Macrophages in periapical lesions: Potential roles and future directions

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Periapical lesions are infectious diseases that occur in the apical region of teeth. They result in the destruction of alveolar bone and are usually accompanied by swelling, pain, and possible systemic impacts. A complex interaction between pathogens and the host immune system determines the development, progression, and outcome of periapical lesions. The lesions, if not treated promptly, may cause resorption of bone tissue, destruction of the periodontal ligament, and loss of the affected teeth, all of which can severely worsen the quality of life of patients, often at considerable economic cost to both patients and medical organizations. Macrophages are a group of heterogeneous cells that have many roles in the development of infections, destruction and reconstruction of bone tissues, and microbe–host interactions. However, the differential and comprehensive polarization of macrophages complicates the understanding of the regulatory mechanism of periapical lesion progression. This report provides a comprehensive review of recent advances in our knowledge of the potential role of macrophages in determining the turnover of human periapical lesions. For example, macrophage differentiation might indicate whether the lesions are stable or progressing while the extent of bacteria invasion could regulate the differentiation and function of macrophages involved in the periapical lesion. In addition, alternative strategies for the treatment of apical periodontitis are discussed.

KEYWORDS
macrophage, periapical lesion, innate immunity, macrophage polarization, oral bacteria

Introduction

Human periapical lesions (PLs) are common infectious diseases that are predominantly induced by endodontic microbial infections, especially by Gram-negative microbes, and affect the tooth and surrounding alveolar bone (1–3). PLs are an osteolytic disease that occurs in the apical region of the root (1). The prevalence of PLs
in the adult population is around 6.3% worldwide; PLs reduce the lifespan and quality of life of those affected (4, 5). The treatment success rate for PLs, including those operated on by endodontic experts or specialists, is only just over 50% after 2 years’ follow-up (4, 5). Thus, despite “optimal treatment”, hundreds of thousands of teeth continue to be affected by PLs, causing symptomatic infection (5, 6). Therefore, it is essential to elucidate the mechanism of development of PLs, and to identify more effective and longer-lasting treatments (2).

Innate immunity is the first line of defence against microbial invaders and plays important roles in the development of the human immune system (7–9). Macrophages are one of the most important innate immune cells and are responsible for the recognition and clearance of exogenous pathogens in microbe-mediated infectious diseases (10–12). Macrophages play a significant role in the response to environmental challenge and in tissue remodeling after injury (9). Autocrine, endocrine, or paracrine cytokines or chemokines from different types of cells modulate the status of macrophages and influence the function and outcomes of macrophages during the progress of infectious diseases (13). In addition, cell–cell interaction through ligand–receptor binding also regulates the metabolic processes of macrophages (14, 15). Consequently, macrophages play diverse roles in regulating the inflammatory response and the reparative process in many diseases (12, 16–18). However, the precise role of macrophages in the development of PLs in humans, and the impact of macrophage dysfunction on the progress of human PLs, remain vague.

This report aims to review the clinical implications of macrophage distribution in PLs from preclinical research and laboratory studies. In addition, we discuss current experimental progress for the treatment of PLs, which might bring us new ideas in the search for new strategies in the treatment of PLs.

The clinical implications of macrophage distribution in periapical legions

Macrophages are one of the most important functional components of the innate immune system (17). They play essential roles in maintaining physiological homeostasis and regulating the turnover of the immune responses in pathological conditions (19, 20). The spatial distribution and the polarization of macrophages might reflect the status of diseases (21, 22). We speculated that the spatial and temporal expression profile of macrophages might indicate the progression of PLs. When periapical tissues are challenged by bacterial invasion, the resident macrophages may respond to foreign material, such as bacteria and the toxins released from bacteria, and regulate the physiological and pathological process (23, 24). In the development of PLs, lipopolysaccharide (LPS) released from bacteria might bind to toll-like receptor 4 (TLR4) to initiate signal transduction and activate immune responses (25). It is believed that macrophages are a group of multifunctional and heterogeneous cells that can be divided into two subsets: M1-like macrophages and M2-like macrophages (26). M1-like macrophages, also known as classically activated macrophages or proinflammatory macrophages, are responsible for mediating and regulating the process of inflammatory responses, with the participation of T helper 1 (Th1) cells and their related cytokines. By contrast, M2-like macrophages usually exhibit anti-inflammatory roles and control the progress of efferocytosis (26–28).

In recent years, multiple groups of researchers have elucidated the distribution phenotype and preliminary function of macrophages in the development of PLs and the roles of macrophages in the different stages of PLs (29–33). Most of the observations of macrophages in PLs were from preclinical studies. Azeredo et al. (34) found that cluster of differentiation 68 (CD68)-positive cells were extensively expressed in radicular cysts (RCs) and periapical granulomas (PGs). However, the number of infiltrating macrophages did not appear to differ between the two types of PL (34). These results could suggest that all macrophages found in RCs and PGs have similar expression characteristics.

Several studies that further explored the relationship between macrophage polarization and the aggressive/recovery phase of apical periodontitis (AP) have shown that the expression of M1-like or M2-like macrophages might be correlated with the pathology of PLs (31, 32). The results of quantitative immunohistochemical (IHC) studies have shown that levels of cluster of differentiation 11c (CD11c)-positive (M1-like) macrophages are higher in RCs, whereas levels of mannose receptor C type 1 (MRC1) (M2-like) macrophages are higher in PGs (31). The higher levels of infiltration of M1-like macrophages in RCs indicate that an aggressive state of RCs could be observed, more proinflammatory cytokines might be secreted to the lesion area, and the activated osteoclasts in the apical region of the affected teeth (33). In addition, a similar expression profile of macrophages was observed in RCs of both primary and permanent teeth (35). These studies suggest that macrophages may exhibit different expression phenotypes at different stages of PL progression. This, in turn, suggests that the expression levels of macrophages could become a detection marker for the classification of PLs, which might help in clinical diagnosis and treatment decision-making. Furthermore, several studies have mentioned a relationships between the subtypes of macrophages and the clinical symptoms of PLs. Veloso et al. (36) evaluated macrophage expression of cluster of differentiation 14 (CD14), 64 (CD64), 80 (CD80), 163 (CD163), and 206 (CD206) in symptomatic apical periodontitis (SAP) and asymptomatic apical periodontitis (AAP) using flow cytometry. The expression profile of the secreted cytokines was determined using...
quantitative polymerase chain reaction (qPCR). The ratio of M1-like to M2-like macrophages was higher in the SAP group than in the AAP group, and macrophages from patients with SAP showed a significant reduction in CD163 expression. The expression of interleukin 6 (IL-6) and 23 (IL-23) was markedly higher in SAP patients than in AAP patients, which indicates that these cytokines might be related to the clinical presentation of PLs (36). In addition, Bracks et al. (37) found that the infiltration of CD68-positive cells was accompanied by increased expression of IL-6. Đzopalić et al. (38) carried out multicolor flow cytometry and further explored the potential relationship between IL-6 expression and the clinical features of PLs. They demonstrated that symptomatic PLs were associated with higher levels of expression of IL-6-positive M2-like macrophages than asymptomatic PLs (38). Elucidation of the relationship between clinical symptoms and the molecular level of specific markers would help us to understand the underlying mechanisms of the presence of pain, and develop specific methods or techniques to detect and resolve the clinical symptoms of PLs.

Although numerous preclinical data have demonstrated that a large number of infiltrating M1-like macrophages might indicate a destructive state of PLs, there is no exact description of how M1-like or M2-like macrophages are distributed in PLs, which needs further biological and biochemical experimentation. In addition, owing to limitations with sample collection during the microsurgery of endodontics and the techniques used in previously mentioned studies, we could not observe the accurate distribution of macrophages and their relationships with the root, inflamed area, and surrounding bones. Therefore, further research should include more comprehensive and valid experiments and provide more solid data to elucidate the precise distribution of different subtypes of macrophages and their relationships with bone resorption area, repaired or regenerative tissues, and other types of immune cells. For example, spatial transcriptomics, single-cell sequencing, and multiple immunechemistry techniques might be applied to investigate the microenvironment of PLs, which might help us to find more specific targets of PLs. These efforts might help us to understand the failure of endodontic treatment and the determining factor in the prognosis or outcomes of PLs.

The impact of bacteria on macrophage polarization

The presence of bacteria and other pathogens is one of the core reasons for the persistence of symptoms and other manifestations of PLs. The emergence of next-generation sequencing for micro-organism detection helped us to understand the distribution of microbial communities of APs. Hou et al. (39) found that the abundance of Porphyromonas gingivalis, Phocaeicola abscessus, Campylobacter showae, and Tannerella forsythia was higher among patients with obvious symptoms than in those without, suggesting that the number and species of microbe might affect the outcome of PLs. Sun et al. (40) found that the dominant bacteria isolated from extraradicular and intraradicular samples from persistent APs were quite different, which suggests that different bacteria play different roles in the pathogenesis of persistent PLs. Considering the fundamental roles of bacteria in the progression of PLs, in this section we summarize the functions of oral bacteria, such as Enterococcus faecalis and P. gingivalis, in the polarization of macrophages (41, 42).

E. faecalis, a Gram-positive bacterium, has been found in many bacterial studies to be closely related to the failure of the treatment of human APs and has a higher detection rate in secondary PLs than that in primary PLs (43, 44). Interestingly, several in vitro studies have found that E. faecalis plays potentially regulatory roles in the differentiation and polarization of macrophages (42, 45, 46). Park et al. (46) found that E. faecalis affected the osteoclastogenesis of macrophages in vitro. In addition, E. faecalis activated the immune responses, promoted proinflammatory cytokine production, and maintained the phagocytic capacity of macrophages. Furthermore, the co-culture of E. faecalis with a human monocyte cell line suggests M2 polarization of infected macrophages, with an increase in the production of reaction oxygen species (ROS) (42). Ran et al. (45) found that the presence of E. faecalis promoted the expression of caspase-1 and nod-like receptor family pyrin domain-containing protein 3 (NLRP3), which are key regulators of pyroptosis. E. faecalis could activate the pyroptosis of macrophages and induce the production and secretion of interleukin 1 beta (IL-1β) (45). These results suggest a link between E. faecalis and APs and endodontic failure.

P. gingivalis has also been recognized as an important oral pathogenic bacterium that plays crucial roles in the development of periodontitis and PLs, as well as several systematic diseases (47). P. gingivalis is thought to have a wider and more complicated effect on the host immune system than E. faecalis. P. gingivalis, its LPS, and its outer membrane vesicles (OMVs) might exert different immune-regulatory roles on macrophage activation (48, 49). P. gingivalis LPS has been reported to weakly activate the polarization of M1- or M2-like macrophages, and to induce the production of inflammatory cytokines and chemotactic cytokines, which might have a great impact on the development and outcome of PLs (50).

Despite multiple studies having preliminarily discussed the potential relationships between the presence or the invasion of different bacteria and macrophage polarization and functions, there is limited evidence demonstrating a connection between the occurrence of bacteria and their pathogenic toxins, the differentiated state of macrophages, and the destruction of...
Strategies for the treatment of periapical lesions

Although root canal therapy (endodontic treatment) is considered to be the most effective treatment for PLs, and has a relatively high success rate, treatment fails in many cases, resulting in refractory inflammation and repeated infections of the affected teeth, accompanied by swelling, pain, and general infection (5). It is essential that we urgently investigate the reasons for persistent infection in alveolar bone tissues and explore new, effective strategies for the treatment of PLs, which should be specific and effective, with few side effects.

From a genetics perspective, several research groups have identified in vitro and/or in vivo experiments molecules that play significant roles in the development of PLs and which might become therapeutic targets for the treatment of PLs in humans. Hypoxia-inducible factor 1 alpha subunit (HIF-1α) is a key molecule in the mediation of oxygen metabolism and homeostasis, and plays important roles in inflammatory reactions and bone reconstruction. Hirai et al. (51) demonstrated that the application of exogenous HIF-1α could protect the progression of APs by inhibiting the secretion of proinflammatory cytokines, attenuating the M1 polarization of macrophages, and down-regulating osteoclastogenic differentiation. In another study, the regulatory role of serum amyloid A (SAA) in the development of PLs was investigated using conditional knockout (KO) mice. In this experimentally induced model of PLs, the number of infiltrating myeloid cells in the periapical region of affected teeth was lower in SAA KO mice than in wild-type (WT) mice. In addition, SAA might regulate the function of macrophages in PLs via toll-like receptor 2 (TLR2) and TLR4, suggesting that SAA might become a regulatory target for the treatment of PLs (52). Similarly, Wang et al. (53) showed that adenovirus-associated virus (AAV)-mediated therapeutic methods can effectively reduce tissue destruction, attenuate the inflammatory reaction, and slow the process of alveolar bone loss. The application of Atp6v1c1 (an AAV specific for C1 silencing) could reduce bone destruction by nearly 70%, decreasing the infiltration of inflammatory cells into the periapical region, and maintain the integrity of periodontal ligament (53). Despite the fact that more data and experiments are needed to ensure the efficiency and safety of this strategy, this study showed that AAV-mediated methods might become an effective strategy for the assistance of endodontic treatment.

As well as uncovering the underlying mechanism of PLs by molecular methods, some research groups have also tried to elucidate the potential roles of immunoregulatory agents or antioxidants in the treatment of PLs, such as melatonin, azithromycin, or metformin (54–56). Saritekin et al. (54) showed that intraperitoneal injection of melatonin could attenuate the progression of PLs, leading to a reduction in inflammatory reactions and a decrease in periapical defects. This indicates that melatonin protects against the development of PLs. The application of azithromycin has also been shown to have a significant beneficial effect on APs, resulting in increased infiltration of neutrophils and M2-like macrophages. These data suggest that azithromycin might become a therapeutic option in the adjunctive treatment of PLs (55). In addition, not only could the general application of drugs help the resolution of PLs, but intracanal administration of some agents could contribute to the recovery of existing periapical lesions. Intracanal metformin could contribute to the healing of APs by suppressing monocyte recruitment and inhibiting inducible nitric oxide synthase (iNOS), which is a representative marker for M1-like macrophages (56). Although these encouraging data raised some potential strategies for the treatment of refractory PLs, more research should focus on elucidating the underlying mechanism of APs and revealing the precise function of the drug or exogenous agents on the healing of PLs. Only in this way can we improve the success rate of endodontic treatment.

Conclusion

With more studies reporting that polarized macrophages are closely correlated with the progressive state of PLs, it is urgent for us to elucidate the precise mechanism of the regulatory roles of macrophages in the development of PLs. As research on the role of macrophages in the pathogenesis of PLs gradually progresses, we must pay more attention to the current limitations and drawbacks in the previously published studies. The predominant subtypes of macrophages at different stages of PLs, the regulatory cytokine networks that macrophages participate in, and the potential therapeutic targets on macrophage regulations need to be settled. In this way, we could understand the pathological mechanisms of PLs better, and we can find more effective strategies to improve the success rate of the treatment of PLs.

Author contributions

HS conceived and designed this review. YS, XL, DH, and HS wrote the first draft of the manuscript and made the literature
review. DH and HS critically revised the manuscript. All authors contributed to this article and approved the final version to be submitted.

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