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Macrophage immunomodulation in chronic osteolytic diseases —the case of periodontitis

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Abstract

Periodontitis (PD) is a chronic osteolytic disease that shares pathogenic inflammatory features with other conditions associated with nonresolving inflammation. A hallmark of PD is inflammation-mediated alveolar bone loss. Myeloid cells, in particular polymorphonuclear neutrophils (PMN) and macrophages (Mac), are essential players in PD by control of gingival biofilm pathogenicity, activation of adaptive immunity, as well as nonresolving inflammation and collateral tissue damage. Despite mounting evidence of significant innate immune implications to PD progression and healing after therapy, myeloid cell markers and targets for immune modulation have not been validated for clinical use. The remarkable plasticity of monocytes/Mac in response to local activation factors enables these cells to play central roles in inflammation and restoration of tissue homeostasis and provides opportunities for biomarker and therapeutic target discovery for management of chronic inflammatory conditions, including osteolytic diseases such as PD and arthritis. Along a wide spectrum of activation states ranging from proinflammatory to pro-resolving, Macs respond to environmental changes in a site-specific manner in virtually all tissues. This review summarizes the existing evidence on Mac immunomodulation therapies for osteolytic diseases in the broader context of conditions associated with nonresolving inflammation, and discusses osteoimmune implications of Macs in PD.

Keywords

bone; macrophage; osteoimmunology; osteolysis

1 | INTRODUCTION

Periodontal diseases are chronic inflammatory conditions characterized by inflammatory destruction of tooth supporting structures that affects almost 50% of adults in the United States.¹ Periodontal diseases, including chronic and aggressive forms of periodontitis (PD),

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AUTHORSHIP

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are associated with local inflammation and bone resorption around teeth that contribute to the systemic inflammatory burden and lead to tooth loss. PD is the most frequent cause of tooth loss in humans and one of the most prevalent diseases causing considerable morbidity that places heavy economic burdens on dental health care systems and significantly impacts the quality of life.²⁻⁴ Diagnosis, treatment, and monitoring of response to therapy for PD relies on various clinical signs and symptoms such as presence of subgingival plaque and calculus, gingival bleeding, periodontal pocket depth, clinical attachment loss, and radiographically visible bone loss around teeth.⁵ Gingivitis, inflammation of gingiva without signs of bone or attachment loss is ubiquitous in children and adults globally.⁶ It represents a risk factor for PD and is considered its initial reversible stage, but not all gingivitis lesions convert to PD.⁷ Major risk factors for PD include poor oral hygiene, greater age, male gender, genetic predisposition, smoking, diabetes mellitus, obesity, low socioeconomic status, and psychosocial stress.^{8,9}

Regardless of contributing factors, PD invariably affects the periodontium comprising of tooth supporting structures: cementum, a functionally oriented periodontal ligament, alveolar bone, and the gingiva.¹⁰ Different genetic, environmental, and behavioral factors contributing to onset of PD lead to a common phenotype dominated by local inflammation around teeth and subsequent loss of alveolar bone. The nonresolving inflammatory response seen in PD is induced by commensal pathogens that thrive in dysbiotic subgingival biofilms, and sustained by inflammatory macrophages (iMac), dendritic cells (DC), and polymorphonuclear neutrophils (PMN).¹¹⁻¹³ Because it is possible to precisely and reproducibly measure alveolar bone loss, and to obtain serial biologic samples noninvasively and repeatedly using animal models of PD, the study of alveolar bone loss in PD has enabled important advances in the means to restore bone health in inflammatory diseases.¹⁴ Immune modulation therapies targeting key cellular players in PD, in particular monocytes and Macs, or reliable markers of disease activity focused on these cells are lacking. In this context, PD can serve as a disease model to identify Mac-modulating drug targets to restore balance in osteoimmune links between host and the human microbiome.

Treatment of PD is tailored to disease severity, aggressiveness, and identified risk factors in an individual. Mild to moderate cases of PD can generally be managed by nonsurgical means, which include scaling and root planing, and adjunctive antibiotics.^{15,16} Scaling and root planing is performed to rid tooth surfaces of bacteria and their byproducts at and below the gingival margin by manual and ultrasonic instrumentation. Antibiotic treatment is implemented during initial phase of treatment in cases associated with suppurating pockets, poorly controlled diabetes, and/or if aggressive and rapid deterioration is noted, despite adhering to good home care and conventional therapy.¹⁷ For severe PD cases or when nonsurgical therapy cannot adequately control disease progression surgical approaches are used to reduce pocket depths and generate anatomic contours at the tooth-periodontium interface that are maintainable through home care and scaling. Surgical therapy may include open flap debridement with pocket reduction, and resective or regenerative surgery to eliminate biofilm retentive periodontal defects and recreate physiologic contours.¹⁸ However, treatment for PD is nonspecific relative to pathogenic mechanisms, healing and regenerative potential, and disease remission predisposition.

Management of PD involves lifelong periodontal maintenance that includes reassessment of risk factors and bone levels, mechanical plaque control, and applicable behavioral modifications. Despite significant advances in understanding the pathogenic pathways in PD, in particular persistent proinflammatory mediator release and failure to resolve inflammation due to PMN and Mac alterations, no therapeutics or cellular markers of disease activity are used in PD management. Advancing precision periodontology aims to classify disease based on pathogenesis rather than progression pattern to achieve the goal of personalized medicine. Similar to other chronic inflammatory diseases sharing pathogenic mechanisms with PD such as rheumatoid arthritis (RA) and ulcerative colitis, anti-cytokine biologics (e.g., adalimumab, anakinra, etanercept, tocilizumab) that block iMac-derived cytokines TNF α or IL-1 or 6 may control inflammation and slow disease progression at the expense of side effects like increased risk of infection.¹⁹ The opposite approach of therapeutically stimulating resolving Mac (rMac) with agents such as specialized pro-resolving lipid mediators (SPMs) or peroxisome proliferator-activated receptor gamma (PPAR- γ) holds promise for chronic inflammatory conditions. This review will cover Mac-modulating approaches to address inflammatory osteolysis in PD.

2 | INFLAMMATORY MEDIATORS IN PATHOGENIC PERIODONTAL OSTEOIMMUNE RESPONSES

PD is associated with oral microbial dysbiosis and altered host immune response to virulence factors in the subgingival biofilm. The current prevailing model for PD pathogenesis proposed by Lamont and Hajishengallis called polymicrobial synergy and dysbiosis (PSD), proposes that physiologically compatible organisms aggregate and coexist in a controlled immuno-inflammatory state that generates proinflammatory and toxic mediators inside periodontal tissues, but the host response controls their overgrowth and overt pathogenicity.²⁰ Both the host response and the bacterial activity contribute to non-resolving periodontal inflammation and bone loss.²¹ Inflammation drives extracellular matrix degradation and bone resorption, but the question how these processes are related in the onset and progression of disease remains unanswered. Pathogenic but not commensal bacteria in dysbiotic biofilms deregulate periodontal inflammatory responses that favor pro-osteolytic environments characterized by release of cytokines and matrix degrading enzymes that mediate the breakdown of connective tissue.²² Considerable attention has been paid to *Porphyromonas gingivalis*, *Treponema denticola*, *Tannerella forsythia*, and *Aggregatibacter actinomycetemcomitans*, which were linked to both chronic and aggressive forms of PD. However, the complex interactions between these keystone periodontal pathogens and dysbiotic subgingival biofilms are beginning to emerge as important factors driving pathogenicity in susceptible individuals. Importantly, putative periodontal pathogens are present in subgingival microbiota of individuals without PD, suggesting that pathogens are required but not sufficient to initiate disease onset.^{23,24} It becomes increasingly clear that multiple bacterial species and their metabolic changes in dysbiotic biofilms combined with altered innate immune defense are involved in initiation of nonresolving periodontal inflammation leading to irreversible breakdown of periodontal tissues.^{25,26} Many gram-negative bacteria involved in PD including *Actinobacillus actinomycetemcomitans*,

Bacterioides, *Porphyromonas*, *Wolinella recta*, and *Fusobacterium nucleatum* are a source of soluble LPS that drives iMac activation.²⁷

LPS and other bacterial factors stimulate PMN recruitment, iMac and DC activation, and local production of proinflammatory mediators, chemokines, matrix metalloproteases (MMPs), and pro-osteoclastic factors. On the other hand, local anti-inflammatory mediators and bone anabolic factors are reduced in PD.²⁸ Whereas the intricate links between innate and adaptive immunity in the context of nonresolving periodontal inflammation are incompletely understood, the PMN competence in controlling biofilm pathogenicity and the Mac ability to limit local inflammation and preserve tissue integrity are thought to play critical roles in preventing onset of PD (Fig. 1). The recognition that PD pathogenesis involves inflammatory factors as well as altered bone metabolism has provided a new insight on the etiology of the disease, categorizing PD under the umbrella of osteoimmunology. This framework that combines bone biology and immunology has made advances in our knowledge of specific cytokines and other mediators involved in the progression of PD and mechanisms underlying bone resorption.^{29,30} Bone loss associated with host inflammatory responses in PD depends on two factors: soluble mediator quantity and location relative to bone. First, the concentration of inflammatory mediators in gingival tissue must be abundant to activate pathways leading to bone resorption. Second, these mediators must be near alveolar bone for resorption to be stimulated.³¹ Accumulating sufficient concentrations of inflammatory mediators that lead to PD depends on the synthesis and secretion of proinflammatory cytokines, such as IL-1, IL-6, IL-8, IL-12, IL-17, IL-23, TNF- α , and leukemia inhibitory factor. Several kinins, thrombin, and chemokines, also play roles in bone resorption. In contrast, the expression of anti-inflammatory cytokines such as IL-4, IL-10, and IL-13 decrease bone resorption.³² Studies have shown that inhibition of proinflammatory mediators can prevent sufficient quantities near alveolar bone for induction of resorption, leading to a reduction in bone loss.³³

Nonresolving inflammation in close proximity to bone is associated with a cascade of events leading to osteoclastogenesis and subsequent bone loss via activation of the receptor activator of NF- κ B (RANK)-RANK ligand (RANKL) axis. During inflammation, cytokines, chemokines, and other proinflammatory mediators alter expression levels of RANKL on the osteoblast surface.^{32,34} Bone coupling is regulated by the ratio of RANKL to osteoprotegerin (OPG), a soluble decoy receptor for RANKL. When RANKL expression is up-regulated relative to OPG, free RANKL binds to RANK on preosteoclasts favoring osteoclastogenesis and subsequent bone resorption. Binding of RANKL to RANK in the presence of Mac colony-stimulating factor (MCSF) stimulates differentiation of the osteoclast precursors into a multinucleated cell that eventually becomes mature osteoclast.³¹ When OPG concentrations are high relative to RANKL expression, OPG competes with and prevents RANKL from binding to RANK, which is associated with net decrease in osteoclastogenesis.³⁵ In PD, excessive bone resorption occurs either due to up-regulation of RANKL or down-regulation of OPG, leading to an overall increase in the RANKL/OPG ratio.^{36,37} Using murine models of PD and genetic manipulation of the RANKL/OPG system, multiple studies found that this axis is a core element of PD, and that an increase in RANKL mRNA expression and protein production leads to high RANKL/OPG ratio and uncoupling of bone formation-resorption cycle through enhanced osteoclastogenesis and

osteoclast survival.^{38–40} Inflammatory mediators released by DC and iMac stimulate differentiation of Th17 lymphocytes, the major immune producers of RANKL, leading to increased RANKL/OPG ratio.²⁹ Thus, the optimal approaches to treat PD seem to be modulation of immune responses to putative periodontal pathogens for efficient nonself clearance and inflammation resolution to control proosteolytic environments and recouple physiologic bone formation and resorption.

3 | EXPERIMENTAL PD MODELING

Animal models are commonly used in periodontology to investigate the impact of risk factors on PD progression, pathogenic mechanisms and the actions of different therapeutics in PD. Most questions of causation cannot be accurately answered in human studies due to ethical considerations for serial tissue sampling and for control subjects. Thus, animal studies are vital for understating the cause and effect relationships and equally important for development of novel therapeutics. Vertebrate animal models can be used to examine the mechanisms governing PD pathogenesis considering that co-expression gene networks involved in bone homeostasis and inflammation seems to be conserved in vertebrates.^{41,42} One approach to simulate human PD in vertebrate animals is to generate subgingival biofilm retentive milieus by placing silk ligatures inside gingival sulci of molars, which facilitate the onset of inflammation mediated alveolar bone loss.⁴³ Changes in ligature-associated biofilms show similar microbial shifts to humans with PD compared to health.^{44,45} The ligature model has been used in rodents and larger vertebrates including dogs or nonhuman primates such as rhesus monkeys, cynomolgus monkeys, and baboons due to similarities with oral human structures that facilitate naturally occurring dental plaque, calculus, putative human periodontal pathogens, and PD.^{46,47} However, larger vertebrates are less frequently used due to ethical and economic constraints, and experimental PD in rodents using ligatures or pathogen inoculation do not fully reproduce human PD.⁴⁸

Ligature-induced PD was first introduced in 1966 and is one of the most widely used animal models in periodontal research. Murine models are often used in studying PD because periodontal anatomy in the molar region shares similarities with that of humans. Further, murine experimental PD mimics human PD by apical migration of junctional epithelium, pocket epithelium proliferation, influx of inflammatory cells, degradation of periodontal ligament fibers, and bone loss. The knowledge of genetic background for each mouse strain and ability to genetically engineer mouse strains for loss or gain of function studies makes them preferable over larger vertebrates.⁴³ Mice are also abundant with reasonable costs in view of the potential value of in vivo findings applicable to improving management of human diseases. Nonetheless, it is technically easy to locate and dissect the inflamed tissue in ligature-induced PD to assess the local inflammatory response.⁴⁹ Serial blood sampling in mice can be used in longitudinal studies to investigate the systemic impact of PD and associated conditions such as diabetes.⁵⁰ However, in small animals like mice, the mechanical trauma caused by the ligatures must be considered as a potential factor contributing to bone loss. Placement of small diameter (30 μ m, ~9.0) ligatures inside gingival sulci is technically challenging but it does not induce mechanical trauma. Nonetheless, experimental PD in rodents is not suitable for studying the natural course of PD due to low susceptibility and continuous growth and migration of teeth. Much about

human pattern of PD progression remains unknown partly because we do not have definitive measures of disease activity. It is generally considered that, in a multilevel system (i.e., individual with disease), PD follows a “random” burst pattern with some diseased sites improving whereas others worsen, which generates simultaneous appearance of sites with active disease and sites in remission in an individual.⁵¹

Experimental PD in mice can be used to investigate pathogenic mechanisms involved in PD and gain insights into critical aspects of disease activity.^{50,52–54} It can further aid in corroborating findings from patients with PD by using genetically modified strains.⁵⁵ The ligature-induced PD model, in which ligatures placed inside the gingival sulci serve as biofilm-retentive milieu mimicking calculus, generates a predictable sequence of events leading to oral dysbiosis, subgingival biofilm maturation, and inflammation-mediated alveolar bone loss.^{43,45} Studies in germfree and conventional mice and rats have confirmed that microorganisms are necessary for onset of inflammatory alveolar bone loss in response to silk ligatures placed around molars.^{56,57} The small diameter (30 μm , ~9.0) silk sutures sit passively inside the gingival sulcus and migrate apically as disease progresses, similar to human calculus-biofilm in PD.^{43,50} Larger diameter (5.0–6.0) sutures used in mice would cause trauma in addition to the intended biofilm retention. This model allows for investigation of immune events preceding bone loss and their relationship to uncoupling of bone resorption and formation applicable to chronic osteolytic diseases. The relative contribution of innate and adaptive immune cells to onset of bone loss can be dissected through selective cell depletion or alterations of critical functions such as recruitment, reactive oxygen/nitrogen species, and cytokine production.⁵⁸ Importantly, ligature experimental PD can be reversed when ligatures are removed after onset of bone loss, allowing for investigation of resolution of periodontal inflammation and bone regeneration, and the testing of therapeutics to enhance the latter.⁵⁹ This is specifically important for understanding the relationship between “constructive” and “destructive” inflammation governed by Mac in view of their central roles in regulation of tissue homeostasis.

4 | MAC FUNCTIONS IN INFLAMMATION, RESOLUTION, AND OSTEOIMMUNOLOGY

Because inflammation-mediated alveolar bone loss is the hallmark of PD and knowing that sustained periodontal inflammation can uncouple bone formation from resorption, effective removal of pathogens followed by active resolution of inflammation are needed to preserve normal tissue structure. Antigen-presenting cells such as Macs and DCs play a major role as sentinels in the first line defense and as mediators that shape the adaptive immune response when the former is inefficient in removing nonself.⁶⁰ Since the discovery of phagocytosis, almost 100 years ago, most phagocyte research has focused on Macs as effector cells that participate in the host’s immune defense.⁶¹ Macs are among the most multifunctional and heterogeneous cell types, present in virtually every mammalian tissue, where they monitor local environment and maintain homeostasis.^{62,63} They are phagocytic cells that rid the body of apoptotic or necrotic cells and debris, tumor cells, viruses, and bacteria. Once activated by bacterial products, Macs acquire microbicidal competence that usually leads to effective neutralization and removal of nonself.⁶⁰ The increasing understanding of the innate and

acquired immunity revealed that Macs possess remarkable plasticity in different tissue environments resulting in adoption of multiple phenotypes with distinct functions as housekeepers that coordinate restoration of tissue structure and function.⁶⁴ They express a broad array of sensing molecules, including scavenger receptors, pattern recognition receptors, nuclear hormone receptors, and cytokine receptors, which allows them to monitor tissue microenvironments, and act to remove nonself and restore tissue integrity. In addition, tissue-specific Macs perform tailored functions to physiology of different organs including the liver, brain, kidney, lung, skin, digestive tract organs, and the bone.

Adult tissue Macs derive from yolk sac embryonic precursors that maintain themselves by self-renewal in steady state and from circulating peripheral blood monocytes during inflammation and its resolution.⁶⁵ Two different monocyte populations, inflammatory (iMo) and patrolling (pMo) with distinct surface chemokine receptor profiles $CCR2^+CX_3CR1^{int}$ (iMo) and $CCR2^-CX_3CR1^{high}$ (pMo) seem to give rise to different Mac phenotypes in the tissues.^{66–68} Regardless of origin, activated Macs have been broadly classified into two main groups—M1 and M2 Macs—having pro- and anti-inflammatory phenotypes, respectively, and mirroring Th1 and Th2 classification of T helper lymphocytes.^{60,61} This classification system that distinguishes between M1-type (“classically activated”) and M2-type (“alternatively activated”) Macs implies directionality when in fact a “polarized” cell can repolarize to a different predominant phenotype.⁶¹ “Classical” activation is triggered by bacterial LPS and Th1-produced IFN- γ that induce a proinflammatory, anti-angiogenic, and anti-tumorigenic phenotype. “Alternative” activation is triggered by Th2-produced IL-4 and/or IL-13, and has been associated with pro-tumorigenic, pro-angiogenic, and tissue remodeling activities.⁶⁹ The in vitro characterized M1 and M2 cells differ in surface receptor expression, cytokine and chemokine production, and effector functions. However, these exclusive activating conditions do not reproduce the in vivo environment where multiple opposing signals are present and change in the course of reestablishing homeostasis. For example, tumor-associated Macs or those acting during the resolution phase of inflammation do not show clear M1 or M2 phenotypes.⁶⁵ Therefore, the dichotomous concept of Mac activation fails to incorporate in time and space the vast complexity and wide array of capabilities the Mac possesses.^{64,70} We will refer hereafter to Macs as activated to preform either predominantly inflammatory (iMac) or resolving (rMac) functions.

iMo and pMo which account for 90% and 10% of human monocytes respectively, share iMac and rMac phenotypes suggesting that Macs may use different modes of tissue trafficking in inflammation and resolution.^{67,71} Existing evidence suggests that iMo reach inflammatory sites and give rise to conventional DCs and iMacs whereas pMo infiltrate tissues and differentiate into rMacs.^{72,73} However, the recently characterized dynamic developmental relationship between the short lived iMo (1 day) and longer lived pMo (7 days) indicates that roughly 50% of iMo become pMo in steady state, a likely conserved pattern in vertebrates.^{65,74,75} Therefore, spatial and temporal relationships between differentially activated monocytes and Macs needs further investigation in both steady state and inflammatory conditions. Additionally, iMo and iMacs share features of osteoclasts indicating that peripheral blood osteoclast precursors likely develop along the iMo-iMac axis in RA and PD.^{76–79} These “cell cycle-arrested quiescent osteoclast precursors” express high RANKL, low CD115 (M-CSF-1, M-CSF receptor), and very low levels of the monocyte/Mac

markers F4/80/Emr1 and CD11b, have low phagocytic activity, and express high levels of the osteoclast markers carbonic anhydrase II, MMP-9, TRAP (tartrate-resistant acid phosphatase), and TFRC (transferrin receptor).⁷⁷ Although the mechanisms underlying monocyte priming to osteoclast precursors in osteolytic diseases are not fully understood, CCL2-mediated recruitment and CD115-mediated differentiation via RANKL seems to play a central role in bone lytic diseases including RA, PD, and osteoporosis.^{76,79–81} Despite increasing evidence that osteoclast precursors may differentiate from circulating monocytes, the current paradigm in osteoclast differentiation considers that osteoclast precursors develop in bone marrow or near bone at sites of bone resorption. Altogether, it is becoming increasingly clear that iMacs play a critical role in regulating bone resorption in an autocrine/paracrine manner, through crosstalk with lymphocytes, and that iMo priming to iMac and osteoclast precursors may be a requirement for osteoclastogenesis in osteolytic diseases.

It is known that Macs can adopt different phenotypes across the pro-to-anti-inflammatory/pro-resolving spectrum in response to diverse tissue microenvironmental signals during the course of neutralizing causes of imbalance.^{82,83} Macs likely undergo sequential functional switches in response to pathogen exposure, and signals from other immune and tissue resident stromal cells, which are essential for coordinated responses, removal of nonself/ altered-self, adaptive immunity and tissue repair.⁶³ In steady state, tissue resident Macs seem to be programmed for uptake of dying cells by phagocytosis and elimination through lymphatics in a process called efferocytosis.⁸⁴ Efferocytosis is thought to be a critical process in which regulatory interactions between target and effector cells determine its outcome.⁸⁵ Efferocytosis induces a phenotypic shift in iMac resulting in conversion to rMac.⁸⁶ During inflammation, activated iMacs (M1-like) produce high levels of proinflammatory cytokines (IL-1 β , IL-6, IL-12, IL-23, TNF- α) by adopting an IL-12^{high}IL-10^{low} phenotype.⁸⁷ They produce reactive nitrogen and oxygen intermediates, express high levels of major histocompatibility class II and co-stimulatory molecules, and display microbicidal activity. iMacs also possess marked iron sequestration properties, which contribute to the cells' bacteriostatic actions.⁸⁸ Synovial iMacs seen in RA are considered the most reliable biomarker of disease severity and response to therapy.⁸⁹ Similarly, periodontal iMacs are likely central to PD progression through production of proinflammatory cytokines and matrix degrading enzymes.^{90,91} iMacs are the major cellular source of gingival proinflammatory cytokines, particularly IL-1 β , IL-6, IL-8, and TNF- α , in chronic PD.^{92,93} iMac-derived cytokines mediate osteoclastogenesis directly through TNF- α , IL-1 β , and indirectly by stimulating recruitment of osteoclast precursors through IL-8 and CCL2, osteoclast differentiation through IL-23-mediated up-regulation of RANK, and production of RANKL and IL-17 by T helper lymphocytes.²⁸ IL-1 β and TNF- α antagonists, and knockout of IL-1 or TNF receptors in mice reduced alveolar bone loss during experimental PD.^{33,94} Nonetheless, periodontal therapy is associated with reduction of IL-1 β and increase of IL-10 levels in the gingival crevicular fluid, and with reduced bone-resorptive activity.^{95,96}

As inflammation enters its resolution phase, recruited monocytes give rise to rMac cells that share M1 and M2 features including reduced production of proinflammatory cytokines, antigen processing/presentation and efferocytosis.^{97,98} Adoption of a resolving phenotype occurs in response to anti-inflammatory cytokines IL-4 and IL-13, primarily produced by

Th2 lymphocytes and plasma/B cells, SPMs that activate surface GPCR receptors (e.g., GPR18, GPR32, GPR120, and ERV1) in Macs, or anti-diabetic therapeutic agents such as thiazolidinediones (TZD; e.g., rosiglitazone) that activate PPAR- γ in monocytes.^{99,100} rMacs adopt a IL-12^{low}IL-10^{high} phenotype with increased phagocytic activity, high surface expression of scavenging receptors, production of ornithine and polyamines through the arginase pathway, reduced proinflammatory cytokine (IL-1 β , IL-6, IL-12, and TNF- α) and cyclooxygenase 2 (COX2) and increased anti-inflammatory cytokine (IL-1RA, IL-10, and TGF β) production.^{101,102} The consequence of Mac phagocytosis of apoptotic cells is suppression of inflammatory responses and uptake of PMN removes a significant source of proinflammatory factors while triggering anti-inflammatory programs in Macs.¹⁰³ These anti-inflammatory, pro-resolution, and pro-remodeling phenotype of rMac closely resemble in vitro generated M2 cells.¹⁰⁴ Disease remission and protection against bone resorption in RA and PD are associated with increased IL-4 and IL-10, which activate rMacs.^{105,106} It is therefore likely that a failure of periodontal Macs to efficiently perform resolving functions, but rather maintain proinflammatory activities leads to progression of gingivitis to PD. The relative contribution of genetic and epigenetic factors to a predominant iMac activation state associated with inflammatory osteolytic diseases is however incompletely understood.

Activated Macs differ greatly in expression of immunoregulatory genes in an input-specific manner that profoundly influence immune responses and maintenance of tissue homeostasis.^{70,88} Transcription factors STAT1, STAT6, C/EBP, IRF-4, IRF-5, and PPAR- γ , regulate transcription programs that control Mac activation across the proinflammatory/pro-resolution spectrum.^{63,83} The response of Mac to bacterial invasion includes the up-regulation of genes responsible for iMac activation.⁶⁰ These include genes encoding for cytokines such as TNF α , IL-1, IL-6, IL-12, cytokine receptors such as IL-7R and IL-15RA, chemokines such as CCL2, CCL5, and IL-8, and the chemokine receptor CCR7. Importantly, Macs pre-activated to a proinflammatory or pro-resolving phenotype seem to dictate responses to putative periodontal pathogens such as *Porphyromonas gingivalis*, which can switch rMacs into iMacs through up-regulation of TNF- α , IL-6, CCL2, CCL3, and CCL5 and induce IL-10 expression in iMacs.¹⁰⁷ Several studies found positive associations between several SNPs in *IL-1A*, *IL-1B*, and *IL-6* genes and chronic PD in systemically healthy and diabetic patients known to be at risk, indicating increased predisposition for iMac activation. *IL-1* and *IL-6* SNPs were also associated with systemic-onset juvenile chronic arthritis and RA. However, SNPs do not indicate a mechanism of action, the cell type in which it acts or the gene affected but definition of expression quantitative trait loci (eQTLs) can relate genetic variants to gene expression.^{108,109} Using eQTL genomics in AXB-BXA recombinant inbred mice subjected to ligature-biofilm experimental PD we found genetic linkage to factors involved in rMac activation (Pdk1, Cobll1, Sp3, and Itga6).⁴³ Further insights into the variance in gene expression may be gained by identifying epigenetic modifications in DNA. Studies on DNA methylation in periodontal tissues identified hypomethylation profiles within the *IFN- γ* , *IL-6*, and *TNF- α* promoters that are associated with increased IFN- γ , IL-6, and TNF- α transcription in gingival biopsies from patients with chronic PD.^{110,111} Interestingly, it was recently reported that periodontal therapy can reduce methylation status of prostaglandin-endoperoxide synthase 2 (PTGS2) but not IFN- γ and TNF- α at healthy and PD sites over time.¹¹² Despite its limitations in

sample size, different sampling sites per time point and relatively short follow-up, this study suggests that local disease activity influences the epigenetics of the tissues, which may be modulated by environmental factors, including the microbiota.

Altogether, this evidence suggests that increased susceptibility to iMac activation favors a tissue-destructive loop in which a continuous influx of PMN and iMo sustains a periodontal niche dominated by microbial and host-derived proinflammatory and matrix degrading mediators (fMLP, LPS, TNF- α , IL-1 β , IL-6, IL-8, IL-12, IL-17, IL-23, CCL2, PGE₂, LTB₄, MMPs, RANKL). On the other hand, in nonsusceptible individuals and in PD remission, resolving inflammation appears to be characterized by predominance of anti-inflammatory and bone anabolic mediators (IL-1RA, IL-4, IL-10, IL-13, TGF β , IGF-1, SPMs, TIMPs, OPG). It becomes increasingly clear that Macs, as master regulators of tissue homeostasis, play a central role in coordination of periodontal responses to nonself and altered self through crosstalk with resident mesenchymal cells, PMN, and lymphocytes (Fig. 2).

5 | MAC DEPLETION

To determine the implications of Macs in different biologic processes, it is necessary to use techniques that allow for their selective depletion. Several approaches had been used to eliminate Macs from tissues including applications of silica, asbestos, and carrageenan.¹¹³ These methods were deemed to be ineffective, as they affected nonphagocytes and were not able to fully deplete tissue Macs. One approach to achieve conditional or inducible Mac depletion is to drive expression of a suicidal gene like the diphtheria toxin receptor using Cre-loxP systems for the *PU.1*, *macrosialin* (*CD68*), *integrin subunit alpha M* (*ITGAM*), *CX3CR1*, lysozyme (*LysM*), or the *MCSF-1 receptor* (*Csf1r*) promoters in transgenic mice.¹¹⁴ The Mac Fas-induced apoptosis (MaFIA) transgenic mouse allows for inducible/reversible apoptosis of Macs and DC using the *Csf1r* promoter to drive expression of a mutant human FK506 binding protein 1A.^{115,116} However, transgenic targeting of Macs using these approaches is either nonspecific for Macs (*CSFr1*, *LysM*, *ITAGM*, *PU.1*) or inefficient in depleting resident Macs (*CX3CR1*, *CD68*).¹¹⁷ Another approach proven to efficiently and specifically deplete Macs is the use of liposomes to deliver clodronate exclusively to these cells.¹¹⁸ Clodronate is therapeutically used to treat osteolytic bone diseases. Clodronate itself is not toxic to cells as it does not readily cross cell membranes. When liposomes are used as distributors of dichloromethylenebisphosphonate (Cl₂MBP) to phagocytes, a metabolite (AppCCI2p) generated intracellularly leads to cell death through irreversible inhibition of ATP/ADP translocase at the inner mitochondrial membrane and subsequent loss of mitochondrial membrane integrity that initiates cell death by apoptosis.^{119,120} Liposomes are effective vehicles for drug delivering into phagocytes, have a short half-life in the body associated with low toxicity, and can be tailored by varying the charge, phospholipid content, and quantity of phospholipid bilayers.¹²¹ The Mac-specific delivery of clodronate is achieved with monoclonal antibodies or another target molecule added on the liposome surface. Once phagocytosed, liposomal phospholipids are disrupted by lysosomal phospholipases, which releases clodronate inside the cell.

The clodronate liposome model can be applied to the field of osteology to study the implications of Macs in regulation of bone homeostasis. Local and intraperitoneal delivery

of clodronate liposomes for 1–3 days results in 75–80% Mac depletion from bone marrow (endosteal) and bone surface (periosteal).^{122,123} Selective Mac depletion studies have brought insights into the contribution of iMacs and bone-lining osteal Macs (osteomacs) to bone fracture healing and intramembranous bone formation.^{123,124} Phenotypically, osteomacs resemble rMacs by sustaining anabolic processes through osteoblast stimulation and control of proinflammatory environments around bone. The timing and relative contribution of iMac and rMac recruitment and activation in osseous regeneration are important to delineate before development of drugs for osteolytic diseases such as PD, RA, and osteoporosis. It was previously reported that Mac depletion abates alveolar bone loss in mice with *Porphyromonas gingivalis* induced PD.¹²⁵ We recently found that iMac depletion during onset of experimental PD is beneficial and that rMac activation is particularly beneficial for healing of osteolytic PD lesions.⁵⁹ The contribution of rMacs to enhanced bone regeneration was attributed in part to secretion of bone anabolic factors that stimulate mineral deposition by osteoblasts suggesting that immune modulation of Macs to gain resolving functions can promote bone formation in osteolytic diseases.

6 | INHIBITORS OF MAC ACTIVATION

Several signaling pathways have been implicated in Mac activation grouped in three main classes based on the sensing receptors and downstream transcription factors : (i) NF- κ B and AP-1 coupled receptors including TLRs, RIG-I-like intracellular receptors (RLRs), TNFR and IL-1R inducing iMac activation; (ii) STAT-coupled receptors including IFN receptors linked to activation of STAT1 and STAT2 for iMac activation, and IL-4R linked to activation of STAT6 for rMac activation; and (iii) nuclear receptors PPAR- γ , liver X receptors (LXRs) and glucocorticoid receptors that counter-regulate the iMac transcription factors.⁸³

Importantly, whereas STAT6 controls much of rMac pheno-type, several studies found that activation of wound healing Macs in vivo is not altered in STAT6, IL4-R, or IL-10 null mice suggesting that IL-4/STAT6 or IL-10 alone are not sufficient to induce rMacs during healing.^{87,126,127} It further demonstrates that inhibition of pathologic Mac activation by targeting one specific signaling pathway is likely to be inefficient. On the other hand, studies with Macs from STAT1 deficient mice clearly show that gene expression induced by both type I IFNs and IFN- γ is dependent on STAT1, and the severe defects of null mice in immunity to intracellular bacterial and viral pathogens, which is dependent on the IFN response, further support the requirement of this transcription factor for iMac activation.¹²⁸ However, type I IFN can have profound anti-inflammatory activity in certain contexts and can promote resolution.¹²⁹ Ongoing research on transcriptional control of Mac activation and ontogeny of monocytic lineage cells aims to understand their regulation in health and disease states. Findings on modulators of Mac activation have shed light on relative the contribution of different phenotypes to the course of inflammation and its failure to resolve in chronic inflammatory diseases (Fig. 3).

Several inhibitors of iMac activation have been tested for multiple inflammatory conditions. One such agent, linomide (quinoline-3-carboxamide) is a potent immunomodulator with well-documented therapeutic effects including inhibition of autoimmunity in models of acute and chronic autoimmune diseases such as autoimmune encephalomyelitis (EAE), type I diabetes, myasthenia gravis, and RA. It was also proven effective in humans.¹³⁰

Linomide's mechanism of action is not fully understood, as the stimulation as well as inhibition of immune cells has been observed in various experimental settings. Linomide was shown to stimulate the Concanavalin A (ConA)-induced proliferation of splenocytes *ex vivo* in part through modulation of Mac function.¹³¹ Previous studies also suggested Linomide's inhibitory effect on autoimmune responses to be due to the inactivation of antigen presenting cells including Macs.¹³² This was supported by the finding that *ex vivo* stimulation of peritoneal Macs from Linomide-treated rats produced less IL-1 β , TNF- α , and IL-6 compared to Macs from untreated rats.¹³³ It was also shown that Linomide-treated PBMCs produce less Th-1 type cytokines following induction with *Staphylococcus aureus* enterotoxin.¹³⁴ Dahlén et al. found that the cell surface expression of the iMac activation markers MHC class II, CD11a, CD54, and CD80 was significantly increased in Linomide-treated animals whereas CD11b, CD40, CD69 and CD86 were not.¹³⁰ Ly-6A/E, a Mac surface marker up-regulated by type I and type II IFN, was up-regulated in Macs of Linomide-treated animals, suggesting that the iMac modulation by Linomide may occur via an IFN-mediated pathway.

Antipsychotic drugs (APDs) used to treat the symptoms of psychiatric disorders are classified into typical or atypical, depending on preferred receptors for high affinity-binding.¹³⁵ Typical APDs chlorpromazine and haloperidol bind with high-affinity to dopamine D2 receptors and with lower affinity to other types of dopamine, serotonin (5-hydroxytryptamine or 5-HT) and adrenergic receptors. Atypical APDs risperidone and clozapine bind to 5-HT and dopamine receptors as well as adrenergic and other receptors. Clozapine has been shown to inhibit cellular production of IFN- γ in human PBMCs through binding of 5HT receptor.¹³⁶ 5-HT can modulate Mac activation by inhibiting LPS-induced release of TNF- α and IL-12, while stimulating IL-10 release.¹³⁷ Further, 5-HT was found to promote maintenance of rMacs through induction of gene expression associated with resolving activation and inhibition of genes associated with inflammatory activation.¹³⁸ Other studies demonstrated that APDs can modulate monocytes by altering expression of IL-2 receptor, IL-6 receptor, and production of TNF- α , IL-1 β , IL-2, IL-10, and IFN- γ measured in serum.^{139–141} Chen et al. showed that risperidone and clozapine reduce Th1 differentiation through inhibition of AKT pathway, whereas haloperidol reduces Th2 differentiation through inhibition of NF- κ B activation.¹⁴² Haloperidol, risperidone or clozapine were also reported to decrease adhesion and phagocytic uptake in monocytes and Macs.¹³⁵ Treatment of peripheral blood monocytes with risperidone or clozapine but not haloperidol also inhibited proinflammatory cytokine production. Together, these findings suggest potential immune modulatory actions of APDs in suppressing iMacs.

Tetracycline group antibiotics such as minocycline, tetracycline and doxycycline have been shown to inhibit rMac activation during wound healing. Of these, doxycycline showed the highest potency as inhibitor of rMac activation both *in vitro* and *in vivo* in a dose-dependent manner at concentrations that do not cause cell toxicity.¹⁴³ These actions are mediated by a reduction in MMP2 and MMP9 activities associated with reduced angiogenesis, and inhibition of fibrosis through the STAT6 pathway.^{144–146} By inhibiting the proangiogenic activities of rMacs, doxycycline has the potential to control pathologic neovascularization associated with chronic rMac activation in nonresolving inflammation. Doxycycline has demonstrated good anti-collagenase activity even when sub-antimicrobial doses are used,

which prompted its use in management of PD, particularly in poor responders to conventional periodontal therapy to prevent relapse.¹⁹ Several randomized clinical trials found sub-antimicrobial dose doxycycline (SDD) (20 mg) to reduce gingival fluid collagenase activity and improve periodontal parameters when used long term (3–9 mo).^{147–150} Golub et al. found significant improvement in attachment levels associated with a decrease in collagenase activity.¹⁵¹ Continuous drug therapy over the 12-wk treatment period was needed to maintain and maximize improvement. Because use of sub-antimicrobial dose antibiotics poses risk of antimicrobial resistance Canton et al. assessed the efficacy and safety of SDD as an adjunct to mechanical therapy over a period of 9 mo, in patients with CP.¹⁴⁷ They found a modest but significant improvement in attachment levels compared to placebo without detrimental shifts in the normal periodontal flora or the acquisition of doxycycline or multiantibiotic resistance. Similar findings were reported by Preshaw et al. who conducted a multi-center doubleblind, randomized, placebo-controlled with parallel treatment groups that included 210 subjects.¹⁵⁰ To confirm the anti-MMP activity of SDD, Emingil et al. measured MMP-8 levels in gingival crevicular fluid of 30 patients with PD during SDD therapy as adjunct to mechanical therapy for 3 mo, with a follow-up of 12 mo.¹⁵² Gingival MMP-8 levels were significantly reduced in the SDD group at 3 and 6 mo compared to placebo but were insignificant compared to mechanical therapy alone at 9- and 12-mo post-therapy. Altogether these findings suggest that use of SDD as adjunct to conventional periodontal therapy may be beneficial in select cases to prevent relapse by inhibiting MMP activity. Nonetheless, it demonstrates that the mechanisms by which drugs such as doxycycline modulate Macs in vivo expand to several pathways that favor resolving functions.

7 | ACTIVATORS OF RMAC

During the physiologic course of inflammation in periodontal and other tissues, myeloid cells actively contribute to inflammation resolution by switching from production of proinflammatory mediators cytokines and prostanoids (e.g., PGE₂ and LTB₄), to anti-inflammatory lipid mediators named SPMs, including lipoxins (e.g., LXA₄), resolvins (e.g., RvE1), maresins (e.g., MaR1), and protectins (e.g., PD1) downstream of arachidonic acid, eicosapentaenoic acid, and docosahexaenoic acids.^{153–155} Lipoxins and resolvins act through lipoxin receptor ALX and LTB₄ receptor BLT1, respectively, to reduce excessive PMN infiltration in response to bacterial chemotactic agents (e.g., fMLP), LTB₄ and IL-8, thus preventing bone loss.^{156–158} In PD, the generation of inflammatory cytokines and lipid mediators of inflammation, in particular the prostanoids, prostacyclins, and leukotrienes, leads to inflammatory cell recruitment, matrix destruction and alveolar bone resorption.²⁶ rMac activation is needed to phagocytose and remove tissue apoptotic PMN, which clears a significant source of proinflammatory factors and triggers anti-inflammatory programs in Macs.¹⁰³ The predominant iMac activation with reduced ability to phagocytose apoptotic cells in periodontal tissues may, in part, explain the failed PMN-Mac crosstalk to actively resolve inflammation, and to maintain alveolar bone levels through coupled resorption and formation. In support of this hypothesis, IL-4 and IL-10 were found associated with disease remission and protection against bone resorption in RA and PD.^{105,106} rMac activation occurs in response to SPMs that activate surface GPCR receptors (e.g., GPR18, GPR32,

GPR120, and ERV1) in Mac, and to anti-diabetic therapeutic agents such as TZD (e.g., rosiglitazone) that activate PPAR- γ in monocytes.^{99,100} Therefore, therapeutic use of SPMs and TZD may be beneficial in patients with chronic osteolytic diseases who have a reduced resolving capacity in Macs.

SPMs binding to their specific GPCR receptors counter-regulate prostaglandins, leukotrienes, platelet-activating factor, and proinflammatory cytokines through NF- κ B down-regulation and increased IL-10.¹⁵⁹ In animal models of PD, LXA₄, and RvE1 protected against inflammation-mediated alveolar bone loss and promoted bone regeneration when used topically.^{160–162} LXA₄ topical in micromolar concentration promoted alveolar bone regeneration in a minipig model of PD by activating osteogenic pathways and promoting endogenous synthesis pro-resolution mediators.¹⁶² There are currently clinical trials in progress evaluating the benefits of resolution molecules (e.g., lipoxin analogue BLXA₄-ME) in the oral cavity, but no data is available to date (<https://clinicaltrials.gov/ct2/show/NCT02342691>). Similar to LXA₄, Hasturk et al. found that topical RvE1 can reduce alveolar bone loss and enhance bone regeneration in a rabbit model of *Porphyromonas gingivalis*-induced CP.¹⁶⁰ Using a critical size defect craniotomy mouse model of bone healing and regeneration Gao et al. have shown RvE1 enhances new bone formation through its receptor ERV1.¹⁶¹ RvE1 binds ERV1 on Mac with higher affinity (K_d of ~11 nM) than the other known ligand chemerin, having a B_{max} of ~4200 binding sites per cell.¹⁶³ RvE1 enhanced phagocytosis in Macs of patients with aggressive forms of PD indicating potent pro-resolving stimulation capable of switching iMacs to rMacs.¹⁶⁴ In other inflammatory models, particularly in the context of obesity and insulin resistance, RvE1 was found to induce pMo recruitment in central metabolic organs, uptake of apoptotic cells by rMacs, production of anti-inflammatory mediators and adiponectin, and expression of insulin-sensitizing genes *PPAR- γ* , *IRS-1*, *GLUT-4*.^{165,166} Because adipocytes and hepatocytes also express high levels of ERV1, it is believed that the insulin-sensitizing actions and induction of adiponectin is due to direct RvE1 actions on these cells.^{166–168} This suggests that the ERV1-RvE1 axis may play a role in regulation of inflammation resolution and energy use/storage in chronic inflammatory conditions associated with metabolic deregulation.

PPAR- γ is a nuclear hormone receptor involved in modulation of various aspects of inflammation and adipogenesis. It is now clear that activation of PPAR- γ reduces inflammation through modulation of monocyte and Mac functions in favor of rMac activation.^{169,170} Deficiency of PPAR- γ in Macs was found critical to pathogenesis atherosclerosis in part through modulation of iMo recruitment via CCR2.¹⁷¹ Mac PPAR- γ is also thought to negatively regulate responses to LPS and IFN- γ .¹⁷² These findings suggest that not only does PPAR- γ induce rMac activation but also diminish iMac activation and recruitment of iMo. The TZD class of synthetic PPAR- γ agonists and anti-diabetic agents, including troglitazone, pioglitazone, and rosiglitazone, have shown anti-inflammatory actions through reduction of IL-1, IL-6, and CCL2.^{170,171,173} PPAR- γ controls iMac activation in part by interfering with proinflammatory signaling pathways AP-1, NF- κ B, or STAT-1 suggesting that TZDs may be particularly efficient for PD management.¹⁷⁴ Indeed, rosiglitazone had a positive impact on alveolar bone preservation and regeneration in experimental PD.^{59,175} The anabolic actions of rosiglitazone were attributed in part to inhibition of osteoclast formation despite favoring adipogenesis over osteoblast

differentiation in bone marrow mesenchymal stem cells.¹⁷⁶ The reduction in bone quality and mass, which is a well-documented side effect of long-term TZDs treatment, is partially explained by induction of adipogenesis in bone marrow stromal cells and decreased osteoblast and aromatase activities.¹⁷⁷ We recently found short-term rosiglitazone treatment in mice with experimental PD to enhance bone regeneration through direct anabolic action on bone cells by inhibition of osteoclast activity and stimulation of bone mineral deposition by osteoblasts.⁵⁹ The positive impact of rMac activation on bone was in part explained by increased production of anti-proteases and bone anabolic proteins. Among anti-proteases induced in rMac in response to IL-4, cystatin C (CysC) was most significantly increased compared to iMac and nonactivated primary bone marrow Macs, and in periodontal tissues.

CysC is cysteine proteinase inhibitor used as a biomarker of kidney function by estimating the glomerular filtration rate, and more recently of cardiovascular risk.^{178–180} CysC was previously found to regulate bone matrix degradation, likely through its potent inhibition of cathepsins.¹⁸¹ It is known that CysC acts on both osteoclasts and osteoblasts, resulting in osteoclast inhibition and osteoblast promotion. CysC inhibits bone matrix degradation by reversibly antagonizing osteoclast formation and cysteine proteinases secretion into resorption lacunae.^{182–184} CysC acts on osteoblasts to promote differentiation and bone mineralization in vitro.¹⁸⁵ CysC-treated osteoblasts increased alkaline phosphatase activity, mineralization of bone matrix, and calvarial bone formation. Osteoblast-specific effects of CysC were attributed to stimulation of the BMP-2/Runx2 cascade. Altogether, these findings suggest that therapeutic rMac induction in inflammatory osteolytic diseases such as RA and PD has the potential to not only control the proinflammatory osteolytic local environment but also stimulate bone formation.

8 | CONCLUSIONS

It is becoming increasingly clear that activation of Macs across the proinflammatory to pro-resolution spectrum has important consequences on inflammatory bone loss and its ability to regenerate. A large body of evidence on iMac implications in bone loss associated with chronic inflammatory diseases such as RA and PD corroborated with knowledge of Mac plasticity and rMac anabolic activities suggest that agents inducing iMac-to-rMac conversion may control disease progression and stimulate bone regeneration. Inflammation resolution failure is likely central to inflammatory osteolytic diseases. Further understanding the causes for altered inflammation resolution and their relationships to clinical phenotypes will help design Mac-targeted immune modulators and estimate response to therapy in a personalized manner.

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Abbreviations:

5-HT Serotonin receptor

APD	Antipsychotic drugs
Arg 1	Arginase 1
BMMs	Bone marrow monocytes
BMP-2	Bone morphogenetic protein 2
CD	Cluster of differentiation
CysC	Cystatin C
iMac	inflammatory macrophage
iMo	inflammatory monocyte
MCSF	Macrophage colony-stimulating factor
OPG	Osteoprotegerin
pMo	patrolling monocyte
PPAR-γ	Peroxisome proliferator-activated receptor gamma
RANKL	Receptor activator of nuclear factor kappa beta
rMac	resolving macrophage
RunX	Runt-related transcription factor 2
TZD	Thiazolidinediones

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FIGURE 1. Overview of the effector cells in the pathogenesis of periodontitis.

In early lesions, bacterial antigens from putative pathogenic bacteria found in the subgingival biofilm activate dendritic cells and tissue resident macrophages, and stimulate recruitment of natural killer cells, T cells, and polymorphonuclear neutrophils (PMN). Antigens are processed and presented to local cells by macrophages, dendritic cells, PMN and lymphocytes. Dendritic cells, macrophages, and B cells are the principal antigen-presenting cells for T cells, whereas follicular dendritic cells are the main antigen-presenting cells for B cells. Inflammatory macrophage (iMac) and neutrophils up-regulate production of proinflammatory mediators, reactive oxygen/nitrogen species, and metalloproteases, which promote extracellular matrix degradation and osteoclastic activity. Resolving macrophages (rMac) mediate inflammation resolution by increasing phagocytosis of dying cells (PMNs and others), clearance of proinflammatory signals, and tissue regeneration likely, in part, through promotion of osteoblastic activity. The imbalance between osteoblast and osteoclast activities is believed to be caused by bacterial products and inflammatory cytokines, and constitutes an important pathologic axis underlying inflammation-mediated bone loss.

FIGURE 2. Periodontal Osteoimmunology.

Nonresolving periodontal inflammation is characterized by a proinflammatory loop sustained by neutrophils and inflammatory monocytes/macrophages (iMo/iMac). Resident macrophages and classical dendritic cells act as professional antigen presenting cells that initiate inflammation when bacterial antigens enter the tissues. IFN- γ and LPS activate iMac. Persistence of chemoattractants for neutrophils (fMLP, IL-8, LTB₄) and inflammatory monocytes (CCL2) results in maintenance of an inflammatory niche dominated by macrophage derived cytokines IL-1 β , IL-8, IL-12, IL-23, TNF- α , and MMPs, which in turn activate Th cells (Th17 and Th1) to produce IL-8, IL-17, and RANKL. This in turn promotes neutrophil recruitment, and differentiation and activation of osteoclasts via MCSF-RANKL. High tissue levels of CCL2 also recruits osteoclast precursors on the bone surface. In nonsusceptible individuals, efficient control of biofilm composition by innate immune cells and disruption of maturing sub gingival biofilms results in active resolution of inflammation coordinated cellularly by resolving macrophages (rMac), and molecularly by SPMs (lipoxins and resolvins) and anti-inflammatory factors IL-1RA, IL-4, IL-10, IL-13, IGF-1, TGF- β , TIMPs, and OPG. This is likely supported by recruitment of patrolling monocytes (pMo) via CX₃CL1, B/plasma cell and Th2 derived cytokines, and osteomacs in the canopy structures surrounding osteoblasts. IL-1RA, TIMPs, and OPG directly block major proinflammatory and osteoclast stimulation pathways. Abbreviations: OB, osteoblast, OC, osteoclast; Th, T helper CD4+ lymphocyte; fMLP, formylmethionyl-leucyl-phenylalanine; IL-1RA, IL-1 receptor antagonist; MMP, matrix metalloproteases; TIMP, tissue inhibitors of MMPs

FIGURE 3. Monocyte (Mo) and Macrophage (Mac) Immune Modulation.

Bone marrow and spleen release classical Mo have inflammatory properties, termed “inflammatory” Mo (iMo) as well as nonclassical Mo that display patrolling properties and are termed “patrolling” Mo (pMo). The short-lived iMo become pMo with extended life span by shedding CCR2 and up-regulating CX₃CR1. pMo that recruit into tissues are thought to generate resolving Mac (rMac) that maintain tissue homeostasis. Importantly, at sites of inflammation, upon stimulus neutralization inflammatory Mac (iMac) switch their metabolism to perform pro-healing/regeneration functions, thus becoming rMac. Therefore, depending on local signals Mac perform complementary functions: iMac coordinate pathogen neutralization whereas rMac coordinate tissue clearance through increased efferocytosis. These actions regulate adaptive immunity mediated by B and T lymphocytes, and restoration of tissue homeostasis by clearance through lymphatics. Mac inhibition by clodronate targets all Mac, whereas Linomide and APDs inhibit iMac by blocking the IFN- γ pathway and the LPS-induced release of iMac activating factors. iMo-to-pMo and iMac-to-rMac conversion is enhanced by specialized pro-resolving mediators (SPMs) and TZDs, IL-4 and IL-10, glucocorticoids and TGF- β