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Macrophages in Oral Tissues

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The balance between cell removal following tissue damage and new cell formation to facilitate repair has long been linked to the behaviour of inflammatory macrophages and their interactions with tissue-resident non-immune cells. The main aim of the inflammatory response is to modulate the tissue environment by removing unwanted cells and recruiting cells and soluble factors from the bloodstream to help protect the damaged tissue against infective foreign bodies. Such processes are essential for remodeling, repair, and forming new tissue in the area of damage. Macrophages play an important role in tissue repair and regeneration by exerting their effects in various tissue repair and regeneration effects by exerting their effects in various tissue repair and regeneration effects by exerting their marks in multiple ways during these processes. Current research shows that depletion of macrophages is detrimental for skin and muscle repair and whole limb regeneration [1-3]. Moreover, resident macrophages are described as regulators of inflammation levels by 'cloaking' microinjuries and regulating neutrophil recruitment [4-5].

Macrophages have been shown to be plastic cells that can act pro-inflammatory (M1) or anti-inflammatory (M2) [6,7]. M1-like macrophages are typically induced by lipopolysaccharide (LPS) or interferon (IFN-γ); they play a pro-inflammatory role by producing cytokines such as tumour necrosis factor (TNF)-a, interleukin (IL)-1β, and IL-6. They express high levels of inducible nitric oxide synthase (iNOS) that results in the production of nitric oxide (NO), thus initiating bacteria-killing processes [8]. M2-like macrophages play an anti-inflammatory role, and they have a function of promoting cell proliferation, tissue remodelling activities, and angiogenesis. M2 macrophages express genes such as CD206 (mannose receptor), Arg1 (Arginase 1), and Transforming growth factor-beta (TGF-β) [9,10]. The expression of arginase competes with iNOS on their common substrate, L-arginine [10]. By competing with iNOS, Arg1+ macrophages inhibit the differentiation of osteoclasts [11]. Notably, recent evidence shows that M1 macrophages and M2 macrophages markers can be detected together in a same cell, suggesting a hybrid state of macrophages [12-14]. A recent study has suggested a lack of memory of M1/M2 polarization, indicating the flexibility of M1/M2 transition, which brings further attention to future therapeutic studies involving macrophage polarization [15].

Due to the high plasticity of macrophages and their existence in various disease conditions, macrophages have been described as having subtypes according to their function. Research over the past decade suggests that M2 macrophages can be further classified as M2a, M2b, M2c, M2d [9]; however, further understanding of these subtypes *in vivo* is needed. With the development of single-cell RNA sequencing (scRNA-seq), an in-depth understating of the sub-populations and the heterogeneity of macrophages is forthcoming, including but not limited to their gene profiles, their functions, and the communication between cell types.

In this commentary, we will present the latest knowledge of macrophages in the soft tissues of the dental organ, namely, the dental pulp, gingiva, and the periodontal ligament.

Dental Pulp

The dental organ is exposed to the outer environment, rendering it susceptible to bacterial infection leading to

caries and possible trauma. Pathological responses to these stimuli are acute or chronic inflammation of the dental pulp, pain, and tertiary dentine secretion.

The dental pulp is a tissue that includes fibroblast cells, resident stem cells, and macrophages in the healthy pulp [16]. These resident macrophages are responsible for homeostasis of the tissue through the clearance of senescent cells, remodelling of the tissue following inflammation, and activators of the immune response by secreting cytokines and chemokines [17]. However, little has been investigated about their behaviour during dental development and damage.

Our research indicates that macrophages are present in developing teeth' mesenchyme; however, their role in development is not yet clear. Research needs to be done to understand when resident macrophages start populating the dental pulp and their role in odontogenesis [18].

Our recent study investigated the effect of macrophage and neutrophil modulation on reparative dentine secretion [18]. We demonstrated that macrophages are key cells for regulating local dental pulp stem cell activation and inflammatory balance, directly impacting reparative dentinogenesis.

It is generally believed that neutrophils are the first cells to reach tissue damage, subsequently recruiting macrophages for tissue healing modulation. Our research on tooth damage indicated that even in the absence of neutrophils, macrophages were still present in the damage and were capable of modulating repair [18]. Whether these are resident macrophages only or recruited macrophages remains unclear, and further understanding of resident and recruited macrophages profile is needed.

During tissue repair, the transition to M2 macrophage indicates that the tissue is ready for stem cell proliferation and differentiation so that repair or regeneration can take place [16,18-20]. Our results demonstrated that M2 CD206+ macrophages play an important part in accelerating the resolution of dental pulp inflammation after damage. The increase of Wnt receiving stem cells in the dental pulp, TGF-β1 expression, and decrease of apoptosis seen by enhancement of Wnt activity suggests a correlation between stem cells and macrophage function in the dental pulp. We further confirmed this by demonstrating that pharmacological ablation of macrophages leads to a decrease in Wnt responsive cells in the pulp. Modulation of stem cell niche and microenvironment using small molecules could be the next generation of dentine/dental pulp treatment; therefore, clinical application of macrophages knowledge may come in handy to increase the life span of a dental organ.

Gingiva

The gingiva is the oral tissue that surrounds the cervical portion of the teeth; it creates a tight junction to the tooth, serving as the first barrier of defence against bacteria entering the body. Suppose bacteria (in the form of plaque) are not removed from the tooth surface and gingival crevice. In that case, dysbiosis occurs, and tissue inflammation is triggered (gingivitis), leading to invasion of bacteria to the mesenchyme, compromising the integrity of the periodontal tissues.

Maintenance of immunological tolerance and tissue homeostasis at the gingival barrier depends on tightly controlled immune cell networks capable of inducing appropriate responses. Macrophages phagocytic capacity and functional plasticity make them a keystone immune population in health and inflammatory disease. However, the ontogeny, heterogeneity, or function in gingival tissues remain largely unknown. In our recent study using single-cell RNA sequencing of human gingival tissues, we identified two macrophage populations that dynamically changed across conditions [21]. We did not observe a clear distinction between M1/M2 classes as both showed a pro-inflammatory phenotype; in fact, recent single-cell studies have argued against a binary definition of macrophages as these are based on simplistic *in vitro* studies. Nevertheless, macrophage polarisation has been studied in gingival tissues in a histological analysis [22], and no changes were found in health, gingivitis and periodontitis. In our study, macrophage 1 (M1) showed higher expression of complement transcripts and a pro-angiogenic phenotype, and macrophage 2 (M2) is enriched for antigen presentation pathways. Transcription factor analysis revealed that both populations show transcriptional regulators' expression in bone metabolisms, such as NFκB and NFAT signalling.

Interestingly, macrophage 1 showed specific expression of oncostatin M (OSM), which is defined as a stem cell niche maintenance factor in skeletal muscle via paracrine signalling [23], and also identified in a dermal macrophage subset [24]. In the mouse skin, ablation of OSM-producing macrophages resulted in hair growth induction [24]. Altogether, these findings suggest that OSM-producing macrophages function as a signalling hub in maintaining quiescence of other stem cell niches and, importantly, reflect the current understanding that macrophages also have homeostatic roles. In the gingiva, further functional studies are required to confirm the role of these OSM-producing macrophages in tissue homeostasis.

Our data also indicates that macrophages express many periodontitis-associated risk genes suggesting that macrophage dysfunction may be linked to disease severity and susceptibility. These findings may inform the development of therapeutic interventions targeted at specialised macrophage subpopulations.

Periodontal Ligament

The most common disease of periodontal ligament (PDL) is periodontitis, a prevalent disease caused by bacteria invading the epithelial barrier of the gingival junction and triggering inflammation in the mesenchyme [25]. This is the advanced stage of gingivitis described in the previous section.

As described previously, macrophages in the dental pulp promote dentin repair [18]. Our findings show that a tissue-resident macrophage population by the gene CX3C chemokine receptor 1 (CX3CR1) is present in dental pulp and the PDL during their homeostasis, indicating the similarity of pulp macrophages and PDL macrophages (unpublished). However, whether these CX3CR1+ tissue-resident macrophages regulate the process of hard tissue (cementum and bone) formation in periodontium is yet to be confirmed.

Macrophages can recognize bacteria metabolites, namely LPS by toll-like receptor (TLR4), CD14 with the help of LPS-binding protein (LBP) [26], leading to a M1 macrophage polarization. The induction of M2 macrophages is beneficial for hard tissue metabolism, it can prevent bone loss in periodontitis [27]. The imbalance of M1/M2 cells in periodontitis leads to further tissue destruction. In the dental pulp, macrophages depletion experiment proves their ability to promote the proliferation of Wnt receiving stem cells, which is constructing the dentin tissue [18]. Similarly, macrophage depletion using clodronate-liposomes in periodontitis results in a reduction in pathogen and bone resorption in a *P. gingivalis*-induced periodontitis mouse model possible identical role of these macrophages in PDL [28]. However, since clodronate depletes all the macrophages populations, it still remains unclear whether a specific sub-population of the macrophages functions as a key player in regulating stem cells in the periodontal tissues.

Axin2 is a direct readout gene of canonical Wnt activation [29] and Wnt receiving cells are stem cells in the dental pulp [18]. We demonstrated that a Wnt-responsive population, Axin2+ stem cells in the PDL region, contribute to cementoblasts formation in PDL homeostasis [30]. Collectively, macrophages in the dental pulp and PDL share some similarities, and the interplay of macrophages and Wnt-responsive stem cells in the dental pulp is a sign of a complex stem cell niche signalling network, however it is still unknown whether Wnt receiving stem cells in the PDL interact with macrophages. Modulating the crosstalk between stem cells and immune cells open up a new research field for potential therapeutic routes, with many questions waiting to be answered.

Conclusions

Macrophages are highly plastic cells subject to the regulation of their micro-environment. Many questions still remain about the role of macrophages in the dental pulp, gingiva, and PDL, most importantly understanding the roles of macrophages in development and these tissues, their molecular profiles in homeostasis, and the subsequent damage, and the various actions and interactions with other cells.

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