontitis, specific immunity to *P. gingivalis* has been linked to protection against this disease in animal models. This might be explained by the role of *P. gingivalis* as a keystone pathogen in periodontitis, although it should be borne in mind that *P. gingivalis* is a risk factor rather than an obligatory factor in periodontal disease pathogenesis. Indeed, the disease depends on a variety of microbial or host factors that could be genetic or acquired (eg, immune deficiencies, immunoregulatory defects, smoking, diet, obesity, diabetes and other systemic diseases, and aging) and may act alone or in combination to modify the host response and the microbiome in a destructive direction.\textsuperscript{22,222,223} Thus, although anti-*P. gingivalis* immunity may be protective in models in which the disease is induced by, or attributed to, this pathogen, such vaccines may be protective only in a subset of human patients in whom periodontitis is primarily driven by the presence of *P. gingivalis*. In this regard, although *P. gingivalis* can be detected in periodontal health,\textsuperscript{234} in many patients, the presence of *P. gingivalis* in subgingival plaque has been shown to predict imminent disease progression (ie, clinical attachment loss).\textsuperscript{225}

Given that some aspects of the adaptive immune response contribute to periodontal tissue destruction and that vaccine adjuvants might cause maladaptive trained immunity, there is currently a need for better understanding of the immunoregulatory mechanisms operating in periodontal disease and for applying this knowledge in designing vaccines, including the selection of adjuvants. Thus, besides enhancing specific immunity to target periodontal bacteria, vaccines should also elicit appropriate noninflammatory modes of immune response that prevent tissue damage and ideally promote inflammation resolution and tissue healing.

### 14 | ANTI-AGING APPROACHES

The elderly display increased susceptibility to infectious and inflammatory diseases, including periodontal disease.\textsuperscript{17,226-242} Aging is thought to affect the immuno-inflammatory status and/or the regenerative potential of the periodontal tissue in a manner that increases susceptibility to periodontitis.\textsuperscript{17,243,244} This “age-altered susceptibility” hypothesis is consistent with findings of aging-related changes in immune and stem cell function that can potentially dysregulate immune responses and impair periodontal tissue repair.\textsuperscript{17,239,245-252}

The aging of hematopoietic stem cells is a fundamental cause of immune senescence,\textsuperscript{250,253} which affects both innate and adaptive immunity.\textsuperscript{237,253,254} In both humans and mice, hematopoietic stem cell function is impaired in old age, resulting in reduced long-term self-renewal capacity attributable to both intrinsic and extrinsic mechanisms of hematopoietic stem cells.\textsuperscript{250,253} Hematopoietic stem cells reside in a specialized microenvironment in the bone marrow, the “hematopoietic stem cell niche,” which maintains stem cells in a quiescent state that reduces DNA damage and also supports their survival and self-renewal, and, upon demand, their proliferation and differentiation.\textsuperscript{250,255-260} Mounting evidence suggests that the hematopoietic stem cell niche is crucial for the regulation of cellular senescence in hematopoietic stem cells.\textsuperscript{250,255} As a consequence, the physiological aging of endothelial cells, a critical cellular component of the hematopoietic stem cell niche, drives functional aging of young hematopoietic stem cells in ex vivo co-culture experiments.\textsuperscript{261}

Intriguingly, the homeostatic function of developmental endothelial locus-1 is not restricted to peripheral tissues such as the periodontium. Developmental endothelial locus-1 is also secreted in the bone marrow (by arteriolar endothelial cells, cells of the osteoblastic lineage, and CXC motif ligand 12 [also known as stromal cell-derived factor 1]-abundant reticular cells) where it serves as a crucial regulator of the hematopoietic stem cell niche.\textsuperscript{262} Specifically, developmental endothelial locus-1 interacts with the alpha-V beta-3 integrin on hematopoietic stem cells and promotes their retention, expansion, and differentiation toward the myeloid lineage, under both steady-state and stress conditions.\textsuperscript{262} Thus, the aging-associated deficiency of developmental endothelial locus-1 discussed earlier may contribute to defective hematopoiesis in old age. Interestingly, transplanted young endothelial cells rejuvenate the functional activity of hematopoietic stem cells in old mice, thus suggesting that senescence is, at least in part, a reversible process.\textsuperscript{261} It is tempting to speculate that the hematopoietic stem cell rejuvenation might, in part, be a result of expression of normal levels of developmental endothelial locus-1 by the transplanted endothelial cells.

Aging-associated alterations in immune function dysregulate the host response in a manner that (a) impairs immunity to pathogens and (b) promotes nonproductive inflammation, the combination of which may increase host susceptibility to a microbe-driven chronic inflammatory disease such as periodontitis.\textsuperscript{68,239,250,254,263-268} At least in principle, molecular pathways involved in aging and senescence could be targeted therapeutically to rejuvenate stem cells and reverse some of the adverse effects of aging, such as increased susceptibility to inflammatory disorders and reduced responsiveness to vaccination. For instance, mammalian target of rapamycin activity is increased with aging in hematopoietic stem cells and contributes to their senescence; inhibition of mammalian target of rapamycin by rapamycin, a US Food and Drug Administration-approved drug, restores the capacity of hematopoietic stem cells for self-renewal
and hematopoiesis and enables effective vaccination of old mice against a lethal influenza virus infection.\(^{269}\) The improved efficacy of vaccination in old mice after hematopoietic stem cell rejuvenation by rapamycin is not surprising given that aging-related alterations in mature immune cells can be traced back to hematopoietic stem cells.\(^{250,253}\) Importantly, moreover, a recent study showed that administration (via the diet) of rapamycin to old mice reversed aging-associated periodontal bone loss, although the effect on the host inflammatory response was not reported.\(^{270}\)

Aging-related alterations occur also to the niche of the mesenchymal stromal/stem cells in the periodontal ligament and other tissues.\(^{251,252,256,258}\) Specifically, the proliferative and differentiative ability of periodontal ligament-mesenchymal stroma/stem cells is altered with aging in ways that impair tissue homeostasis and repair.\(^{251,252,256}\) Importantly, this periodontal ligament-mesenchymal stroma/stem cell defect is reversible and regulated by the extrinsic microenvironment.\(^{252,256}\) Overall, there is a growing interest in approaches to rejuvenate hematopoietic stem cells or mesenchymal stroma/stem cells (eg, by targeting stem cell niches).\(^{227,230,247}\) These emerging therapeutic options may counteract the adverse effects of aging on a number of diseases and perhaps periodontitis, although more data are necessary to determine the feasibility of these approaches.\(^{250,253,271}\)

## CONCLUSIONS AND PERSPECTIVE

Progress in understanding the mechanisms driving periodontal disease pathogenesis has been paralleled with the development of many and diverse approaches to control the disease (Figure 2). Host-modulation therapies involving biologics to target specific components of the immune response may have potential safety issues, including increased risk for infections. However, potential risks and adverse effects are more likely when therapeutics are administered systemically and are prescribed for long-term use, as discussed above for nonsteroidal anti-inflammatory drugs. For instance, potential adverse effects on thymocyte and lymph node development by the use of retinoic acid-related orphan receptor gamma t inhibitors\(^{272,273}\) are less likely if these inhibitors are administered locally.

Being a local inflammatory disease, periodontitis is amenable to local host-modulation treatments. Importantly, local anti-inflammatory therapies, such as complement inhibition, in animal models of periodontitis not only do not predispose to defective immune surveillance in the periodontal tissue but also appear to suppress dysbiotic microbial communities that rely on inflammation for growth and persistence.\(^{52,120,121,169,274}\) In line with this, the bacterial biomass of periodontitis-associated dental plaque increases with increasing clinical inflammation.\(^{19}\) Of course, more reliable information regarding safety can be obtained when candidate drugs enter clinical trials.

At least in principle, the use of endogenous molecules that can both inhibit inflammation and promote its resolution appears to be both safe and effective. For instance, because expression of developmental endothelial locus-1 is diminished under inflammatory conditions or in old age, restoring its levels by exogenous administration could therapeutically reinstate tissue homeostasis. Therapies utilizing endogenous molecule administration have increased in recent years and endogenous molecule replacement is well tolerated.\(^{275,276}\) The promotion of tissue homeostasis can also be achieved by biologics as long as they are safe and effective. In this regard, although the C3 inhibitor AMY-101 was administered locally in the gingiva of nonhuman primates as infrequently as once every 3 weeks and was withdrawn at 6 weeks, the treated animals maintained significantly reduced clinical periodontal/inflammatory indices for at least an additional 6-week period.\(^{151}\) This long-lasting protective effect was unexpected for a small-molecule inhibitor that was not used throughout the experimental period. One explanation may involve the positive-feedback loop between inflammation and dysbiosis\(^{31,32,277}\) (Figure 1): it is possible that inhibition of inflammation by AMY-101 tips the balance toward host-microbe homeostasis, which might be resilient to pathological processes that would tend to reinstate active periodontitis.

With the possible exception of probiotics, most of the aforementioned interventions are intended to be used for the treatment, rather than the prevention, of periodontitis. However, at least in principle, most of the above-discussed host-modulation approaches would not necessarily be relevant only in a therapeutic setting; they could also be implemented on a preventive basis (ie, prior to the onset of periodontitis) to high-risk individuals, such as cigarette smokers and patients with diabetes.\(^{278,279}\)

The great complexity of pathogenic mechanisms in periodontitis and the involvement of polymicrobial dysbiotic communities, rather than specific pathogens, appear to complicate the development of a periodontitis vaccine. A major challenge is to fine-tune the vaccine-induced host response to maximize protective immunity while minimizing its potentially destructive aspects.

Although our mechanistic understanding of periodontal disease pathogenesis is still incomplete, available knowledge has facilitated promising preclinical studies such as those featured here. Despite the demonstrated potential of rational targeted approaches to inhibit periodontitis, a greater challenge is to clinically evaluate candidate drugs in terms of their risks and benefits, as adjunctive therapies for the treatment of human periodontitis.

ACKNOWLEDGEMENTS

The authors’ research is supported by U.S. Public Health Service grants from the National Institutes of Health (DE024153, DE024716, DE015254 to GH; DE026152 to GH and TC; and AI068730 and AI030040 to JDL), Deutsche Forschungsgemeinschaft (SFB-TR 127 to TC), and the European Commission (FP7-DIREKT 602699 to JDL).

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How to cite this article: Hajishengallis G, Chavakis T, Lambris JD. Current understanding of periodontal disease pathogenesis and targets for host-modulation therapy. *Periodontology 2000*. 2020;84:14-34. https://doi.org/10.1111/prd.12331