Alterations in Macrophage Polarization Play a Key Role in Control and Development of Periodontal Diseases

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Abstract

Periodontitis is a chronic inflammatory disease driven by complex interplays between a dysbiotic oral microbiome and a dysregulated host inflammatory response that results in the destruction of the tooth-supporting apparatus. Among the inflammatory cells involved in the pathogenesis of periodontitis, macrophages are recruited early on to sites of periodontal infection. These cells can polarize in different phenotypes that mediate the initiation and resolution of inflammatory responses, as well as in tissue healing. Macrophage phenotypic plasticity is thought to play a critical role in the induction and resolution of inflammation and may be compromised in patients with chronic inflammatory diseases. Here, we reviewed the role of macrophage polarization in periodontal disease and therapy.

Key words: Gingivitis, Immunomodulation, Inflammation, Macrophages, Periodontitis

Introduction

Clinically healthy periodontal tissues are associated with symbiotic relationships between the health-promoting resident microbial biofilms and host tissues [1-3]. However, the relationship between the oral microbiome and the host is dynamic and can be disturbed by dysbiotic shifts in the microbiome and alterations in host defense mechanisms. Dysbiotic ecological changes in the supragingival biofilm result in gingivitis [4]. In gingivitis, the host's inflammatory response to the dysbiotic biofilm does not resolve due to its inability to eradicate microbial organisms arranged in mature biofilms [2, 3]. This results in a chronic inflammatory state, in which inflammation and microbial dysbiosis are reciprocally reinforced [2, 3, 5]. In susceptible individuals, a disproportionate host inflammatory response underlies the progression of gingivitis to periodontitis [2, 3, 6, 7].

Macrophages are recruited early on to sites of periodontal infection where they play critical roles in nonspecific innate immune responses and initiating adaptive responses [8]. Until recently, macrophages were thought to be a relatively uniform population of cells. However, remarkable progress has been made in identifying that macrophages may assume a range of heterogeneous phenotypes and functions depending on local signals, the extremes of which have been classified as pro-inflammatory (M1) and anti-inflammatory or pro-healing (M2) macrophages [9, 10]. Macrophage phenotypic plasticity is thought to play a critical role in the induction and resolution of inflammation and may be compromised in patients with

chronic inflammatory diseases [8, 11]. Here, we will review the role of macrophage polarization in periodontal disease and therapy.

Macrophage phenotypic transition in gingivitis

Macrophages undergo phenotypic and functional changes in response to a range of local microbial and inflammatory cues within the tissue microenvironment. Unsurprisingly, gingival tissues with inflammation have shown alterations in macrophage polarization [12, 13]. Two recent studies have reported differences in M1/M2 macrophage polarization in gingivitis, albeit with some discrepancy between their results. While it has been reported that both M1 and M2 macrophages are more abundant in gingival tissues of patients with gingivitis compared to those from periodontally healthy individuals or those from patients with periodontitis [12], it has also been shown that gingival tissues from patients with gingivitis have fewer M1 macrophages than those from periodontitis patients but exhibit higher M2 counts than healthy gingival tissues [13]. The reason for these conflicting results remain unknown but may be attributed to the definitions of M1 and M2 cells in tissues. Indeed, in these two studies, macrophages have been defined by different markers. While the study by Zhou *et al.* [13] defined M1 macrophages as F4/80+/inducible nitric oxide synthase (iNOS+) cells, the 2019 study by Garaicoa-Pazmino *et al.* [12] defined them as CD68+/iNOS+ cells. Of interest, CD68 can be expressed in monocytes and basophils, and these two cell types can be seen in inflamed gingival tissue sections.

Emerging evidence also indicates that the relative proportion of M2 cells in gingival tissues is influenced by local inflammation [12]. Accordingly, M2 macrophages account for 72% of all macrophages in gingival health, 45% in gingivitis, and 48% in periodontitis [12]. By contrast, M1 macrophages encompass approximately 20% of all macrophages in gingival/periodontal tissues derived from patients diagnosed with health, gingivitis, and periodontitis [12]. This, in turn, agrees with studies on other infectious diseases that have reported imbalances in M1/M2 macrophage ratios, with M2 macrophages being often downregulated [14].

Finally, and perhaps most interestingly, macrophages presenting a hybrid phenotype that simultaneously expresses M1 and M2 markers have been shown in inflamed gingival tissues [12]. Such hybrids account for about 15% of macrophages in gingivitis and periodontitis samples [12], which suggests the existence of a more complex scenario, beyond the conventional views on the M1/M2 binary macrophage polarization. These findings support the idea that the hybrid M1/M2 phenotype can be a transient phenotypic result of macrophages constantly undergoing M1–M2 transitions during chronic inflammatory states [15]. This mechanism could allow the M1-directed killing of invading organisms and activation of innate and adaptive immunological responses, followed by M2 responses to counteract the excessive inflammation and tissue injury. In turn, as M2 macrophages induce a local immunosuppressive state, these cells could further enhance microbial dysbiosis. The antimicrobial activity displayed by M1 macrophages involves not only the phagocytosis of microbial organisms but also the release of reactive oxygen and nitrogen species, cytokines, and chemokines, which, in turn, recruit and activate additional inflammatory cells. This whole process, while important for pathogen elimination, can also cause tissue injury. On the other hand, M2 macrophages are involved in phagocytic removal of dead cells, angiogenesis, and secretion of growth factors and cytokines that, as mentioned before, inhibit immune and inflammatory responses but also induce cellular proliferation for tissue healing. It is important to point out that M1 and M2 are the two extremes of a biological gradient. Therefore, it is not surprising that researchers have described mixed phenotypes in a complex and dynamic environment that is the inflamed gingiva.

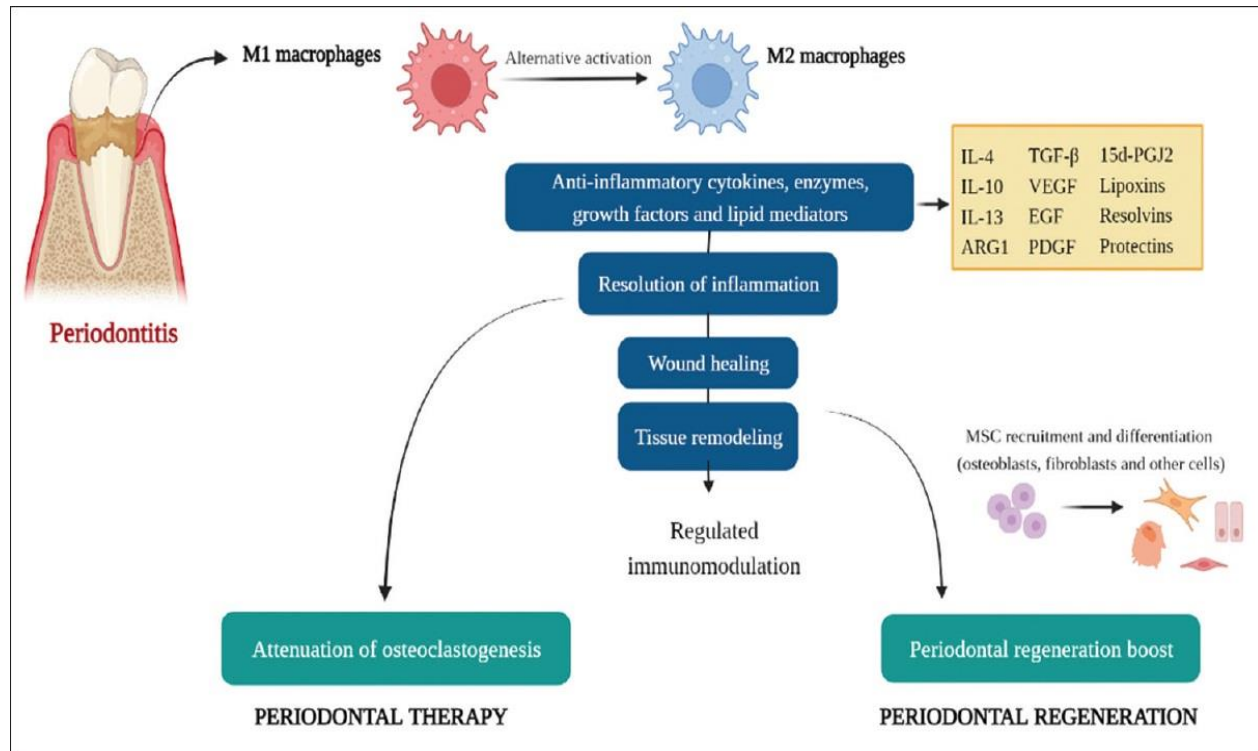*Macrophage phenotypic transition in periodontitis*

There is some evidence for an increase in the abundance of M1 macrophages in periodontal tissues from patients with periodontitis compared to M1 counts in periodontal tissues from healthy controls [13, 16], although M1 macrophages found in patients with periodontitis may have impaired phagocytic activity [16]. However, adding to the complexity of the M1/M2 paradigm, M1 polarization in periodontitis lesions has also been reported to be reduced [17] or to have similar levels to those observed in gingival tissues from periodontally healthy controls [12]. The basis for these contrasting results is still largely unknown but might be related to methodological differences among studies. In addition to the issues raised above about the M1 and M2 definition criteria, differences in clinical sampling and disease definition may explain the discrepant data. For example, only the free marginal gingiva was sampled in the Zhou *et al.*'s study [13], which means that the sampling site for their periodontitis cases did not represent the real pathogenesis of periodontitis. Finally, Li *et al.* [17] used online Gene

Expression Omnibus databases that lacked the clinical data required to determine the appropriateness of the diagnosis. In the context of M2 macrophages, there is evidence from a clinical study of the reduction in M2 counts in periodontal tissues from patients with periodontitis, compared to counts in periodontal tissues from healthy individuals [16], although one study reported no changes in M2 counts during periodontitis [13].

With the exception of one study that showed no alterations in the M1/M2 ratio in periodontal tissues from patients with periodontitis [17], all clinical studies found increases in the M1/M2 macrophage ratio in periodontitis compared with those found in gingivitis [18] and healthy gingiva [13, 16]. It is important to point out that the only study that suggested that the M1/M2 ratio remains unaltered during periodontitis was the one that lacked clinical data [17]. Supporting these observations, a study using a mouse experimental model showed that macrophages from periodontitis lesions were positive for iNOS expression, while macrophages from healing tissues were positive for arginase 1, suggesting M1 and M2 phenotypes, respectively [19]. Moreover, other preclinical studies demonstrated that the intraoral inoculation of rodents with *Porphyromonas gingivalis* enhances M1/M2 ratio [20-22] and macrophage-mediated alveolar bone loss [20]. Of interest, increased M1/M2 ratios in periodontitis samples led to imbalances between local inflammatory and repair mechanisms characterized by increased expression of multiple pro-inflammatory cytokines (i.e., interferon-gamma, tumor necrosis factor-alpha, interleukin [IL]-6, and IL-12) and metalloproteinases in gingival samples [13, 18]. In turn, this M1/M2 misbalance-induced hyperinflammation is expected to result in progressive destruction of periodontal supporting tissues. In support of this hypothesis, high M1/M2 macrophage ratios correlate positively with periodontitis severity [13].

Macrophage phenotypic modulation as a strategy in periodontal therapy

The current standard treatment of periodontitis is based on the mechanical elimination of the dysbiotic biofilm to allow the reestablishment of a symbiotic biofilm and the resolution of inflammation [23]. However, this strategy does not always succeed in halting disease progression [24]. Over the past few years, there has been a great deal of interest in modulating M1 and M2 responses to optimally eliminate pathogens without the development of untoward pathologies associated with severe or persistent inflammation [25]. Although the therapeutic modulation of macrophage phenotypes has not been employed for treating patients with periodontitis, it has shown promising results in preclinical models of periodontitis. In this regard, the local delivery of controlled-release microparticles containing the chemokine C-C motif chemokine ligand 2 (CCL2) was shown to increase the numbers and ratio of M2 macrophages and the expression of the anti-inflammatory marker IL-1 receptor antagonist in periodontal tissues [26]. Furthermore, CCL2 administration reduced receptor activator of nuclear factor kappa-beta expression along with osteoclast numbers and activity and thereby prevented alveolar bone loss in a mouse model of periodontitis [26]. Likewise, by inducing M2 macrophage polarization, exosomes derived from gingival tissue-derived mesenchymal stem cells (MSCs) [27] and bone marrow MSCs (BMSCs) [21] promoted increased expression of transforming growth factor-beta 1 (TGF-b1) and resolution of inflammation which, in turn, attenuated collagen destruction, osteoclastogenesis, and ligature-induced bone loss in murine models of periodontitis. Finally, it was demonstrated that local administration of gold nanoparticles inhibits iNOS expression, osteoclastic activity, collagen degradation, and alveolar bone loss in a rat ligature-induced periodontitis model through regulation of macrophage M2 polarization [28]. Based on the available evidence, it is reasonable to suggest that therapeutic strategies that foster M2 development are likely to reduce the negative impacts of sustained inflammation on the periodontium **(Figure 1)**. Nonetheless, these findings have yet to be validated in prospective clinical trials.

Figure 1. Macrophage phenotypic modulation as a strategy in periodontal therapy and regeneration. The immunomodulation of macrophage phenotypes from M1 to M2 (alternative activation) promotes the release of anti-inflammatory cytokines, growth factors and lipid mediators, and arginase 1 expression, which are involved in the resolution of the inflammatory process and wound healing. Just as a sustained increase in the M1 macrophage population can lead to exaggerated inflammation and inflammation-driven periodontal destruction, immunomodulation toward the M2 phenotype favors the resolution of inflammation and osteoclastogenesis and improves the outcomes of regenerative periodontal treatments through recruitment and activation of multiple mesenchymal cell types. IL-4 – Interleukin-4, IL-10 – Interleukin-10; IL-13 – Interleukin-13; ARG1 – Arginase 1; TGF-β – Transforming growth factor-β, VEGF – Vascular endothelial growth factor, EGF – Epidermal growth factor; PDGF – Platelet-derived growth factor; 15d-PGJ2 – Anti-inflammatory lipid mediators 15-deoxy-delta-12,14-prostaglandin J2; MSC – Mesenchymal stem cells

Macrophage phenotypic modulation as a therapeutic strategy in periodontal regeneration

In addition to promoting inflammation, which may result in cellular and tissue damage, macrophages play a pivotal role in tissue repair and regeneration during injury resolution [29]. Macrophage contributions to wound healing are unlikely to be restricted to the initial inflammatory phase of healing. Instead, emerging evidence has demonstrated that once macrophages assume the M2-resolving phenotype, they first contribute to the resolution of inflammation by removing apoptotic cells and secreting anti-inflammatory factors [30]. Subsequently, M2 macrophages secrete factors that enhance the mobilization and recruitment of MSCs and stimulate the proliferation, differentiation, and activation of multiple cell types, including fibroblasts, epithelial cells, endothelial cells, and MSCs [30, 31] **(Figure 1)**.

Macrophages have long been implicated in the formation of mineralized tissues during both normal bone homeostasis and fracture healing [32]. However, the recent identification of the molecular mechanisms that underlie the cross talk between macrophages, MSCs, and bone-forming cells [32] has opened the possibility of targeting macrophages as an attractive and novel therapeutic strategy to enhance periodontal regeneration. In line with this, the impact of macrophages on periodontal regeneration has already been tested in preclinical studies. One of these studies demonstrated that the application of high-stiffness hydrogels containing IL-4 and stromal cell-derived factor-alpha in critical-sized periodontal defects promoted macrophage M2 polarization at an early stage of healing and the recruitment of BMSCs into these defects [33]. In turn, the

coordinated cross talk between BMSCs and M2 macrophages favored BMSC osteogenic differentiation and the formation of a functionally oriented periodontal ligament (PDL) interposed between the new bone and the new cementum [33]. Similar results were reported in another study in which the interaction between PDL stem cells (PDLSCs) and macrophages induced enhanced M2 polarization within periodontal defects which, in turn, reduced local inflammation and boosted periodontal regeneration in a rat fenestration defect model [34]. Adding to the body of evidence that macrophages play an essential role in periodontal regeneration, one of the latest studies showed that by inducing M2 polarization through gold nanoparticles, there was an increase in macrophage bone morphogenetic protein-2 expression and the osteogenic differentiation of human PDLSCs, which contributed to improved regenerative outcomes in a rat fenestration defect model [28]. Altogether, these findings suggest that M2 macrophage modulation is a potential therapeutic strategy for periodontal tissue engineering.

Network model of macrophage function in inflammatory periodontal disease
Although variations in the M1/M2 macrophage ratio in both healthy states and in periodontal disease have been observed, the traditional M1–M2 paradigm, which classifies macrophages into these two categories, is now being reevaluated in light of a more comprehensive network model of macrophage function, particularly in the context of inflammation. This network model acknowledges the complexity of macrophage roles, especially in their transition from an inflammatory (M1) to a pro-resolving or regulatory (M2) phenotype, and vice versa. Such transitions are crucial in determining the progression, resolution, or persistence of inflammatory diseases, including periodontal diseases. Further insights come from studies on macrophages infected by *P. gingivalis*. These studies suggest that macrophages can be viewed as a communication network, linking infection to systemic diseases [35]. They also highlight similarities in macrophage molecular signatures across different diseases, such as rheumatoid arthritis, osteoarthritis, and periodontal disease [36]. As macrophages are cells that recognize invading pathogens early through phagocytosis, assuming functions such as antigen presentation and cytokine release, the investigation of genes expressed in these cells during pathogen–host interactions can reveal the underlying molecular network that contributes to their functional plasticity in inflammatory conditions. Understanding these mechanisms could be pivotal in the prevention, early diagnosis, and treatment of various infectious and inflammatory diseases, including periodontal disease.

Conclusion

Macrophage polarization plays a crucial role in the pathogenesis of periodontal disease and impacts periodontal therapy outcomes in preclinical models, with M1 macrophages enriched in disease and M2 macrophages preventing disease progression and favoring periodontal regeneration. However, a couple of questions remain to be answered: (a) Can we locally turn off M1 polarization to limit periodontitis progression in humans, without interfering with host protection or causing microbial dysbiosis? (b) What are the best strategies to regulate macrophage polarization during periodontal regenerative therapy? A better understanding of context-dependent macrophage polarization in the periodontium will provide new opportunities for improving patient outcomes.

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