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Immuno-Pathogenesis of Periodontal Disease: Current and Emerging Paradigms

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Abstract Periodontal disease (PD) is a highly complex disease involving many factors; however, two principal facets central to initiation and progression of the majority of PD are the composition of the microbes in the subgingival plaque, and the host immune response to these organisms. Numerous studies point to the complexity of PD, and to the fact that despite innate and adaptive immune activation, and resultant inflammation, our immune response fails to cure disease. Stunning new findings have begun to clarify several complexities of the host-pathogen interaction of PD pointing to key roles for microbial dysbiosis and immune imbalance in the pathogenesis of disease. Furthermore, these investigations have identified novel translational opportunities to intercede in PD treatment. In this review we will highlight a select few recent findings in innate and adaptive immunity, and host pathogen interactions of PD at a micro-environmental level that may have profound impact on PD progression.

Keywords Periodontal disease · Innate immunity · Adaptive immunity · Bacteria · Microbial dysbiosis · Porphyromonas gingivalis · Cell receptors · Cytokines · Chemokines · Resolving · Interferon · Complement · T cell imbalance · Macrophages · Dendritic cells · Micro-environment · Osteoclast · Bone remodeling

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Introduction

Periodontal disease (PD) is one of the most common chronic inflammatory diseases of humans. PD is multi-factorial, with significant involvement of host, environment, and bacterial factors [1]; however, it is the host inflammatory response that drives much of the soft and hard tissue destruction. In severe disease this can lead to tooth loss. Localized forms of PD are associated with *Aggregatibacter actinomycetemcomitans*, while chronic generalized forms of disease are associated with a number of bacteria including *Porphyromonas gingivalis*, *Tannerella forsythia*, *Treponema denticola*, and others. The microbial diversity of the oral cavity is immense, and it is clear that the host response during PD is complex, with innate and adaptive elements driving chronic inflammation and bone loss. Yet, from a mechanistic level our understanding of the disease process is still emerging. Indeed, acquisition of select pathogens, with their impact on microbial communities; emergence of important new T cell subsets, and their implication in bone loss and preservation of oral bone; as well as the implication of the local micro-environment in PD, may have significant impact on the way the host recognizes and responds to periodontal pathogens. In PD, erosion of the bone supporting the teeth does not appear to occur in a predictable manner. Indeed, it is not clear what factors are absolutely required to define where bone loss will occur. In this review we will focus on a select few contemporary findings of the host innate and adaptive immune response present during PD, or elicited by periodontal bacteria, and discuss their impacts on inflammation and oral bone homeostasis. Generalized chronic PD represents the majority of PD, and will be the primary focus. We will illustrate novel hypotheses and findings that have recently changed our understanding of the host immune response in PD and its pathogenesis.

Bacterial Complexity of PD and Microbial Dysbiosis

Early descriptions of PD note the importance of dental plaque in the progression of disease. Through detailed culture-based evaluations of dental plaque samples, it became clear that there are differences in the microbes residing in dental plaque of healthy individuals and those with PD. In PD, frequently, Gram negative organisms of the "Red complex" including *P. gingivalis*, *T. forsythia*, and *T. denticola* are detected [2]. Indeed, the seminal finding in non-human primates that *P. gingivalis* oral challenge stimulated oral bone loss [3], solidified this organism as a pathogen. Yet, insult with multi-organism complexes of periodontal pathogens can stimulate more severe forms of oral bone loss than mono-microbial challenge, thus supporting the importance of microbial complexity in disease pathogenesis [4, 5]. Now, more powerful analysis techniques, including 16S sequence analysis, provide a more thorough definition of the microbial composition of the oral cavity. Not only do these contemporary approaches better clarify the range of microbial diversity in sub-gingival plaque, but may shed light on new bacteria or groups of micro-organisms associated with disease. The bacteria that inhabit the sub-gingival crevice are predominantly associated with biofilm [6]. Dewhirst et al. [7] generated sequence information and have begun to define the human oral microbiome, which consists of over 600 distinct species. Employing this tool investigators identified organisms that either increased or persisted in refractory cases of PD and identified bacteria previously not associated with disease [8•].

Recent findings suggest that periodontal pathogens can sufficiently modify their environment in a manner favorable for their survival, and stimulate microbial dysbiosis. It is suggested that this dysbiosis may be a critical element in the switch from periodontal health to disease [9]. Based on this, organisms capable of modifying their local environment such as *P. gingivalis* may serve as keystone pathogens, driving complex microbial changes that shift the host response during disease [10, 11]. Indeed, *P. gingivalis* possesses an array of virulence factors including major and minor fimbriae, LPS, ceramides, gingipains, and others [12–15]. Gingipains are cysteine proteases, and are grouped based on enzymatic activity for arginine or lysine residues [15]. Gingipains are known to cleave a variety of host proteins including cytokines, cell adhesion molecules, complement components [15], and thus possess the ability to modify the local inflammatory environment, possibly tailoring it to one that is more favorable for *P. gingivalis* survival [16].

PD Inflammatory Mediators and Immune Cell Mileau

It is generally accepted that the host inflammatory response mounted during PD drives much of the hard and soft tissue destruction that accompanies PD. Innate and adaptive immune activation is evident in PD, with numerous immune mediators including cytokines, chemokines, acute phase mediators, and antibodies detected at levels different than in periodontal health to PD-associated organisms such as *P. gingivalis* [17–20]. Here, we will not detail the contributions of known cells to immune outcomes of PD, but will focus on a few interesting emerging themes.

Inflammation

Cellular infiltration and expression of a complex array of cytokines, chemokines, and lipid mediators are key characteristics of PD. In early / active lesions neutrophils predominate the cellular infiltrate, while in advanced chronic lesions, there is a switch to monocyte rich lesions characterized with T cells, B cells, dendritic cells (DC), and macrophages (MØ) [21]. These cells migrate to the nidus of infection via gradients of complement breakdown, and chemokines using an array of cell adhesion molecules.

In PD the presence of elevated inflammatory cytokines including tumor necrosis factor (TNF)-α, interleukin (IL)-1, IL-6, interferon (IFN)-γ, and IL-12 is considered a central force, when coupled with cell activation and receptor activator of nuclear factor kappa-B ligand (RANKL) activation in driving pathogen elicited bone loss [22, 23]. In addition to pro-inflammatory cytokines, an array of chemokines including IL-8, monocyte chemotactic protein (MCP)-1, and others are frequently elevated in PD [24]. In addition, the presence of anti-inflammatory and regulatory cytokines, such as transforming growth factor (TGF)-β, IL-10, and IL-4, has been reported [20, 25]. In light of the complexity of this response, early investigations into the type of T helper (Th) cell guiding the host response to infection were undertaken, however despite an array of studies, no clear consensus has emerged (see review [26], and other reviews in this issue). Historically, differentiated T cells were divided to either Th1-type, or Th2-type cells [27], and many studies have shown that both types play a role in PD progression [28, 29]. Recently however, it has become clear that additional Th cell subsets exist, and many new studies have begun addressing the role of these T cell subsets in PD.

Among the earliest host response molecules found in response to infection are lipid mediators [30]. Resolution of inflammation involves the production of lipid mediators termed immunoresolvents, and includes the resolvins, protectins, lipoxins, and maresins (see recent reviews [21, 30, 31]). Resolvins are synthesized from dietary precursor essential ω-3 polyunsaturated fatty acids eicosapentaenoic acid and docosahexaenoic acid [32]. Functionally, resolvins limit inflammation in part through prevention of neutrophil penetration, limiting inflammation at the local level, and promote tissue regeneration [33, 34]. Topical treatment with

resolvin R1 limits experimental periodontitis [35]. Based upon these important findings, targeting the reduction of inflammation through use of resolvin-based approaches may represent a novel strategy to potentially augment PD treatment approaches.

Novel T Cell Subsets and Immune Imbalance in PD

T cells are well known as key regulating cells that orchestrate the host immune response, and they can be classified based on function, including Th cells, T cytotoxic (Tc) cells, and T regulatory (Treg) cells. Th cells arise from the $CD4^+$ population and based on cytokine profiling early studies showed them to segregate into Th1 and Th2 populations [27].

Seymour et al. [28] suggested that the early periodontal lesion is associated with a Th1 response, while other studies support that progressive lesions possess a Th1 profile, where stable lesions possess Th2 characteristics [29]. Indeed, it is clear that there is no consensus on the contribution of T cell help in PD that is consistent with the Th1 versus Th2 paradigm. Modern cytokine profiling and transcription factor analysis has led to a much more detailed classification of Th cells, and the emergence of the Th17, Treg, T_{FH}, Th9, and Th22 subsets [36, 37]. Recent studies in PD report that not only do these T cell subsets exist, but also that a complex interaction between T cell subsets and an imbalance between key subsets may be crucial to PD pathogenesis [38–40, 41••]. At the heart of this imbalance lie the opposing roles of Th17 and Treg cells. Indeed, an inverse relationship between Th17 and Treg cell populations has been suggested in PD [42••]. Th17 cells associate with periodontal inflammation and tissue destruction [43], while Treg cells are implicated in protection from the development of periodontitis [40]. Employing mouse models of bone loss, it was reported that levels of both Th17 and Treg cell-related cytokines increased in response to infection, and that inhibiting IL-17 blocked periodontal destruction; whereas inhibiting the function of Treg cells exacerbated periodontal lesions [40, 41••].

Emerging information regarding DCs and DC / T cell interaction has shed light on aspects of the adaptive immune response during PD. Antigen presenting cells utilize MHCII coupled with co-stimulatory molecules to present antigen to T cells. *P. gingivalis* LPS can induce endotoxin tolerance [44]. DC treated with *P. gingivalis* LPS led to shifts in the expression of the T cell co-stimulatory molecules CD80 and CD86 [45]. Furthermore, chronic periodontitis may involve activation and in situ maturation of DCs in response to *P. gingivalis* with production of regulatory cytokines and the formation of T cell-DC foci [46]. In the context of poly-microbial challenge of mature DCs *P. gingivalis* / *F. nucleatum* and *P. intermedia* / *F. nucleatum* challenge resulted in synergized IL-6 and TNF-α expression. *P. intermedia* / *F. nucleatum* combination synergized IL-12 production. These results indicate that poly-bacterial challenge of cells results in varied response profiles associated with antigen presentation and immune activation that is dependent on the specific characteristics of the microbiota present in the sub-gingival plaque [47].

With these polar groups of T cells present in gingival tissues, it is highly likely that the fine balance between these subsets at the micro-environmental level may be a key factor in the host transition from periodontal health to disease. Modulation of the T cell imbalance may provide important new clinical approaches to PD therapy (see therapy section). Furthermore, as has been reported with polymicrobial challenge, the sub-gingival microbiota likely serves as an important driver of the local inflammatory lesion, possibly by influencing shifts in T cells, T cell subsets, and other inflammatory cells present at these sites (Fig. 1).

Innate Sensing of Periodontal Pathogens

Innate recognition of bacteria and their products by the host involves a sophisticated array of receptors providing specificity to pathogen detection. Through these receptors cells can directly respond to conserved pathogen-associated microbial patterns (PAMPS), and host danger-associated molecular patterns (DAMPS).

Toll-Like Receptors (TLRs) and TLR Signaling

To understand PD, it is critical to focus on the role played by TLRs and other pathogen recognition receptors in tuning the host immune response. In the context of PD, TLR2 and TLR4 play important roles in bacterial antigen sensing [48, 49], and oral bone loss [50–52]. TLR engagement initiates intracellular signaling via adaptor molecules culminating in nuclear factor kappa-light-chain-enhancer of activated B cells (NFκB) activation and gene expression [53]. TLR signaling occurs in a manner that is dependent on the adaptor molecule myeloid differentiation primary response gene (MyD88), or occurs independent of MyD88 via TIR-domain-containing adapter-inducing interferon-β (TRIF) [53, 54]. All TLRs with the exception of TLR3 signal via MyD88; however, TLR4 engages both MyD88 and TRIF signaling pathways. To date, TLR2 has been shown to be an important sensor of *P. gingivalis* and other periodontal pathogens. As such, MyD88 is expected to play a key role in tailoring the host innate response to periodontal pathogens. However, emerging data suggests that MyD88-independent pathways (TRIF-dependent and others) may contribute significantly to PD. A seminal study from Burns et al. [55] demonstrated that although the host cytokine response to *P. gingivalis* challenge is dependent on TLR2, MyD88 is not critical. In contrast, the ability of the host to clear *P. gingivalis* depends on MyD88. Other studies have identified an important role for TRIF in the

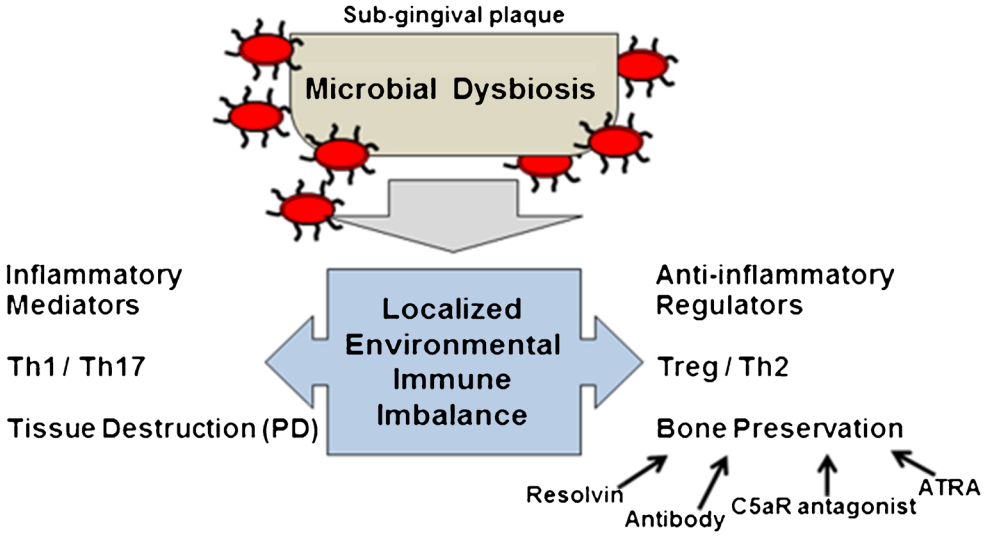

Fig. 1 Microbial dysbiosis and immune imbalance. In periodontal health the presence of defined periodontal pathogens is low. Emerging data support that rather than the presence of specific organisms, it may be that keystone pathogens such as *P. gingivalis* (red ovals) generate a microbial dysbiosis in the sub-gingival plaque. In response, at a micro-environmental level, immune imbalance ensues as a consequence of the immune response, pathogen effects, and infiltration of specific T cell subsets that shift the balance locally towards inflammation (characterized by pro-inflammatory mediators and Th1 and Th17 cells), and eventually leading to tissue destruction (PD). Numerous factors are at play to counterbalance the destructive inflammation, such as anti-inflammatory and immuno-regulatory cytokine expression, Treg and Th2 cells. Although not fully defined, contributions of antibody, resolvin [35], C5aR antagonist [81], and all-trans retinoic acid (ATRA) treatments [42] may represent important therapeutic advances, by shifting the balance of the host response toward oral bone preservation

response to *P. gingivalis*. TRIF participates in shaping CD4 T cell responses to *P. gingivalis* HagB [56], and using mouse MØ we recently observed a role for TRIF in full development of cellular TNF-α responses to *P. gingivalis* [57]. Thus, MyD88-dependent and MyD88-independent signaling both appear to play important roles in different phases of the host response during PD.

TRIF, Interferon Regulatory Factors and Type 1 Interferon

CXCL10 (IP-10) and CCL5 (RANTES) are detected in PD [58]. The expression of these chemokines is dependent on interferon regulatory factor 3 (IRF3) [59]. In the context of TLR-signaling activation of IRF3 occurs via TLR3 and TLR4 through TRIF via TBK-1 [60]. The TLR / TRIF / IRF3 axis is essential in the production of type 1 interferon (IFNα and IFNβ) [61]. IFNβ serves as an activator of additional gene products in an autocrine manner through type 1 interferon receptor (T1IFNr), STAT1/2 pathway to drive expression of interferon inducible genes such as IP-10 and RANTES [61]. Thus it is highly plausible that factors under the control of IRF3 play an important role in PD. Indeed, elevated IFNα have been measured in tissue samples from periodontitis patients [18, 62], and following periodontal therapy type 1 interferon levels dropped to those observed in healthy subjects [63]. Gaddis et al. [64] reported that recombinant *P. gingivalis* HagB stimulates bone marrow derived DCs to express IRF3 and IFNβ. Recently we reported that IRF3 participates in the development of a full immune response to *P. gingivalis* [57]. Furthermore, IRF7 participates in TLR2 and TLR7 signaling by MØ cultured with *P. gingivalis* or its LPS [65]. To date, the contribution of the TRIF-signaling pathway to the key clinically relevant endpoint of oral bone loss has not been reported. A recent paper supports that type 1 interferon may be important to the pathogenesis of PD. Nowak and colleagues identified an important mechanistic difference between local and generalized PD based on presence of type I NKT cells in aggressive, but not chronic, periodontitis lesions in vivo, and in vitro modeling using DCs identified that *A. actinomycetemcomitans*, but not *P. gingivalis*, elicited a type 1 IFN signature [66•]. Collectively, these findings bring together important elements of MyD88 and TRIF signaling pathways as critical to the development of PD. The implications of the TRIF pathway in the progression of PD and oral bone homeostasis will require continued investigation.

Other Pattern Recognition Receptors and PD

Scavenger Receptors

Scavenger receptors are a family of membrane bound innate immune PRRs [67]. These receptors are best defined by their roles in lipid scavenging by MØ [67]; however, two of these receptors, scavenger receptor (SR)-A and CD36, participate in bone remodeling and the host response to periodontal pathogens. Lin et al. [68] reported that mice deficient in SR-A had greater levels of bone mineral density than WT mice. Our studies support that *P. gingivalis* stimulates SR-A (*msr*1) gene

and surface protein expression and that the presence of SR- A in part limits host TNF-α production to this organism [69]. Triantafilou et al. [70] showed that CD36 was present in the lipid rafts of vascular endothelial cells and was one of multiple receptors used by human vascular endothelial cells to bind *P. gingivalis*, including TLR1, TLR2, and CD11b/CD18. To date, no studies have been performed to broadly assess scavenger receptor expression in human PD lesions, define the contribution of scavenger receptors in PD bone remodeling, or follow-up the role these receptors may have in shaping subgingival microbial communities; and as such follow-up studies are necessary.

Nucleotide-Binding Oligimerization Domain (NOD)-Like Receptors

The nucleotide-binding oligomerization domain or (NOD)-like receptors (NLRs) constitute a family of intracellular sensors of microbial PAMPs and host DAMPs that enter cells following uptake [71]. In one scheme, NLRs are divided into 3 groups: NODs (NOD1, NOD2, and others); NLRPs (also called NALPs); and IPAF (recently reviewed [72]). In the context of PD, NOD utilization has emerged as an important sensing mechanism. NOD1 and NOD2 are associated with ICAM-1 expression in human gingival fibroblasts responding to P. gingivalis challenge [73]. In addition to *P. gingivalis*, *A. actinomycetemcomitans*, and *F. nucleatum* stimulate NODs, and interestingly, in comparison to the other organisms, *P. gingivalis* was a less potent activator [74]. In a mouse model of oral bone loss, mice lacking NOD1 exhibit reduced bone loss, impaired recruitment of neutrophils to gingival tissues, and reduced osteoclasts in alveolar bone [75]. NOD2 deficiency in hyperlipidemic ApoE gene knockout mice led to significant increases in inflammatory cytokine production and alveolar bone loss, compared with ApoE-knockout mice, in response to *P. gingivalis* oral challenge [76]. Interestingly NOD-mediated evasion by periodontal pathogens may serve as important mechanisms to their survival. Madrigal et al. [77] reported that *P. gingivalis* likely evades a NOD-mediated host response through the ability to degrade RIPK1 and RIPK2 via its lysine-specific gingipain, Kgp. When taken collectively these findings point to NODs as important mediators of pathogen-elicited inflammation and oral bone loss.

Complement and Antibody

Complement

Complement is an important molecular system that provides antimicrobial activity. Indeed, in the context of PD, complement components are found in crevicular fluids [78], and new findings support an important role for the complement system in the pathogenesis of PD. We will not go in depth into the role of complement here, as this has recently been expertly reviewed [79]. However, from a translational perspective the observation that C5a receptor (C5aR)-deficient mice are protected from *P. gingivalis*-elicited oral bone loss [80], and that treatment with a C5aR antagonist protects mice from bone loss induced by oral *P. gingivalis* challenge, is very exciting [81].

Antibody

Evidence of adaptive immune activation in PD is generally established [82]; however, what these antibodies are doing in the context of disease is not clear. Approaches looking deeper into antibody function support their ability to opsonize, and in the context of *P. gingivalis*, treatment with IgG purified from patient sera support opsonophagocytic uptake by neutrophils [83]. Yet the presence of elevated levels of antibodies to bacteria associated with PD does not stop the progression of disease [84]. Therefore, the antibodies in PD may be generated to pathogen epitopes that are not highly expressed or are poorly accessible, or are unable to effectively interact with bacteria when they reside in biofilm. Indeed, a myriad of factors may be involved, and future studies in this area will impact on the ability to design an effective vaccine to the major periodontal pathogens.

Macrophages as Effectors at the Microenvironmental Level

Although neutrophils and lymphocytes are important cells in the pathogenesis of PD [21, 85], roles for other immune cells such as MØ are less well understood [86–88]. MØ comprise between 5 % and 30 % of the inflammatory cells identified in PD lesions [89]. These cells are recruited in response to inflammatory and chemotactic signaling, and upon entering diseased periodontal tissues express pro-inflammatory and anti-inflammatory mediators [20, 25], and may influence a variety of other effector functions.

MØ are heterogeneous and highly dynamic cells that provide important homeostatic and immune functions [90]. Early studies identified MØ subsets employing CD14 and CD16 [90]; however, recent studies now clearly depict a highly dynamic and flexible plasticity of MØ to adjust to the microenvironmental needs on entering local tissues [91]. The term "polarization" has been used generically to describe this functional adaptation; however, this terminology is restrictive as it suggests antitheses of what is thought a dynamic continuum of MØ activation types [92]. Early studies showed that classical MØ activation in response to pathogen is replicated by IFN-γ + TLR agonist (LPS) treatment. Gordon and colleagues showed that MØ were also activated by IL-4 treatment;

however, this activation was different than classical activation, as in addition to other molecules, nitric oxide (NO) production was not detected— and was coined alternative activation [93]. Three groups of alternatively activated MØ are frequently studied and include M2a, M2b, and M2c, based on differences in MØ response to IL-4, immune complexes, and IL-10, respectively [94, 95]. Broadly, alternatively activated MØ are associated with resolution of inflammation, tissue repair, and interestingly in chronic infections such as brucellosis, plague, and Q fever [96].

In the study of PD and other diseases, MØ are often used as model cells to define immune function. Indeed, transfer of TLR2 expressing MØ to TLR2-deficient mice restored host sensitivity to *P. gingivalis* oral challenge [97]. However, at the subset level, the contribution of these cells to PD is poorly understood. An elevated level of IFN-γ, a prototypical stimulus for classical MØ activation is detected in PD samples providing an environment potentially supporting classically activated MØ (25). Employing immunohistochemical approaches Chapple et al. [98] detected acute inflammatory MØ, resident histiocytes, and tissue reparative MØ, and of these, the most prevalent were reparative MØ. Topoll et al. [99] reported significant shifts in anti-inflammatory MØ population. In the context of prototypical stimulus for alternative activation, IL-4 levels appear low during active PD, and if provided at high levels, IL-4 can drive MØ apoptosis [100]. An inverse relationship between IL-4 levels and periodontal status has been observed with elevated IL-4 associated with remission/improvement of periodontal status [101]. The potential for IL-10 to influence alternative activation of MØ in PD is not well understood. Taken together, these data support that MØ phenotypic variation may be associated with different stages of PD based on micro-environmental levels of inflammatory and regulatory mediators and bacterial composition of sub-gingival plaque. Future studies directed to better understand the impact MØ have on PD inflammation, tissue repair, and oral bone homeostasis may provide important insights into the dynamics of the underlying mechanisms.

Novel Therapeutic Approaches

Although a vaccine for PD is attractive, to date, none exists. Filling this void is an array of novel and exciting therapeutic approaches aimed at interceding in the progression of PD by targeting the host immune response. In a rabbit model, application of resolvin R1 prevented periodontitis [35]. In a mouse model, C5aR antagonist significantly limited *P. gingivalis*-elicited oral bone loss [81]. Furthermore, treatment of mice with all-trans retinoic acid (ATRA), as an activator of Treg cells, reduced levels of alveolar bone loss by modulating the Th17/Treg imbalance to Treg predominance [42••]. Clinical studies are needed to determine the translational capacity of these treatment approaches to limit human disease.

Conclusions

Despite the advances made in the field of PD pathogenesis and the roles played by innate and adaptive immunity, much more needs to be understood. The implication that periodontal pathogens cause an immune imbalance that leads to a general microbial dysbiosis is an important new concept backed by compelling findings. The implications this new information may have at the micro-environmental level may provide a new window to better predict where oral bone homeostasis may be disrupted. Indeed, with advances in potentially relevant biomarkers, better targeting of disease therapy based on novel pathogen composition and immunologic understanding, in the near future better control of PD progression may be on the horizon.

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Compliance with Ethics Guidelines

Conflict of Interest Dr. Nasi Huang and Dr. Frank C. Gibson received a grant from NIH for ongoing research projects.

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