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Determinants of Periodontal/Periapical Lesion Stability and Progression

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Abstract

Periodontal and periapical lesions are infectious inflammatory osteolytic conditions in which a complex inflammatory immune response mediates bone destruction. However, the uncertainty of a lesion's progressive or stable phenotype complicates understanding of the cellular and molecular mechanisms triggering lesion activity. Evidence from clinical and preclinical studies of both periodontal and periapical lesions points to a high receptor activator of NF-κB ligand/osteoprotegerin (RANKL/OPG) ratio as the primary determinant of osteolytic activity, while a low RANKL/OPG ratio is often observed in inactive lesions. Proinflammatory cytokines directly modulate RANKL/OPG expression and consequently drive lesion progression, along with pro-osteoclastogenic support provided by Th1, Th17, and B cells. Conversely, the cooperative action between Th2 and Tregs subsets creates an anti-inflammatory and proreparative milieu associated with lesion stability. Interestingly, the trigger for lesion status switch from active to inactive can originate from an unanticipated RANKL immunoregulatory feedback, involving the induction of Tregs and a host response outcome with immunological tolerance features. In this context, dendritic cells (DCs) appear as potential determinants of host response switch, since RANKL imprint a tolerogenic phenotype in DCs, described to be involved in both Tregs and immunological tolerance generation. The tolerance state systemically and locally suppresses the development of exacerbated and pathogenic responses and contributes to lesions stability. However, immunological tolerance break by comorbidities or dysbiosis could explain lesions relapse toward activity. Therefore, this article will provide a critical review of the current knowledge concerning periodontal and periapical lesions activity and the underlying molecular mechanisms associated with the host response. Further studies are required to unravel the role of immunological responsiveness or tolerance in the determination of lesion status, as well as the potential cooperative and/or inhibitory interplay among effector cells and their impact on RANKL/OPG balance and lesion outcome.

Keywords: periodontal disease, apical periodontitis, innate immunity, adaptive immunity, T helper, cytokines

Introduction

Periodontal and periapical lesions are infectious inflammatory osteolytic conditions that affect the periodontal tissues and alveolar bone. While different entities, periodontal and periapical lesions present with overlapping pathogenesis mechanisms; both develop from a complex inflammatory immune response triggered by microbial elements, which ultimately results in bone destruction (Alvarez et al. 2019). While treatment procedures differ significantly, the ultimate treatment goal in both conditions is to control the microbial factors; this would allow for a cessation of the local chronic inflammatory osteolysis, thereby prompting the host response toward the repair of damaged tissues (Alvarez et al. 2019). While an established lesion is understood as the cumulative result of the host response over time, its development does not necessarily follow a unique and defined pattern, and determining the status of these lesions remains challenging (Teles et al. 2016; Nomura et al. 2017; Teles et al. 2018). Furthermore, difficulty in determining a particular lesion's phenotype not only affects its clinical management but also translates into critical confounders in studies focused on lesion immunopathogenesis. Moreover, the combination of an unclear phenotype with the inherent complexity of inflammatory and immunological networks and their numerous effector cells and soluble mediators may account for the high variability observed in host response elements observed in each lesion (Dutzan, Vernal, et al. 2009; Garlet 2010; Araujo-Pires, Francisconi, et al. 2014; de Campos Soriani

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Azevedo et al. 2019). In this review, we present a critical appraisal of the current knowledge on host determinants of periodontal and periapical lesion progression and stability.

Lesion Progression Patterns: Evidence from Clinical and Laboratory Studies

Numerous studies have attempted to define periodontal disease progression patterns, and burst and linear theories for periodontal disease progression were proposed with a major focus on bone and clinical attachment loss. A better understanding of the multilevel complexity in periodontitis (individual, tooth, and site level) clarified the limitations of previous hypotheses (Teles et al. 2016; Nomura et al. 2017; Teles et al. 2018). Furthermore, novel models demonstrate that lesions can present progressing, stable, regressing, and intermediate phenotypes (Teles et al. 2016; Nomura et al. 2017; Teles et al. 2018). Similarly, periapical lesions can remain stable, decrease, or increase over time, and determining its status could contribute to enhanced diagnosis to better determine the need for potential reintervention (Yu et al. 2012). It is plausible that the variability in lesion phenotypes can account for the heterogeneity of host response–related factors involved in lesion development, therefore limiting our understanding of the role of these factors in disease pathogenesis (Garlet 2010; Araujo-Pires, Francisconi, et al. 2014; Alvarez et al. 2019). While longitudinal studies with large patient cohorts would be ideal to assess lesion progression patterns and their associated host responses, challenges imposed by ethical, logistic, and financial factors limit feasibility of such studies. Intrinsic limitations such as sample collection timing and frequency are additional challenging factors to be considered.

Nevertheless, clinical and preclinical studies have provided important evidence about the host factors that are associated with either a progressive or a stable nature of inflammatory osteolytic lesions. Induction of oral infection in mice was demonstrated to trigger an exacerbated periodontal inflammatory response characterized by a high receptor activator of NF-κB ligand/osteoprotegerin (RANKL/OPG) ratio, along with a marked increase in bone resorption (Trombone et al. 2009; Garlet 2010; Araujo-Pires et al. 2015; Francisconi et al. 2018). This progressive period was then followed by a steadiness of inflammatory cell influx, with arrest of bone resorptive activity and a low RANKL/OPG ratio. These events are similar during the development of periapical lesions in mice (Francisconi et al. 2016, 2018; de Campos Soriani Azevedo et al. 2019). These findings suggest that, depending on specific host elements of lesion activity, lesions can be considered of a progressive or stable nature, coherent with the key role of RANKL during osteoclastogenesis, acting in opposition to its decoy receptor OPG (Yuan et al. 2011; Francisconi et al. 2018).

Accordingly, chronic periodontitis samples (collected from periodontal therapy unresponsive sites) show a more frequent RANKL > OPG profile, suggestive of progressive lesions (Menezes et al. 2008). Conversely, chronic gingivitis samples (from patients with a clinical history of gingival inflammation without significant bone loss) show a predominant RANKL < OPG profile, suggestive of lesion stability (Menezes et al. 2008). In this context, considering the aforementioned restrictions regarding long-term longitudinal studies, the analysis of short-term responsiveness/unresponsiveness to periodontal therapy may comprise a convenient indicator of lesion phenotype (Menezes et al. 2008; Dutzan, Vernal, et al. 2009; Colavite et al. 2019). Periapical granuloma samples also present variable RANKL/OPG ratio profiles, suggestive of the existence of both progressive and stable lesions (Menezes et al. 2008; de Campos Soriani Azevedo et al. 2019). In order to limit analysis bias, a hypothesis-free cluster analysis of periapical lesion data revealed 2 main clusters, with a high match (>95%) with the active/inactive lesions' theoretical segregation derived from RANKL/OPG ratio analysis (Araujo-Pires, Francisconi, et al. 2014). RANKL/OPG patterns from orthodontic tooth movement sites, which are well established with regards to bone resorption status, provide additional evidence for the lesion activity/inactivity profile. The pressure side, characterized by bone resorption, displays a high RANKL/OPG profile similar to that observed in chronic periodontitis, whereas the tension side, characterized by new bone formation, presents a RANKL < OPG profile similar to gingivitis sites (Menezes et al. 2008). Accordingly, previous studies also described an increased RANKL/OPG ratio in periodontitis samples in comparison to healthy or gingivitis tissues, with increased RANKL levels associated with progressive tissue destruction (Bostanci et al. 2007; Bi, Sun, et al. 2019; Lopez Roldan et al. 2020). Nevertheless, longitudinal prospective studies are required to further determine if a RANKL/OPG ratio comprises an effective biomarker of disease activity as described for other bone-related conditions. The clear cause-and-effect association between RANKL and OPG with the development of experimental lesions provides a strong support to understanding the mechanisms underlying lesion progression or stability (Yuan et al. 2011; Francisconi et al. 2018). The next sections will discuss the host response mediators that can modulate RANKL/OPG and their potential roles in lesion pathogenesis and treatment outcomes.

Host Response Determinants of Lesion Progression and Stability

Among the plethora of host mediators involved in the progression of inflammatory osteolysis, numerous cytokines have been regarded as potential determinants of lesion activity via RANKL upregulation (Fig. 1). Classic proinflammatory mediators such as tumor necrosis factor (TNF), interleukin (IL) 1, and IL6 have been implicated in lesion development by directing stimulating osteoclastogenesis (Assuma et al. 1998; Alvarez et al. 2019). Indeed, mice strains selected for maximal inflammatory response, characterized by high levels of multiple proinflammatory cytokines, present an accelerated lesion progression rate (Trombone et al. 2010). The prototypic Th1 cytokine interferon-γ (IFN-γ) inhibits osteoclastogenesis in vitro; however, its prominent proinflammatory effect in vivo

seems to overcome the inhibitory effect, leading to increased bone resorption (Garlet et al. 2008; Sommer et al. 2019). Indeed, IFN-γ contributes to lesion progression via induction of chemokine expression and the consequent chemoattraction of RANKL-producing cells and osteoclast precursors to the lesion environment (Garlet et al. 2008; Sommer et al. 2019). In addition, Th17 and B cells are described as osteoclastogenic subsets implicated in inflammatory osteolytic conditions via RANKL upregulation and boost of neutrophils and/or macrophages activity (Dutzan, Gamonal, et al. 2009; Abe et al. 2015; Sommer et al. 2019).

Thus far, most of the studies in the field have focused on individual cytokines or cell types, and their potential interplay in the determination of lesion progression remains unclear. Interestingly, when human periapical lesions are stratified based on RANKL/OPG ratio, the association of proinflammatory cytokines with high RANKL levels in the putative active clusters in evidenced by associative and mechanistic studies (Araujo-Pires, Francisconi, et al. 2014; Colavite et al. 2019; de Campos Soriani Azevedo et al. 2019). IFN-γ and IL17 were independently associated with increased RANKL expression in distinct Th1 and Th17 clusters, suggesting that each subset individually mediates lesion progression (Araujo-Pires, Francisconi, et al. 2014; Colavite et al. 2019; de Campos Soriani Azevedo et al. 2019). Indeed, while IFN-γ and IL17 can amplify host response by the induction of proinflammatory cytokines and mediate RANKL production by resident cells of periapical lesions, Th1 and Th17 responses can be mutually inhibitory (Colavite et al. 2019; Duka et al. 2019). In contrast, the simultaneous detection of high levels of both IL17 and IFN-γ in diseased periodontium is also described, suggesting a possible interplay toward disease progression (Dutzan, Gamonal, et al. 2009; Bi, Sun, et al. 2019; Sommer et al. 2019). Considering that even cytokines that are routinely used as Th polarization surrogates (i.e., IFN-γ, IL17) can also be produced by other cell types and that Th plasticity has been described in periodontal tissues (with the occurrence of $Tbet^{+}IL10^{+}$ and $FOXp3^{+}IL17^{+}$ cells) (Okui et al. 2012), it becomes clear that comprehensive studies, not exclusively focused on a single mediator or cell subset, are required to unravel the immunological complexity underlying lesion progression.

Although multiple pathways can supposedly account for periodontal/periapical lesion progression by increasing RANKL levels, accumulated evidence suggests that a cooperative action between Th2 and Treg subsets is implicated in the determination of lesion stability (Araujo-Pires et al. 2015; Francisconi et al. 2016) (Fig. 2). Accordingly, putative Th2 and Tregs products are known for their inhibitory activities on proinflammatory cytokine and RANKL production, as well as the upregulation of OPG, therefore supporting their involvement in lesion inactivity (Araujo-Pires et al. 2015; Francisconi et al. 2016). Indeed, immunization protocols that result in Th2-polarized response and immunoregulatory strategies that increase Treg activity halted lesion progression (Wang et al. 2014; Wilensky et al. 2017; Bi, Wang, et al. 2019). Furthermore, the adoptive transfer (i.e., transfer of cells originated from another individual with the goal of improving immune functionality; the cells extracted from the donor can be sorted, cultured in vitro, and/or genetically modified for specific subpopulation selection/generation before transfer) of Th2 cells, as well of Tregs, also limits experimental lesion progression (Wang et al. 2014; Wilensky et al. 2017; Bi, Wang, et al. 2019). Recent evidence suggests that the link between Th2 and Tregs involves triggering CCL22 production by IL4, which in turn allows for CCR4-dependent Treg migration to the periodontal environment (Araujo-Pires et al. 2015). The Th2/Treg interplay is required for the switch of the experimental lesion phenotype into the late inactive stage, which is characterized by the influx of Th2 and Tregs in parallel with increased levels of IL4, IL10, and transforming growth factor β (TGFβ) and a low RANKL/OPG ratio (Trombone et al. 2010; Araujo-Pires et al. 2015; Francisconi et al. 2016). Noteworthy, IL10 and TGFβ can also be produced by mesenchymal stem cells (MSCs) and contribute to local immunosuppression (Liu et al. 2012; Araujo-Pires, Biguetti, et al. 2014). However, a chronically inflamed tissue milieu limits the immunoregulatory

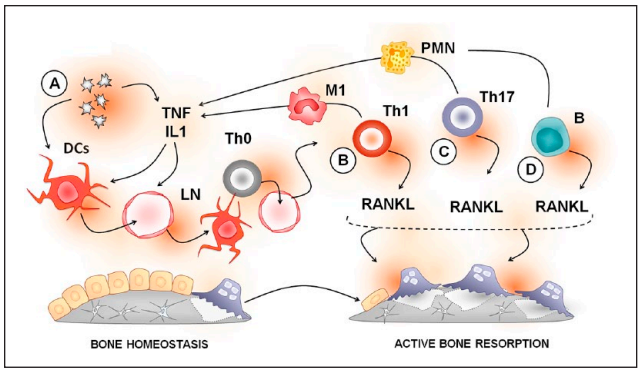

Figure 1. Host inflammatory immune response elements responsible for periodontal and periapical lesion progression. Oral bacteria or their products in the periodontal and periapical environment trigger an initial proinflammatory response (**A**), involving cytokines such as tumor necrosis factor (TNF) and interleukin (IL) 1, which trigger the activation and emigration of dendritic cells (DCs) to draining lymph nodes, with the subsequent activation of lymphocyte subsets with different properties. (**B**) The influx of Th1 cells into the periodontal/periapical environment can upregulate receptor activator of NF-κB ligand (RANKL) levels and proinflammatory cytokine levels, possibly in a proinflammatory loop mediated by M1 macrophage polarization by interferon-γ (IFN-γ). Therefore, the proinflammatory and pro-osteoclastogenic effects of Th1 cells can result in unremitting inflammation and a high RANKL/osteoprotegerin (OPG) ratio that ultimately drives lesion progression. (**C**) The influx of Th17 cells into the periodontal/periapical environment can upregulate RANKL and proinflammatory cytokine levels, possibly in a proinflammatory loop mediated by neutrophil chemoattraction and activation by IL17. Therefore, the proinflammatory and pro-osteoclastogenic effects of Th17 cells can result in unremitting inflammation and a high RANKL/OPG that ultimately drives lesion progression. (**D**) The influx of B cells into the periodontal/periapical environment can upregulate RANKL levels and proinflammatory cytokine levels, possibly in a proinflammatory loop mediated by opsonization mechanisms. Therefore, the proinflammatory and pro-osteoclastogenic effects of B cells can result in unremitting inflammation and a high RANKL/OPG that ultimately drives lesion progression.

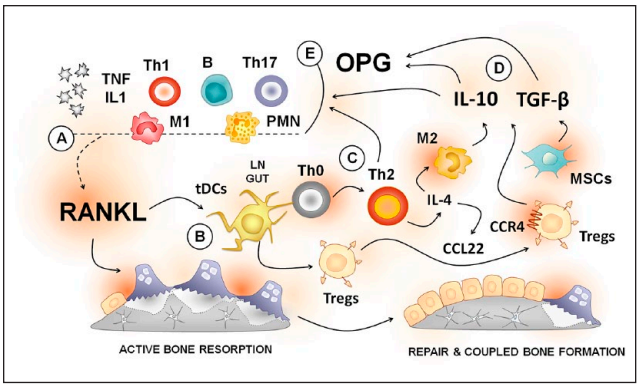

Figure 2. Host inflammatory immune response elements responsible for periodontal and periapical lesion stability. (**A**) Oral bacteria or their products in the periodontal and periapical environment trigger an initial proinflammatory response, which involves cytokines such as tumor necrosis factor (TNF) and interleukin (IL) 1 and Th1, Th17, or B-cell subsets with which they can upregulate local receptor activator of NF-κB ligand (RANKL) levels and drive lesion progression. (**B**) High RANKL levels in the lesions may affect dendritic cell (DC) polarization, resulting in a tolerogenic phenotype and in the induction of Tregs in draining lymph nodes or in the gut. (**C**) In parallel with Tregs induction, RANKL/DCs interaction can influence Th polarization toward a Th2 pattern in draining lymph nodes. The presence of Th2 in the lesions results in IL4 production, which can lead to the polarization of macrophages into an M2 phenotype and also trigger the local expression of the chemokine CCL22, responsible for Treg chemoattraction via the CCR4 receptor. (**D**) Tregs and M2 macrophages can produce immunoregulatory cytokines such as IL10 and transforming growth factor β (TGFβ), generating an anti-inflammatory and proreparative milieu, which can support further local immunosuppressive activity by mesenchymal stem cells (MSCs). (**E**) The cooperative action of Th2, Tregs, M2, and MSCs may be responsible for limiting host immunological hyperresponsiveness, which ultimately results in a low RANKL/osteoprotegerin (OPG) ratio and lesion stability.

capacity of MSCs, suggesting that MSC immunosuppressive properties require a proper environment, which could derive from the cooperative Th2/Treg axis (Liu et al. 2012; Araujo-Pires, Biguetti, et al. 2014).

Of note, it is also important to consider that the lesion environment can also affect local macrophage and neutrophil functions (Figs. 1, 2). While macrophages acquire the M1 phenotype under IFN-γ influence, IL4 drives macrophage polarization into an anti-inflammatory and proreparative M2 phenotype. The M1 subset prevails in human periodontitis lesions in comparison with M2 dominance in gingivitis, likely indicating the contribution of each macrophage subset to disease progression and stability phenotypes (Zhou et al. 2019). Accordingly, induced migration of M2 macrophages into periodontal tissues was shown to prevent bone loss in an experimental model of periodontitis (Zhuang et al. 2019). A similar profile was described in periapical lesions, where an inflammatory state associated with M1 macrophages was related to lesion progression. Conversely, an immunomodulatory environment associated with M2 macrophages was associated with lesion stability (Franca et al. 2019). Noteworthy, macrophage polarization at the lesions can account for progression/stability not only by the contrasting M1/M2 immunoregulatory properties but also by its differential potential as osteoclast precursors. Although the exact nature of osteoclast precursors in osteolytic lesions remains unclear, evidence suggests that putative monocytic osteoclast precursors can function as inflammatory cells or differentiate into osteoclasts (Zhang et al. 2011). In this framework, the M1 subset presents a higher osteoclastogenic differentiation capacity than M2 cells (Fukui et al. 2017). While Th1 and Th2 subsets can directly module macrophage polarization and osteoclastogenic potential, Th17 cells increase neutrophil infiltration and function, which is essential for bone resorption (Eskan et al. 2012). Collectively, these findings highlight the differential impact of polarized adaptive immunity cells over innate immunity cellular components.

However, due to the overlapping production of cytokines by multiple cell types and the potential redundancy or compensating roles of each cytokine or cell subset, defining the individual contributions of each element in supporting or counteracting inflammation and RANKL-mediated osteoclastogenesis is still impossible. Furthermore, the contribution of each individual element to lesion progression or stability and the factor(s) responsible for the conversion of an active lesion into an inactive phenotype remain unknown. Surprisingly, evidence points to RANKL as an unexpected immunoregulatory trigger (Francisconi et al. 2018).

RANKL as a Double-Edged Sword: Its Dual Role in Triggering Lesion Progression and Stability

RANKL has been regarded as the master osteoclastogenic factor and a key determinant of periodontal/periapical lesion progression (Yuan et al. 2011; Francisconi et al. 2018; Alvarez et al. 2019). However, the RANK (receptor activator of nuclear factor kappa B)/RANKL/OPG system plays important roles in the regulation of the immune system, modulating lymphoid organ microenvironments and influencing immune response outcomes (Lin et al. 2016; Kimura et al. 2020). In peripheral tissues, RANKL exerts an immunoregulatory role by modulating the phenotype and function of dendritic cells (DCs) (Williamson et al. 2002; Loser et al. 2006; Izawa et al. 2007; Lin et al. 2016). Upon activation, DCs mature and migrate to secondary lymphoid organs, which express costimulatory molecules and produce distinct patterns of cytokines, which control the subsequent lymphocyte response (Song et al. 2018). A lower density of immature DCs is observed in inflamed gingival tissue, suggesting that DCs follow typical maturation and migration patterns in the periodontal environment (Souto et al. 2014). Furthermore, periodontal pathogens and/or their products are generally described to induce DC activation, which trigger the host inflammatory response and subsequent lesion development (Souto et al. 2014). Indeed, studies demonstrated that activated DCs may contribute to lesion development through the induction of Th1 or Th17 lymphocytes (Huang et al. 2011; Diaz-Zuniga et al. 2015; Song et al. 2018). However, significant phenotypic and functional variations in DC-induced Th response have been reported depending on the microbial

serotypes, virulence factors, and/or receptors involved, which might be relevant to periodontitis pathogenesis (Huang et al. 2011; Diaz-Zuniga et al. 2015) (Fig. 1). Accordingly, activated DCs may present a protective role via induction of Th2 or Treg subsets (Huang et al. 2011; Diaz-Zuniga et al. 2015; Song et al. 2018). Despite a central role of DCs in immune response outcomes, studies focused on DCs in periodontal/periapical lesion pathogenesis are scarce, and the exact contribution of DCs to periodontal/periapical lesion pathogenesis remains to be determined.

While currently available studies usually focus on DC activation by bacteria or their products, additional signaling molecules, such as RANKL, can also modulate DC activity. In previous studies, RANKL-stimulated DCs were shown to enhance a T-cell effector response, albeit recent evidence points toward a prevalent imprint of an immunoregulatory phenotype in DCs by RANKL (Williamson et al. 2002; Loser et al. 2006; Izawa et al. 2007; Kimura et al. 2020). In an experimental periapical lesion model, as expected, RANKL inhibition limited the osteolytic response while also impairing the natural immunoregulatory process over time (Francisconi et al. 2018). Specifically, RANKL inhibition resulted in a sustained immunological high responsiveness, characterized by continuous proinflammatory response in the periapical region (Francisconi et al. 2016, 2018). In addition, RANKL inhibition resulted in systemic effects such as high delayed-type hypersensitivity (DTH) and T CD4 proliferative response, opposing the natural immunoregulation process (Francisconi et al. 2016, 2018). Noteworthy, such a natural immunoregulatory process involves the decrease in DTH and T-cell proliferative response and the concomitant decrease in Th1 and Th17 responses along with an increase in Th2 and Tregs (Araujo-Pires et al. 2015; Francisconi et al. 2016, 2018). Interestingly, the immunoregulatory process observed is compatible with the development of immunological peripheral tolerance, a complex state of active hyporesponsiveness (Wambre and Jeong 2018) (Fig. 2).

Immunological tolerance takes place at thymic and peripheral levels as a T-cell-focused mechanism of protection. Central tolerance occurs in the thymus to control autoreactive T-cell clones and involves clonal deletion, clonal anergy, and the generation of regulatory T cells, mediated by cooperative action of thymic DCs and medullary epithelial cells (Wambre and Jeong 2018). In the thymic environment, RANKL and RANK are critical modulators of the antigen presentation that mediates the negative T-cell selection and Treg generation (Lin et al. 2016). Peripheral immunological tolerance is a complex process, which can involve T-cell clonal deletion and anergy, as well as the generation of Tregs targeting innocuous dietary protein and microbial antigens by tolerogenic DCs (Weiner et al. 2011; Wambre and Jeong 2018). Importantly, immunological tolerance differs from the endotoxin tolerance, which takes place at an individual cellular level, where previous exposure to bacteria and/or its products leads to transient refractory responses to further stimuli (Foey and Crean 2013). Within the inherent complexity of peripheral tolerance, Tregs play a central role by limiting adaptive immunity at secondary lymphoid organs and peripheral tissues (Weiner et al. 2011; Wambre and Jeong 2018). While evidence linking RANKL with the development of immunological tolerance is still scarce, studies suggest tolerogenic DCs and Tregs as the connecting elements (Williamson et al. 2002; Izawa et al. 2007). In cutaneous and mucosal peripheral tissues, RANKL induces tolerogenic DCs, which in turn mediate oral tolerance via Treg induction (Williamson et al. 2002; Izawa et al. 2007; Matteoli et al. 2010). Noteworthy, the sustained response in periapical lesions due to RANKL inhibition recapitulated the phenotype derived from Treg inhibition (Francisconi et al. 2016). Also, unremitting response derived from RANKL inhibition is reversed by Treg adoptive transfer, reinforcing the RANKL/Treg interplay in this process (Francisconi et al. 2018).

Hence, the high RANKL levels observed in active periodontal/periapical lesions could serve as a trigger for an immunoregulatory feedback, mediated by the modulation of the DC phenotype before its migration to draining regional lymph nodes (Figs. 2, 3). Accordingly, DCs are known to have a protective role in experimental periodontitis, which is dependent on the transcription factor FOXO-1, whose expression and nuclear translocation are triggered by RANKL (Wang et al. 2015; Xiao et al. 2015), strengthening the immunoregulatory outcome of RANKL-stimulated DCs. Furthermore, the RANKL production peak in active lesions was temporally followed by changes in cervical lymph node expression patterns and the rise of the Th2/Tregs axis, concomitant with a switch from active to inactive lesions (Trombone et al. 2010; Francisconi et al. 2016, 2018). In line with these findings, DCs skew T-cell polarization toward a Th2 pattern at draining lymph nodes after tolerance induction (van Wilsem et al. 1995), supporting the potential connection between RANKL, DCs, and the overall immunoregulation that confer lesion stability.

While cumulative experimental evidence supports the RANKL-DC-Treg-tolerance axis hypothesis, to date, no previous study has systematically addressed the multiples features of immunological tolerance in the host-microbe interplay in the oral cavity. Nonetheless, such hypothesis is also supported by the oral-gut connection. Since oral bacteria are constantly swallowed and abundant in the gastrointestinal system, and the gut lamina propria is described as a highly tolerogenic environment, oral bacteria-specific Tregs could be also generated in the gut environment (Weiner et al. 2011; Wambre and Jeong 2018). Accordingly, the delivery of antigens via the oral route results in Treg-mediated tolerance, a strategy currently used to avoid and control exacerbated immune responses in different distal tissues (Weiner et al. 2011; Wambre and Jeong 2018). Furthermore, gastrointestinal mucosal presentation induces systemic T-cell tolerance to *Fusobacterium nucleatum* (Keys et al. 1986). Thus, targeted delivery of oral microbe antigens via the oral route, aiming at the development of Treg-mediated tolerance, could comprise a preventive or therapeutic strategy to avoid and/or control exacerbated immune responses, mirroring strategies to manage pathological processes in other tissues (Weiner et al. 2011; Wambre and Jeong 2018). Interestingly, gut homeostasis is required for immunological tolerance

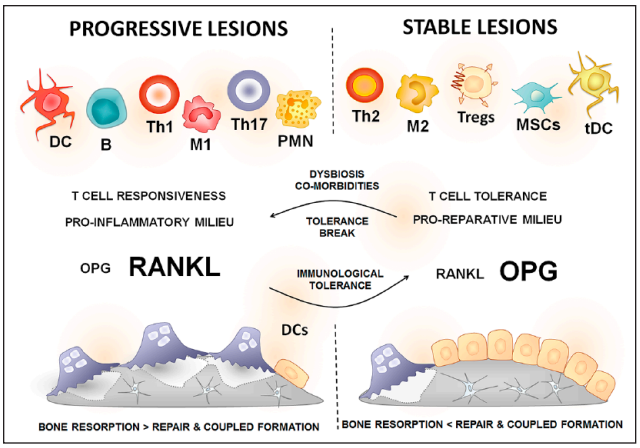

Figure 3. Distinct progressive and stable periodontal/periapical lesion features and lesion phenotype switches. Evidence from human and experimental lesions points to a high receptor activator of NF-κB ligand/osteoprotegerin (RANKL/OPG) ratio as a primary determinant of lesion osteolytic activity, coherent with the RANKL osteoclastogenic role as opposed to its antagonist OPG. Conversely, low RANKL/OPG ratio is observed in nonprogressive lesions, being associated with a stability phenotype. Proinflammatory cytokines can mediate lesion progression modulating RANKL/OPG balance toward osteoclastogenesis and by inducing the action of dendritic cells (DCs) toward the polarization and subsequent migration of Th1 and Th17 subsets, which can independently mediate lesion progression by boosting the production of RANKL and by respectively boosting proinflammatory response via M1 and neutrophil activity. B cells also appear to be a substantial RANKL source and a lesion progression factor. Conversely, Th2/Treg interplay determines lesion stability by counteracting proinflammatory and pro-osteoclastogenic pathways and by creating a proreparative environment, with this response associated with M2 polarization and immunosuppression by mesenchymal stem cells (MSCs). RANKL appears as an unexpected switch of a host response pattern, possibly triggering immunoregulatory feedback via tolerogenic DCs and Treg induction, which contributes to the development of immunological tolerance to oral bacteria. The tolerance state systemically suppresses the development of exacerbated and pathogenic responses and contributes to lesion stability. Conversely, immunological tolerance break by comorbidities or dysbiosis could explain relapse toward lesion activity.

induction, and its disruption by chemical agents used to trigger experimental gut inflammation results in alveolar bone loss in response to commensal oral microbiota in mice (Oz and Ebersole 2010). Accordingly, inflammatory gut conditions were also associated with increased periodontitis risk (Vavricka et al. 2013). Similarly, other conditions that interfere with an immunological responsiveness/hyporesponsiveness balance, such as arthritis, have been associated with periodontitis as a comorbidity or modifying factor. Indeed, different arthritis models were shown to trigger alveolar bone loss in mice, which can be prevented by oral bacteria control (Trombone et al. 2010), thus supporting the possible break of the preexisting immunological tolerance to commensal microbes (Francisconi et al. 2018). Therefore, while immunological tolerance development appears to contribute to lesion stability, tolerance break could explain a potential relapse toward lesion progression. Since comorbidities likely to disrupt immunological tolerance could also modify oral microbiota toward dysbiosis (Graves et al. 2019), the mechanisms linking such conditions with host responsiveness in the periodontal environment remain to be unraveled (Fig. 3).

Interestingly, the development of immunological tolerance upon oral infection resembles the evolving "disease tolerance" concept, which relies on immunoregulatory mechanisms that limit the damage imposed on host tissues during infection (Martins et al. 2019). Noteworthy, the strict translation of the original disease tolerance definition (which refers to "disease variation without a direct correlation with pathogen load") (Martins et al. 2019) to oral conditions may be limited by its inherent host-microbe interplay complexity. However, the relative stability and similarity of bacterial levels in active and inactive experimental periodontal/periapical lesions (Trombone et al. 2009; Araujo-Pires, Biguetti, et al. 2014; Araujo-Pires et al. 2015; Francisconi et al. 2016, 2018) appear to fit with the disease tolerance definition. Furthermore, disease tolerance mechanisms include the immunoregulation by Tregs, which restrain innate and adaptive immunity from operating beyond damage response thresholds (Martins et al. 2019), resembling the role of Tregs in periodontal and periapical lesions (Francisconi et al. 2016, 2018). Importantly, although immunological tolerance and disease tolerance concepts differ, the involvement of Tregs in both scenarios suggests potential functional interplay in these regulatory processes (Wambre and Jeong 2018; Martins et al. 2019). Therefore, in situations characterized by the impossibility to eliminate microbial elements (i.e., without clinical intervention), irrespective of the nature and intensity of the host immune response, the development of immunological and disease tolerance seems to converge as part of a host regulatory process aiming to limit periodontal/periapical tissue damage. Indeed, the exacerbated response conferenced by genetic selection for maximal inflammatory response increases lesion severity but does not improve bacterial clearance, whereas a moderate response provides an effective protection with minor tissue damage (Trombone et al. 2009). Even in a clinical situation where the clinical procedures aim to control the microbial factors, the development of a regulated host response would result in a more favorable outcome in case of potential reinfection. Noteworthy, while the development of immunological tolerance theoretically results in a homeostatic host-microbe interplay with absent or minimal tissue damage even in the presence of inflammatory cells, other therapeutic strategies aim to resolve the inflammatory response. Proresolving mediators (e.g., resolvin E1) were described to effectively shut down the inflammatory process and lesion progression and to counteract biofilm dysbiosis by limiting the tissue breakdown that supports some pathogens' emergence (Hasturk et al. 2007).

Concluding Remarks

Multiple lines of evidence point to the RANKL/OPG ratio as a key determinant of periodontal and periapical lesion progression/stability. Proinflammatory cytokines directly modulate RANKL/OPG expression and consequently drive lesion progression, along with pro-osteoclastogenic support provided by Th1, Th17, and B cells. Conversely, the cooperative action

between Th2 and Treg subsets creates an anti-inflammatory and proreparative milieu associated with lesion stability. Interestingly, the trigger for status switch from active to inactive lesions can originate from an unanticipated RANKL immunoregulatory feedback, involving the induction of Tregs and a host response outcome with immunological tolerance features. In this context, DCs appear as potential determinants of this switch, since RANKL imprints a tolerogenic phenotype in DCs, as described in both Treg and immunological tolerance generation. Further studies are required to unravel the role of immunological responsiveness or tolerance in the determination of lesion status, as well as the potential cooperative and/or inhibitory interplay among effector cells and their impact on RANKL/OPG balance and lesion outcome.

Author Contributions

F. Cavalla, A. Letra, R.M. Silva, contributed to conception, design, and data interpretation, critically revised the manuscript; G.P. Garlet, contributed to conception, design, data acquisition, analysis, and interpretation, drafted and critically revised the manuscript. All authors gave final approval and agree to be accountable for all aspects of the work.

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