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

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Abstract

Recent advances indicate that periodontitis is driven by reciprocally reinforced interactions between a dysbiotic microbiome and dysregulated inflammation. Inflammation is not only a consequence of dysbiosis but, via mediating tissue dysfunction and damage, fuels further growth of selectively dysbiotic communities of bacteria (inflammophiles), thereby generating a self-sustained feed-forward loop that perpetuates the disease. These considerations provide a strong rationale for developing adjunctive host-modulation therapies for the treatment of periodontitis. Such host-modulation approaches aim to inhibit harmful inflammation and promote its resolution or to directly interfere with downstream effectors of connective tissue and bone destruction. This paper reviews diverse strategies to modulate the host periodontal response and discusses their mechanisms of action, perceived safety and potential for clinical application.

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 ¹. 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 ¹. However, it should be noted that gingivitis is a major risk factor and a necessary pre-requisite for periodontitis

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and, moreover, can increase the serum levels of inflammatory biomarkers such as C-reactive protein ²⁻⁵.

The first systematic model to describe the temporal development of host response events leading to the development of periodontitis dates back in the 1970s when Roy Page and Hubert Schroeder defined four types of histopathologic lesions: the ‘initial’, ‘early’ and ‘established’ lesions exemplifying distinct and sequential stages of gingivitis and the ‘advanced’ lesion featuring bone loss and clinically manifested as periodontitis⁶. The development of periodontitis correlated with increased complexity of the cellular infiltrate composition from one dominated by neutrophils (initial lesion) to one containing elevated numbers of macrophages and T cells (early lesion) and, additionally, B and plasma cells that predominate in the established and advanced lesions ⁶. 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 ⁷.

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’) ⁸. Besides the microbiota, additional challenges or potential insults that may contribute to the induction of local immunity include ongoing damage from mastication and perhaps 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. In contrast, a clinically healthy periodontium contains minimal numbers of B cells and plasma cells and, moreover, the neutrophils present are at lower numbers than in gingivitis or periodontitis. A small population of $\gamma\delta$ T cells and of innate lymphoid cells may also be seen in healthy gingiva and are thought to contribute to protective immunity and maintenance of tissue homeostasis ^{8,9}.

However, in individuals with susceptibility to periodontitis, the host response is ineffective, dysregulated, and destructive ¹⁰. Whereas the 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 ^{7,11}. Resistance or susceptibility to periodontitis appears to be determined by multiple factors that may modify the host response in either a protective or a destructive direction. Such factors include genetic, epigenetic, environmental (such as smoking, stress, and diet), aging, and systemic diseases such as diabetes ¹²⁻¹⁸ (Figure 1). Observations that gingivitis in certain individuals may remain stable indefinitely, *i.e.*, without necessarily progressing to periodontitis ⁶, 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 (i) the periodontitis-associated microbiota is considerably more diverse and complex than previously thought and (ii) the bacteria involved act in disease through polymicrobial synergy and dysbiosis ¹⁹⁻³⁰. 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 respect, inflammatory tissue breakdown products (*e.g.*, degraded collagen and heme-containing compounds, 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 microbiota imbalance (dysbiosis) ^{30–32}. 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 ³³. This conversion of the original ‘homeostatic’ community into a ‘dysbiotic’ one can also increase the community’s proinflammatory potential ³³. Thus, an initial inflammatory response to subgingival biofilm development (for instance, due to absence of adequate oral hygiene) may select for ‘inflammophilic’ pathobiotic bacteria (incipient dysbiosis), which, upon further growth in a nutritionally favorable inflammatory environment, can further exacerbate inflammation. This feed-forward loop of dysbiosis and inflammation can ultimately cause overt periodontitis in susceptible individuals (Figure 1).

If left untreated, periodontitis can not only lead to tooth loss but may also affect mastication, esthetics and the quality of life ^{34–36}. Almost 50% adults are affected by some form of periodontal disease (ranging from mild to severe) with nearly 10% being afflicted by severe periodontitis ^{37–39}. Although highly variable between different communities, the prevalence of gingivitis is quite high (can reach above 80% in some communities) ^{40–42}. Current standard-of-care therapy in 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 the periodontitis patients and some individuals (‘refractory periodontitis patients’) do not respond favorably to scaling and root planing ⁴³. Therefore, periodontitis represents a significant health and economic burden ^{35,44,45}. Since tissue damage in periodontitis is mediated primarily by the host inflammatory response, which moreover is 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, whereas many more are presently being pursued at a preclinical level. An emphasis will be placed in their mechanisms of action and on their perceived safety and potential for clinical application. The relevance and rationale of the discussed therapeutic interventions can be better understood after a brief outline of the mechanisms of inflammation induction and resolution.

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: (i) elimination of the initial cause of infection or cell injury; (ii) clearance of apoptotic and necrotic cells and debris; and (iii) 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 their interaction with chemokines deposited on the luminal surface of endothelial cells⁴⁹. Chemokine- and selectin-induced signalling in neutrophils cooperatively induces their β_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 β_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 β_2 integrins LFA-1 (CD11a/CD18) and Mac-1 (CD11b/CD18), respectively^{46–48}, and genetic deficiency in β_2 integrins (owing to CD18 mutations; leukocyte adhesion deficiency) results in few or no neutrophils in peripheral tissues such as the gingiva⁵². The function of β_2 integrins and hence neutrophil extravasation can be physiologically regulated. A major such mechanism is mediated by a protein secreted by endothelial and other cells, the developmental endothelial locus-1 (Del-1). Del-1 binds LFA-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 C5a⁵⁶. The complement system contains some 50 fluid-phase, cell surface-associated, or intracellular proteins that trigger and regulate signaling pathways 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 (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 periodontitis⁵⁸.

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^{48,59,62,63}. For instance, by releasing the chemokines (C-C motif) ligand 2 (CCL2) and ligand 20 (CCL20), neutrophils can recruit Th17 cells⁶⁴. Th17 are CD4+ T helper cells that release interleukin-17 at sites of inflammation and, in periodontitis, represent an osteoclastogenic subset that links T-cell activation to inflammatory bone loss⁶⁵. Moreover, neutrophils secrete B-lymphocyte stimulator and a proliferation-inducing ligand (APRIL), two key cytokines that promote the survival, proliferation, and maturation of B lymphocytes into plasma cells^{66,67}.

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^{68–73}. 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 regulate the activation and differentiation of B cells and T-cell subsets^{56,57}. In periodontitis, these complex interactions lead to inflammation-induced bone loss, which is largely mediated by a triad of proteins consisting of the receptor activator of nuclear factor- κ B ligand (RANKL), its functional receptor RANK, and its decoy receptor osteoprotegerin. RANKL is produced by activated T and B lymphocytes as well as by osteoblasts in the inflamed periodontium⁷⁴. The binding of cell-surface or soluble RANKL to RANK on osteoclast precursors triggers osteoclast maturation and activation, although this RANKL/RANK-driven process is antagonized by the decoy receptor osteoprotegerin^{7,75}.

The ideal outcome of an inflammatory response is its timely termination so that it does not become chronic with potentially adverse effects. Indeed, non-resolving inflammation underlies the pathogenesis of many chronic conditions, including periodontitis⁷⁶. The successful resolution of inflammation is an active and well-coordinated process that involves a series of steps. These include down-regulation 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^{77,78}.

A ‘lipid-mediator class switching’ takes place during the resolving phase of inflammation. This temporal switch signifies a transition from an environment rich in pro-inflammatory prostaglandins and leukotrienes to one with high levels of pro-resolving mediators, which include arachidonic acid-derived lipoxins and omega-3 polyunsaturated fatty acid-derived resolvins and protectins^{11,79,80}. 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 peptidoleukotriene-dependent neutrophil adhesion by down-regulating the expression of P-selectin on endothelial cells and of Mac-1 (CD11b/CD18) on neutrophils^{81,82}. Additionally, lipoxins and resolvins can inhibit neutrophil recruitment by down-regulating β 2 integrin and ICAM-1 expression and upregulating endothelial cell production of nitric oxide, an inhibitor of leukocyte adhesion to

vascular endothelium^{78,83,84}. In addition to restraining neutrophil infiltration, pro-resolving lipid mediators promote neutrophil apoptosis and their phagocytosis by macrophages at the inflamed site⁸⁵.

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, c-Mer tyrosine kinase receptor (MerTK), and the integrins $\alpha v \beta 3$, $\alpha v \beta 5$ and CD11b/CD18, also known as complement receptor-3. These receptors do not necessarily work independently of each other but actually appear to engage in cooperative interactions^{86–88}. 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 with the help of ‘bridging molecules’, with different types of efferocytic receptors on macrophages⁸⁷. An example of the former mechanism involves the efferocytic receptor T-cell immunoglobulin- and mucin-domain-containing molecule-4 which can directly bind phosphatidylserine⁸⁹. An example of indirect interaction involves the bridging molecules Del-1 and the related milk fat globule epidermal growth factor-8 (MFG-E8; also designated lactadherin), which promote efferocytosis by each binding to $\alpha v \beta 3$ integrin (via an RGD motif on the *N*-terminal part of the molecules) and to phosphatidylserine (via discoidin-I like domains in the *C*-terminal part of the molecules)^{90–92}. Moreover, MerTK 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, Mac-1 (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 cells⁹³.

Efferocytosis serves more than a mechanism of waste disposal, that is, to clear apoptotic cells and prevent 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 re-programmed to reduce the expression of proinflammatory cytokines (*e.g.*, interleukin-6 and interleukin-23) and increase the expression of regulatory cytokines, such as transforming growth factor- β and interleukin-10^{78,80,91,94}. Liver X receptors (LXRs) and peroxisome proliferator-activated receptors (PPARs) are ligand-activated transcription factors belonging to the nuclear receptor superfamily and play important roles in efferocytosis and the resolution of inflammation^{95,96}. LXRs comprise two isoforms (LXR α and LXR β) and are

activated by endogenous oxysterols, *i.e.*, oxidized derivatives of cholesterol. Endogenous ligands of PPARs (existing in distinct isoforms α , β/δ , and γ) include fatty acids and other lipids⁹⁷. Engulfment of apoptotic neutrophils triggers LXR signaling in the efferocytic macrophage in response to the sterol lipids of the apoptotic plasma membrane. LXR signaling in turn upregulates the expression of MerTK, thereby enhancing further efferocytosis, and furthermore inhibits proinflammatory gene expression^{91,96,98,99}. PPAR β/δ signaling is also induced during apoptotic cell uptake, apparently by polyunsaturated fatty acid ligands derived from the apoptotic cell plasma membrane. PPAR β/δ signaling induces the expression of C1q (a complement component that binds phosphatidylserine) and MGF-E8, both of which can promote the phagocytosis of apoptotic cells by macrophages^{90,100}. Consistent with this, PPAR β/δ -deficient macrophages have impaired capacity to perform efferocytosis and to switch to an anti-inflammatory and pro-resolving phenotype¹⁰¹. Overall, macrophages exhibit remarkable plasticity, which is crucial for successful resolution of inflammation, hence an important target for host modulation in inflammatory diseases.

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 pro-inflammatory 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 where the mechanisms of action are adequately understood (Figure 2).

Non-steroidal anti-inflammatory drugs

Non-steroidal anti-inflammatory drugs block the cyclooxygenase pathway of arachidonic acid metabolism and have been considered for the treatment of periodontitis^{102,103}. Non-steroidal anti-inflammatory drugs can be non-selective or selective depending on their ability to block the activity of both enzyme isoforms, cyclooxygenase-1 and -2 or only 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 (*e.g.*, fibroblasts) and recruited cell types (*e.g.*, macrophages, and neutrophils) in the periodontium in response to lipopolysaccharide or pro-inflammatory cytokines. Prostaglandin E2 functions as a

vasodilator and promotes vascular permeability as well as bone resorption in periodontitis^{104,105}. Animal model-based studies and clinical trials have shown that non-steroidal anti-inflammatory drugs could inhibit alveolar bone resorption. Although these drugs could improve the clinical outcome of mechanical periodontal treatment, the results decline rapidly after drug withdrawal^{102,103,106,107,108}. Moreover, current formulations have serious adverse effects that preclude their prolonged use for periodontal therapy. Non-selective non-steroidal anti-inflammatory drugs were associated with gastrointestinal mucosal damage and renal toxicity^{109,110}. The use of selective cyclooxygenase-2 inhibitors is also problematic as they can induce prothrombotic side-effects¹¹¹, which may be attributed to reversal of cyclooxygenase-2-dependent attenuation of expression of tissue factor, a molecule that activates blood clotting¹¹².

Anti-cytokine therapy

Anti-cytokine treatments involve the use of neutralizing monoclonal antibodies or receptor antagonists to block the action of pro-inflammatory 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^{53,113,114}. 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 periodontitis¹¹⁵. Such drugs include infliximab (monoclonal antibody to tumor necrosis factor), etanercept (soluble form of tumor necrosis factor receptor) and anakinra (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 due to the small number of studies performed, which moreover were not specifically designed to address efficacy in periodontitis. Anti-cytokine therapy has potential adverse effects on immunity, which may be less serious for local than for systemic administration. 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 network¹¹⁶. 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 axis⁵². Indeed, anti-interleukin-23 therapy (through ustekinumab, a monoclonal antibody that blocks the common interleukin-12/interleukin-23 p40 subunit) in a patient with LAD inhibited gingival expression of interleukin-17 and resolved inflammatory lesions without adverse reactions after one year of systemic treatment¹¹⁷.

Specialized pro-resolution mediators

As discussed in more detail above, specialized pro-resolving 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^{78,83,84}. A newly dissected pro-resolving 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 $\beta 2$ integrin antagonist Del-1, thus potentially contributing to the resurgent expression of Del-1 during the resolution of inflammation^{91,118}. A number of studies have established the ability of specialized pro-resolving 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 acid¹²⁶. To date, these intervention studies have involved small sample sizes. Larger-scale clinical trials are required to substantiate the initial promising results.

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 appear to modulate both the microbiota and the host response^{127,128}. Several probiotic preparations were 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 treatment¹²⁹. This might be due to 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 pro-inflammatory 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 loss¹³⁰. In mice, moreover, topical application of *Lactobacillus brevis* CD2 was shown to inhibit ligature-induced gingival inflammation and bone loss and reduce the counts of gram-negative bacteria¹³¹. A major mechanism by which *L. brevis* CD2 exerts anti-inflammatory action is through suppression of nitric oxide synthesis.

In a subsequent human study, *L. brevis* CD2 or placebo lozenges were applied topically in the mouth (3 times daily for 14 days) and allowed to slowly dissolve. The probiotic had a modest effect in reducing signs of experimental gingivitis in *L. brevis* CD2-treated volunteers as compared to placebo-treated group¹³². 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 they cause significant improvement in clinical indicators of periodontal disease (reviewed in refs.^{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 involved small sample size and are quite heterogeneous (*e.g.*, use of different probiotic strains, doses, and mode of

administration). Therefore, strong conclusions cannot be readily drawn. Indeed, a recent meta-analysis concluded that more clinical studies, especially long-term, are required to strengthen the notion that *L. reuteri*-based probiotics have clinical efficacy as an adjunct to scaling and root planing¹³³. Thus, long-term efficacy as well as safety of probiotics need to be established before reliable clinical recommendations could be made¹²⁹.

Complement

Early clinical studies have shown considerably higher abundance of complement activation products in the gingival tissue and the gingival crevicular fluid of periodontitis patients 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 inflammation¹⁴¹. Conversely, successful periodontal treatment which resolved clinical inflammation inhibited complement activation in the gingival crevicular fluid of the treated patients¹⁴². 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), which were found to be protected against experimental periodontitis as compared to 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 as to whether C3 inhibition could provide a therapeutic benefit for periodontitis patients. 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 is a 13-residue, cyclic peptide (I[CVVQDWGHHRC]T-NH₂)¹⁴⁸. All members of the compstatin family block the activation of C3 exclusively in humans and non-human 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 to the original version of compstatin, Cp40 has several improved features, including stronger inhibitory action, higher binding affinity for the C3 target and better pharmacokinetic parameters¹⁴⁶. Specifically, Cp40 has subnanomolar affinity for C3 ($K_D = 0.5$ nM) and a plasma half-life (~40 h) that exceeds expectations for most peptidic drugs^{146,147,149}.

When locally administered by intra-gingival injection in a preventive setting, Cp40 inhibited inflammation and bone loss in young adult non-human primates subjected to ligature-induced periodontitis¹⁴³. Cp40 was effective also in a therapeutic setting in multiple subsequent studies. Importantly, in the absence of additional treatments such as scaling and root planing, the drug inhibited pre-existing, naturally occurring periodontitis in aged non-human primates, even when given once every 3 weeks, and its protective effects lasted for at least 6 weeks after drug withdrawal^{150,151}. In the above-discussed studies^{143,150,151}, C3 inhibition by Cp40 exerted a strong suppressive effect on interleukin-17, a potent proinflammatory and pro-osteoclastogenic cytokine (Figure 3). In this respect, complement and Toll-like receptors are cooperatively involved in the regulation of interleukin-17 production by both innate and adaptive immune cells, such as Th17^{152–157}. Although complement has in general complex regulatory effects on interleukin-17 expression, in the

periodontium, complement enhances interleukin-17 production in synergy with Toll-like receptor signaling¹⁵³. Th17-derived interleukin-17 can mediate bone immunopathology by inducing matrix metalloproteinases and RANKL, thereby contributing to degradation of both connective tissue and the underlying alveolar bone in both human periodontitis and experimental periodontitis in animal models^{65,74,158,159}(Figure 3). Inhibition of RANKL expression by Cp40 in non-human primate periodontitis^{143,150} may in part be attributed to the ability of this C3 inhibitor to suppress interleukin-17 production.

Cp40 has been licensed by Amyndas Pharmaceuticals and formed the basis for the development of AMY-101, a clinical candidate drug intended for therapeutic intervention in complement-mediated diseases including periodontitis^{160,161}. AMY-101 has been extensively tested for safety. Monitoring of non-human primates under prolonged (up to 3 months) systemic treatment with AMY-101 revealed no significant differences in hematological, biochemical or immunological parameters in their blood or tissues relative to those of control animals, despite complete inhibition of C3 in the plasma. Furthermore, wounds inflicted in the skin of the AMY-101-treated monkeys did not show signs of infection but in fact showed a trend towards faster wound healing as compared to controls¹⁶². Moreover, AMY-101 was shown to be free of local irritation when intra-gingivally injected in non-human primates¹⁵¹. More recently, AMY-101 was evaluated in a first-in-human clinical trial in healthy volunteers, in which AMY-101 was shown to be safe and well tolerated^{161,163}. Overall, the safety and efficacy features of locally administered AMY-101^{143,150,151} suggest that this is a promising approach that merits investigation as a host-modulation therapy in human periodontitis.

Homeostatic proteins comprising epidermal growth factor-like and discoidin-like domains

The secreted homologous proteins Del-1 and MFG-E8, 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 (Del-1 has three and MFG-E8 has two such repeats), the second of which contains an integrin-binding RGD motif. At the *C*-terminus, both Del-1 and MFG-E8 have two discoidin I-like domains which can bind phospholipids and glycosaminoglycans^{90,92,164–166}. Both proteins were shown to have important homeostatic functions and to inhibit periodontitis in mouse and non-human primate models^{53,167–169}.

As mentioned earlier, Del-1 can regulate β_2 integrin-dependent neutrophil functions, including firm adhesion to the endothelium, thereby suppressing neutrophil recruitment to sites of inflammation^{53,54,170} (Figure 3). As a consequence, Del-1-deficient mice display excessive neutrophil infiltration in the gingiva and develop spontaneous periodontal inflammation⁵³. Moreover, Del-1 is expressed by osteoclasts and regulates their RANKL-induced differentiation as well as restrains their resorptive function¹⁶⁷. More recently, Del-1 was shown to contribute to the resolution of periodontal inflammation⁹¹. In both human and murine periodontitis, inflammation clearance correlated with restoration of Del-1 expression

levels after the initial downregulation of Del-1 during initiation of inflammation. Resolution of experimental periodontitis in mice failed in Del-1 deficiency, and Del-1 was functionally connected with resolvins. Indeed, Del-1 was shown to act as a non-redundant downstream effector of inflammation resolution mediated by resolvin D1 and was required for optimal production of resolvin D1 and resolvin E1 ⁹¹. Therefore, Del-1 is not only a crucial effector of periodontal inflammation resolution but is also positively cross-regulated with resolvins ^{91,118}, likely leading to the generation of a positive-feedback loop that fortifies inflammation resolution. Del-1 was also shown to promote the resolution of acute monosodium urate crystal-induced peritoneal inflammation through its ability to promote effective apoptotic neutrophil clearance (efferocytosis) through an $\alpha\text{v}\beta 3$ integrin-dependent mechanism that was discussed above ⁹¹.

Consistent with its regulatory actions, recombinant Del-1 (fused to the Fc part of IgG; Del-1-Fc) was shown to inhibit periodontal tissue inflammation and bone loss in both mice and non-human NHPs subjected to ligature-induced periodontitis ¹⁶⁷. Apparently, Del-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 since the immune system and periodontal tissue anatomy of monkeys is similar to that of humans; moreover, monkey periodontitis shares important clinical, microbiological, and immunohistological features with the human disease ¹⁷¹.

MFG-E8 expressed by macrophages was shown to promote efferocytosis and hence the resolution of inflammation ⁹⁰. As noted above, MFG-E8 is structurally similar to Del-1, thus it contains the critical features (RGD motif in the *N*-terminal part and discoidin-like domains in the *C*-terminal part) that enhance apoptotic cell phagocytosis. Moreover, MFG-E8 has direct anti-inflammatory activity in macrophages. Specifically, it can bind $\alpha\text{v}\beta 3/5$ -integrins and induce signal transducer and activator of transcription 3 (STAT3)-mediated activation of suppressor of cytokine signaling-3 in macrophages ¹⁷². Accordingly, recombinant MFG-E8 inhibits inflammation in animal models of sepsis or cerebral ischemic injury ^{173,174}. More recently, and similar to Del-1, MFG-E8 was shown to suppress the RANKL-induced differentiation of osteoclasts ¹⁶⁹. Consistent with its ability to regulate inflammation and osteoclastogenesis, locally administered recombinant mouse MFG-E8 was shown to inhibit periodontal inflammation and bone loss in ligature-induced periodontitis in mice ¹⁶⁹. Moreover, recombinant human MFG-E8 inhibited periodontal inflammation and bone loss in non-human primates subjected to ligature-induced periodontitis ¹⁶⁸. In humans, the gingival crevicular fluid levels of MFG-E8 are significantly lower in periodontitis as compared to health. However, the gingival crevicular fluid levels of MFG-E8 are significantly elevated in periodontitis patients following non-surgical (scaling and root planing) or surgical periodontal treatment. These findings support the potential utility of MFG-E8 as both a biomarker and therapeutic agent in periodontal disease.

Nuclear metabolic receptor agonists

Nuclear receptors, such as the LXRs and PPARs mentioned above, are ligand-activated transcription factors involved in the expression of genes that regulate cell growth and

differentiation, and metabolic homeostasis¹⁷⁵. LXRs and PPARs are constitutively found in the nucleus where they form heterodimers with retinoid x receptors (RXRs) that bind to specific response elements in target genes even in the absence of ligand binding. However, when not occupied by ligands, LXR/RXR and PPAR/RXR heterodimers are transcriptionally inactive as they are complexed with corepressor proteins. In 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 expression¹⁷⁵. More recently, LXRs and PPARs were linked to the regulation of inflammation, thus placing them at the intersection of metabolism and immunity^{99,175}. As alluded to earlier, LXRs and PPARs play important roles in the clearance of apoptotic neutrophils (efferocytosis) by tissue phagocytes^{95,96}. LXRs are also critical for the ability of Del-1 to induce a pro-resolving macrophage phenotype in the context of efferocytosis⁹¹.

Accumulating evidence suggests that PPAR activation may have protective effects in periodontitis. The activation of PPAR δ (also known as PPAR β hence often designated PPAR β/δ) was shown to suppress the ability of *P. gingivalis* lipopolysaccharide to activate matrix metalloproteinase-2 in human gingival fibroblasts¹⁷⁶. Mechanistically, PPAR β/δ signaling – triggered by the selective agonist GW501516 – inhibited mRNA and protein expression of NADPH oxidase 4 (Nox4), thereby attenuating the production of reactive oxygen species that would in turn induce matrix metalloproteinase-2 activation. Consequently, GW501516-induced activation of PPAR β/δ 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 pathogenesis¹⁷⁷, these findings may provide mechanistic support for an in vivo rat study showing that systemic administration of a selective PPAR β/δ agonist (GW0742) attenuated ligature-induced periodontal inflammation and tissue damage¹⁷⁸. Protective results in ligature-induced periodontitis in rats were obtained also by administering synthetic agonists of PPAR α ¹⁷⁹ or PPAR γ ¹⁸⁰.

Targeting adaptive immune cells

B lymphocytes and plasma cells are abundant in the inflammatory infiltrate of advanced chronic periodontitis in humans^{6,73,181}. The survival, proliferation, and maturation of B lymphocytes depends on two cytokines of the tumor necrosis factor ligand superfamily, namely APRIL and B-lymphocyte stimulator^{182,183}, which are both upregulated in human periodontitis¹⁸⁴. Antibody-mediated neutralization of APRIL or B-lymphocyte stimulator in mice was shown to diminish the B cell numbers in the gingival tissue and inhibit periodontal bone loss¹⁸⁴. Therapeutic approaches targeting APRIL 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 FDA for the treatment of systemic lupus erythematosus^{185,186}. 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 destruction¹⁸⁷. Thus, although more studies are required for reliable conclusions, limited evidence suggests the potential of B cell-targeted therapies for the treatment of periodontitis.

CD4⁺ Foxp3⁺ regulatory T cells (Tregs) downregulate the induction and proliferation of effector T cells and can be protective in inflammatory and autoimmune conditions ¹⁸⁸. In experimental periodontitis in mice, Tregs appear in high numbers after the peak appearance of RANKL-expressing CD4⁺ T cells ¹⁸⁹ and selective Treg depletion exacerbates inflammation and bone loss ⁷⁰. Tregs therefore mitigate inflammatory tissue damage. However, their protective potential may be compromised in inflamed tissues. For instance, interleukin-23-activated $\gamma\delta$ T cells restrain Tregs and shift the balance in favor of effector T helper cells and, moreover, render effector T cells refractory to suppression by Tregs ¹⁹⁰. However, local treatment of mice or dogs with a chemoattractant formulation for Tregs, consisting of the chemokine CCL22 encapsulated in degradable polymer, enhanced the recruitment of Tregs and inhibited experimental periodontitis in these models ¹⁹¹.

A recent study has implicated Th17 cells in inflammatory tissue destruction of experimental periodontitis ⁶⁵. Specific genetic inhibition of Th17 cell differentiation (by knocking out the critical transcription factors Stat3 or ROR γ t) not only led to the absence of Th17 cells from the gingival tissues but also significantly blocked inflammatory bone loss in a murine model of ligature-induced periodontitis. Human relevance for the importance of Th17 in periodontal disease pathogenesis was established by showing that patients with genetically impaired Th17 cell development (due to STAT3 loss-of-function mutations; autosomal dominant hyper IgE syndrome ¹⁹²) exhibited significantly decreased periodontal inflammation and bone loss as compared to age- and gender-matched healthy controls and periodontitis patients ⁶⁵. From a translational perspective, pharmacological inhibition of ROR γ t using the small-molecule inhibitor GSK805, which inhibits Th17 development and function ¹⁹³, resulted in significantly decreased periodontal inflammation and bone loss in mice ⁶⁵. GSK805 preferentially targeted the expansion of Th17 cells without affecting other interleukin-17-secreting cellular sources, such as $\gamma\delta$ 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 Th17 inhibitor in periodontitis that can provide protection against this inflammatory disease.

Approaches for direct inhibition of periodontal tissue destruction

A persisting, non-resolving 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 receptor activator of nuclear factor- κ B ligand (RANKL), 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 sub-antimicrobial doses of these antibiotics could block the activity of matrix metalloproteinases, 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 and 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 a placebo and scaling and root planing combination. Sub-antimicrobial doxycycline (20 mg, taken twice daily) has received FDA approval and has been marketed as Periostat for the treatment of human periodontitis ^{196,197}.

Bisphosphonates are anti-resorptive 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. 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 periodontitis patients 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 ^{200,201,202}.

Another approach to directly inhibit bone loss is to inhibit the differentiation of osteoclasts by blocking the interaction of RANKL with its receptor RANK on the surface of osteoclast precursors. Proof-of-concept was obtained by showing that the natural inhibitor of RANKL, osteoprotegerin (administered as a fusion protein with the Fc portion of IgG) and anti-RANKL antibody both inhibit periodontal bone loss in rats ^{203,204}. Denosumab is a humanized monoclonal antibody that binds RANKL and prevents its interaction with RANK and has been used for treating osteoporosis ^{205,206}. The efficacy of denosumab has not been specifically tested in periodontitis patients, 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 (sFRPs) ^{208,209}. For instance, sFRP1 was shown to bind RANKL, thereby preventing its interaction with RANK and hence inhibiting osteoclastogenesis ²⁰⁹. The main targets of sFRPs are, nevertheless, the Wingless/integrase-1 (Wnt) family of proteins. This is because sFRPs are structurally related to the frizzled receptors of Wnt proteins and can act as decoy receptors that antagonize Wnt protein-frizzled receptor interactions ²¹⁰. In this context, Wnt5a and sFRP5 constitute a typical ligand/antagonist pair ²¹⁰. The binding of osteoblast-derived Wnt5a to a co-receptor complex involving receptor tyrosine kinase-like orphan receptor-2 and frizzled on osteoclast precursors induces noncanonical Wnt signaling that upregulates RANK expression. This in turn sensitizes the precursors to the action of RANKL, thereby promoting osteoclast differentiation ²⁰⁸. Importantly in this regard, local intralingival injection of sFRP5 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 sFRP5 could additionally, or alternatively, be attributed to its anti-inflammatory properties ^{211–213}. Interestingly, there is a reciprocal relationship between sFRP5 and Wnt5a expression in human periodontal health

and disease, with Wnt5a dominating in diseased and sFRP5 in healthy tissue²¹¹. In principle, sFRP5 might potentially be both a biomarker and a therapeutic agent in periodontitis.

Vaccination

The first essential condition for immunization against any microbially induced disease is to define the causative agent(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 1980s²¹⁴. A challenge in vaccine development for periodontitis is that the disease primarily results from collateral 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^{215–219}. Another challenge for vaccine development is that periodontitis is initiated by synergistic and dysbiotic microbial communities rather than by a few select ‘periodontopathogens’^{19–28}.

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, *i.e.*, exerts community-wide effects that promote the pathogenicity of the entire biofilm⁴⁷. 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^{23,28,220,221}. If *P. gingivalis* exerts similar effects in human periodontitis, at least in a subset of patients (more below), 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, due to the undesirable reactogenicity of these types of vaccines, subsequent attempts in animal models have focused on defined microbial proteins or genetically engineered subunits thereof²¹⁴. Such subunit vaccine approaches 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^{222–226}. 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 (*e.g.*, 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 ²¹⁴.

Subcutaneous immunization of rats with the hemoglobin-binding domain of *P. gingivalis* gingipain could induce specific IgG antibodies and moderate protection against alveolar bone loss ²²⁷. The same immunogen, when given with Freund's complete adjuvant, was able to potentiate the antibody responses but failed to confer protection against bone loss ²²⁷. This finding may have been due to 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 antibody responses and protection against *P. gingivalis*-induced periodontal bone loss ²²⁵. 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 Fc γ RII-dependent phagocytosis and killing. Alternatively, specific IgG2a antibodies could inhibit colonization by *P. gingivalis*. More recently, a chimera vaccine combining key gingipain sequences formulated in alum induced antigen-specific IgG1 antibody and Th2-biased response that protected mice against *P. gingivalis*-induced periodontitis ²²⁸. Although the role of Th2 cells in periodontitis is incompletely understood, this subset does not seem to be involved in periodontitis pathogenesis, in contrast to the Th17 subset ^{65,69,229}. 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 antibody responses and protection against subsequent *P. gingivalis*-induced periodontal bone loss ²²².

Vaccination studies in macaque monkeys are particularly valuable as periodontitis in these animals more closely resembles the human diseases than the more convenient and relatively inexpensive rodent models and, importantly, 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* ²³⁰. Moreover, there were no significant differences between immunized and control animals with regard to periodontal bone loss ²³⁰. 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 polyoxyethylene/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 non-immunized controls ¹⁰⁵. This study suggests that suppression of inflammatory mediators by immunization, possibly due to reduced pathogenic challenge as a consequence of antibody-dependent inhibition of colonization or killing, is a potential 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 load²³¹. These findings are consistent with the concept that the presence of *P. gingivalis* benefits the entire microbial community, as anticipated by the keystone-pathogen hypothesis⁴⁷.

Additional studies are needed to better define vaccination strategies and the immune response mechanisms that may be effective in controlling periodontal disease in primates. Specifically, much more has to be done to define adjuvant formulations and immunization routes (mucosal routes are considered 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 (immune deficiencies, immunoregulatory defects smoking, diet, obesity, diabetes and other systemic diseases, aging) and may act alone or in combination to modify the host response and the microbiome in a destructive direction^{22,232,233}. Thus, although anti-*P. gingivalis* immunity may be protective in models where 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 health²³⁴, in many patients, the presence of *P. gingivalis* in subgingival plaque has been shown to predict imminent disease progression (clinical attachment loss)²³⁵.

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 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 non-inflammatory modes of immune response that prevent tissue damage and ideally promote inflammation resolution and tissue healing.

Anti-aging approaches

The elderly display increased susceptibility to infectious and inflammatory diseases including periodontal disease^{17,236–242}. 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^{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^{17,239,245–252}.

The aging of hematopoietic stem cells is a fundamental cause of immune senescence^{250,253}, which affects both innate and adaptive immunity^{237,253,254}. In both humans and mice, hematopoietic stem cell function is impaired in old age, including reduced long-term self-renewal capacity, attributable to both hematopoietic stem cell-intrinsic and -extrinsic mechanisms^{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, stem cell proliferation and differentiation^{250,255–260}. Mounting evidence suggests that the hematopoietic stem cell niche is crucial for the regulation of cellular senescence in hematopoietic stem cells^{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 experiments²⁶¹.

Intriguingly, the homeostatic function of Del-1 is not restricted to peripheral tissues such as the periodontium. Del-1 is also secreted in the bone marrow (by arteriolar endothelial cells, cells of the osteoblastic lineage and by CXCL12-abundant reticular cells) where it serves as a crucial regulator of the hematopoietic stem cell niche²⁶². Specifically, Del-1 interacts with the $\alpha v\beta 3$ integrin on hematopoietic stem cells and promotes their retention, expansion and differentiation toward the myeloid lineage, both under steady-state and stress conditions²⁶². Thus, the aging-associated deficiency of Del-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 process²⁶¹. It is tempting to speculate that the hematopoietic stem cell rejuvenation might, in part, be due to expression of normal levels of Del-1 by the transplanted endothelial cells.

Aging-associated alterations in immune function dysregulate the host response in a manner that (i) impairs immunity to pathogens and (ii) promotes non-productive inflammation, the combination of which may increase host susceptibility to a microbe-driven chronic inflammatory disease such as periodontitis^{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 (mTOR) activity is increased with aging in hematopoietic stem cells and contributes to their senescence; inhibition of mTOR by rapamycin, an FDA-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 infection²⁶⁹. The improved efficacy of vaccination in old mice after hematopoietic stem cell rejuvenation by rapamycin treatment 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 reported²⁷⁰.

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 (*e.g.*, 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 non-steroidal anti-inflammatory drugs. For instance, potential adverse effects on thymocyte and lymph node development by the use of ROR γ 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, since Del-1 expression 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 non-human 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 inflammation inhibition by AMY-101 tips

the balance towards host-microbe homeostasis, which might be resilient to pathological processes that would tend to re-instate active periodontitis.

With the possible exception of probiotics, most of the aforementioned interventions are intended to be used for treatment rather than 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, i.e., prior to onset of periodontitis, to high-risk individuals, such as cigarette smokers and diabetic patients^{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.

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Figure 1. Periodontal disease pathogenesis.

Periodontal health is maintained by homeostatic immunity and is associated with symbiotic microbiota. Periodontitis is associated with a dysbiotic polymicrobial community, in which different members have distinct and synergistic roles that promote destructive inflammation. Keystone pathogens — which are aided by accessory pathogens in terms of nutritional and/or colonization support — initially subvert host immunity leading to the emergence of dysbiotic microbiota, in which commensal-turned pathobionts overactivate the inflammatory response and cause tissue destruction. Inflammation in turn can exacerbate dysbiosis through provision of nutrients for the bacteria (derived from tissue breakdown products; *e.g.*, collagen peptides and heme-containing compounds). Therefore, inflammation and dysbiosis are reciprocally reinforced and generate a positive-feedback loop. This self-sustaining loop may underlie the chronicity of periodontitis, the development of which requires a susceptible host. Risk factors include (but are not limited to) the presence of bacteria that subvert the host response, systemic disease, smoking, aging, high-fat diet, and immune deficiencies. These factors could promote dysbiosis by acting individually or more effectively in combination.

Figure 3: Proinflammatory functions of interleukin-17 with potential for periodontal tissue destruction.

Synergistic complement and Toll-like receptor signaling in antigen-presenting cells enhances interleukin-17 production by adaptive immune cells (Th17). Interleukin-17, in turn, acts predominantly on innate immune and stromal cells to promote inflammatory responses. By upregulating G-CSF, 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 Del-1, a homeostatic protein that suppresses neutrophil adhesion and extravasation by blocking the interaction between the LFA-1 integrin on neutrophils and the adhesion molecule ICAM-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.