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Review

Oral health and pathology: a macrophage account

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Abstract

Macrophages are present in healthy oral mucosa and their numbers increase dramatically during disease. They can exhibit a diverse range of phenotypes characterised as a functional spectrum from pro-inflammatory to anti-inflammatory (regulatory) subsets. This review illustrates the role of these subsets in the oral inflammatory disease lichen planus, and the immunosuppressive disease oral squamous cell carcinoma (SCC). We conclude that the role of macrophages in driving progression in oral disease identifies them as potential therapeutic targets for a range of oral pathologies.

Keywords: Oral pathology; Immunology; Macrophages

Introduction

The oral cavity is a non-sterile, dirty environment that contains millions of foreign antigens from sources that are harmless (food and commensal micro-organisms), or potentially detrimental (pathogenic micro-organisms) to the host. Host tissues must therefore be segregated from the oral cavity by the oral mucosa, which consists of two layers. Stratified squamous epithelium acts as a barrier to separate the host tissues from the environment of the oral cavity, and the lamina propria lies beneath the epithelial layer and contains a collection of immune cells (macrophages, dendritic cells, B cells, and T cells) that either initiate a response to pathogenic micro-organisms, or induce a state of immune non-responsiveness (tolerance).[1–3] Induction and maintenance of tolerance is essential to avoid inappropriate reactions to food, and to commensal micro-organisms that benefit the host. The choice between immune activation and induction of tolerance depends on the nature of the antigen and the context by which it is presented by antigen-presenting cells to cells of the adaptive immune system (T cells). Two major antigen-presenting cells predominate in the oral mucosa: dendritic cells and macrophages. Dendritic cells can taste antigen in the oral cavity by extending their dendrites between the epithelial cells into the lumen.[4] An immune response is elicited if the antigen-presenting cells present antigen to the T cells in the presence of co-stimulatory molecules (B7-1, B7-2).[5] Conversely, presentation of antigen in the absence of such molecules, or through the ligation of inhibitory receptors expressed on T cells – for example, cytotoxic T-lymphocyte antigen 4 (CTLA-4), can induce a state of tolerance.[6] Unlike dendritic cells that move in and out of the oral mucosa, macrophages remain within it, and can exhibit an array of diverse functions that depend on factors encountered in their microenvironment. Their distinct effector phenotypes can be considered as a spectrum ranging from pro-inflammatory or host defence (M1), to anti-inflammatory or regulatory (M2).[7] The relative balance of macrophage subsets is likely to influence disease; a disruption in favour of M1

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macrophages (inflammation) could exacerbate oral inflammatory disorders, whereas a predominance in M2 subsets (immunosuppression) will favour the progression of cancers. Macrophages increase in number in oral disease, so they could have a key role in progression.[8,9] This review focuses on the role of macrophage subsets in driving inflammatory oral diseases and oral cancers, and specifically investigates their role in oral lichen planus and oral squamous cell carcinoma (SCC).

The macrophage

Macrophages are phagocytic cells, most commonly known for forming the first line of defence against pathogens. They are derived from blood monocytes, which are recruited into the tissues by chemokine signals such as monocyte chemotactic protein-1 (MCP-1).[10] On delivery to target tissues, they differentiate into tissue macrophages, which protect against potential invasion, or fight against an existing infection. Macrophages have an array of important functions; they recognise and kill pathogens, initiate and resolve inflammation, and heal and prime the adaptive immune system. Their function therefore is essential for the survival of the host (Fig. 1). During infection they can engulf pathogens by a process known as phagocytosis and can subsequently kill the pathogen through direct attack by reactive oxygen and nitrogen species, and non-oxidative mechanisms that include exclusion of nutrients, and lowered pH and digestive enzymes such as lysozymes. Macrophages also instruct cells of the adaptive immune system to activate and prime the T cells by presenting fragments of the pathogen (in the presence of co-stimulatory molecules and differentiating cytokines), which initiates a memory response designed specifically for their clearance. Of equal importance is the role macrophages have in halting the immune response after pathogens have been cleared. They produce the anti-inflammatory cytokines interleukin-10 (IL-10) and transforming growth factor-β (TGF-β), which dampen down an inflammatory episode by downregulation of pro-inflammatory cytokines and antigens, and through the induction of regulatory T cells that suppress antigen-presenting cells and T cell effector responses. They also aid tissue regeneration after inflammatory damage through the production of matrix metalloproteinases (MMPs) and their inhibitors, TIMPs (tissue inhibitors of metalloproteinases) that remodel tissue. This diverse array of macrophage effector functions has prompted the classification of distinct subsets along a phenotypic spectrum defined by their effector functions; pro-inflammatory or host defence (M1) to anti-inflammatory or regulatory (M2).[11,12]

M1 macrophages develop in an inflammatory setting; interferon gamma (IFN-γ) with microbial products– for example, lipopolysaccharide, or differentiation factors such as granulocyte macrophage colony-stimulating factor (GM-CSF). They are characterised as IL-12^{HIGH}, IL-23^{HIGH}, and IL-10^{LOW}, and produce high levels of pro-inflammatory cytokines such as tumour necrosis factor-α (TNF-α) and chemokines (IL-8 – neutrophil recruitment, and MCP-1 – monocyte recruitment).[13–15] M1 macrophages have potent antimicrobial potential through the generation of reactive nitrogen species by the induction of inducible nitric oxide synthase and by increased production of reactive oxygen species.[16] Conversely, macrophages exposed to an immunosuppressive or anti-inflammatory environment (IL-4 and IL-13 immune complexes; IL-10 macrophage colony-stimulating factor (M-CSF) or glucocorticoids, or both) adopt an anti-inflammatory or regulatory M2 phenotype.[7,13,17,18] They are predominantly anti-inflammatory through their secretion of anti-inflammatory cytokines such as IL-10, TGF-β, and IL-1 receptor antagonist (IL-1Ra), and are characterised as IL-12^{LOW}, IL-23^{LOW}, and IL-10^{HIGH}.[14] M2 macrophages express lower antimicrobial activity than M1 macrophages, but express higher levels of scavenger receptors such as mannose receptors, which correlates with their role in tissue repair, homeostasis, and clearance of cell debris.[12]

To induce a state of tolerance the balance of macrophage subsets in the lamina propria of healthy oral mucosa is likely to be tipped in favour of an M2 phenotype. As disruption to this balance can lead to an inappropriate or exaggerated response to particular stimuli, macrophages can potentially aid the progression of inflammatory and immunosuppressive oral diseases, and are therefore promising candidates for cell-based therapeutic targets. This review focuses on the role of pro-inflammatory M1 macrophages in driving the inflammatory disease oral lichen planus, and on the role of regulatory M2 macrophages in favouring progression in oral SCC (Fig. 2).

Oral lichen planus

M1 macrophages can exacerbate chronic oral inflammatory diseases such as oral lichen planus, which presents clinically as white striations, with papules or plaques, or both, that principally involve the buccal mucosa, tongue, and gingiva.[19] Histologically, it is characterised by a dense subepithelial lymphocytic infiltrate, with disruption of the basal membrane,[20] and is predominantly mediated by T cells. The interplay between macrophages and T cells emphasises the importance of macrophages in the progression of the disease. Infiltrating monocytes recruited into the lesion develop a pro-inflammatory M1 phenotype because of the high levels of GM-CSF, TNF-α, and IFN-γ produced at the site.[21,22] M1 macrophages can aid progression by three main mechanisms: initiation of inflammation, activation and priming of T cells, and direct destruction of the basal membrane. They can exacerbate inflammation through the production of pro-inflammatory cytokines (TNF-α, and IL-1β), which can upregulate cell adhesion molecules on endothelial and keratinocyte surfaces and induce chemokine expression (RANTES (regulated upon activation, normal T

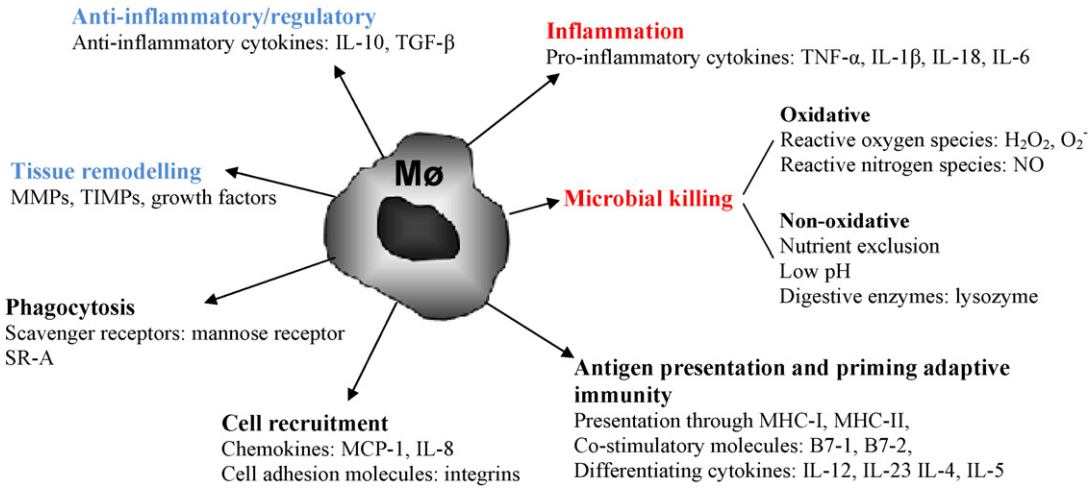

Fig. 1. Macrophages are functionally diverse, and mediate several important processes. Depending on activation and differentiation factors encountered in their microenvironment, they will express a pro-inflammatory phenotype (red), which has a predominant role in host defence (phagocytosis, microbial killing, inflammation, cell recruitment, antigen presentation, and priming of T cells), or an anti-inflammatory or regulatory phenotype (blue), which has a chief role in regulatory or tissue reparative mechanisms involved in homeostasis (phagocytose cellular debris, anti-inflammatory cytokine secretion, tissue repair, cell recruitment, and antigen presentation to induce tolerance).

expressed and secreted) for T cells; MCP-1 for monocytes) by oral keratinocytes, which results in increased recruitment of inflammatory cells into the lesion.[21,23] At the site macrophages can activate antigen-specific T cells (antigen unknown in oral lichen planus) and influence the polarisation of T cells through the secretion of differentiation cytokines (IL-12 → Th1 or IL-4, IL-5 → Th2).[24] T cells in the disease have been found to secrete IFN-γ,[25] which is typical of Th1 subsets, and is indicative of IL-12 production by the macrophages in oral lichen planus. IFN-γ and IL-2 are cytokines produced by activated Th1 cells, and they function to permit the full activation of $CD8^+$ cytotoxic T cells, which are hypothesised to kill basal keratinocytes.[26] IFN-γ can also feed back and activate the M1 macrophages to produce TNF-α which can directly initiate basal keratinocyte apoptosis, and indirectly increase the rate of destruction of the basal membrane through the upregulation of MMP-9 from lesional T cells.[27] MMP-9 cleaves type IV collagen causing the membrane to be destroyed and the subsequent loss of attachment of basal keratinocytes, which potentially results in keratinocyte apoptosis and further damage.[19] Macrophages are distributed close to the damaged basal layer and can therefore contribute

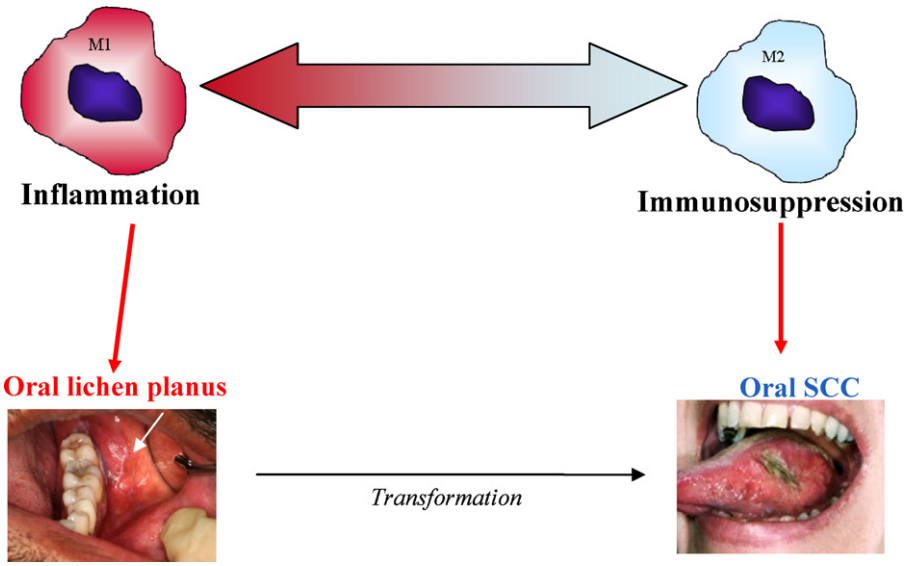

Fig. 2. The role of macrophages in oral disease. Macrophages display a diverse array of functions represented as a phenotypic spectrum from pro-inflammatory M1 to anti-inflammatory or regulatory M2 subsets. They can be activated to give an exaggerated or inappropriate response that can perpetuate oral disease. M1 macrophages can aid the progression of oral lichen planus and potentially induce malignant transformation. Conversely, M2 macrophages aid immunosuppressive disease such as oral squamous cell carcinoma (SCC).

to the destruction of the basal membrane.[28] There seems to be a vicious cycle of perpetuating inflammation and damage to the basal membrane, as this destruction can further initiate inflammation through the release of danger-associated molecular patterns (DAMPs).[29] M1 macrophages can therefore aid the progression of lichen planus by activating T cells and exacerbating inflammation at the site.

Malignant transformation

A link between inflammation and cancer has been known for decades. As a chronic inflammatory disease, lichen planus has been defined as a premalignant lesion by the World Health Organization, although there is controversy regarding the rate of transformation.[30] M1 macrophages have the ability to aid initiation of the transformation process, and M1 cells produce reactive oxygen and nitrogen species (superoxide, hydrogen peroxide). Although this is beneficial in the short term, these reactive species have mutagenic capabilities because of their cytotoxicity to many pathogens, and in chronic inflammation (as in lichen planus) they can potentially cause the disease to progress and epithelial cells to transform. Reactive oxygen species can directly oxidise DNA, while reactive nitrogen species can cause nitration and deamination reactions of DNA bases that lead to changes in the DNA, and increase the rate of mutation.[31] Pro-inflammatory cytokines (TNF-α, and IL-1β) produced by M1 macrophages can also activate specific signal transduction pathways that can affect the expression of genes that control cellular processes such as proliferation and apoptosis.[32] Nuclear factor κB (NFκB) is a major transcription factor that is activated and is a link between inflammation and cancer.[33] It can promote oncogenic cell transformation by upregulating the expression of anti-apoptotic proteins (X-linked inhibitor of apoptosis protein (XIAP), and TNFR-associated factor 1 (TRAF-1), which increases cell survival), cell cycle mediators (cyclin D, which increases proliferation), and angiogenic factors (vascular endothelial growth factor (VEGF), which forms new blood vessels to supply additional nutrients), so tipping the balance in favour of cell proliferation and survival.[34] The M1 macrophage phenotype can therefore aid the malignant transformation of cells in chronic inflammatory conditions such as lichen planus, and result in oral cancer.

Oral squamous cell carcinoma

Oral SCC presents a serious public health issue and constitutes over 90% of all malignancies of the oral cavity, and holds the eighth position in the ranking of cancer incidence worldwide.[35] Leukocyte infiltration (monocytes, neutrophils, eosinophils, lymphocytes) is a characteristic of oral SCC and, from knowledge of other solid tumours, can account for around half of the tumour mass.[8,36–38] Macrophages constitute a substantial portion of these leukocytes and are termed tumour-associated macrophages (TAMs). Monocytes are recruited to the tumour site by monocyte chemotactic factors such as MCP-1, which is produced by head and neck SCCs and their associated TAMs; the level of MCP-1 correlates with the amount of infiltration by the TAMs.[39] On migration into the tissues the monocytes differentiate into TAMs in response to tumour-derived factors. The phenotype of TAMs varies with the status of tumour development; M1 macrophages may aid initiation, whereas M2 cells, dictated by the ability of cancer cells to secrete M2 priming factors such as M-CSF and IL-10, can aid progression in the advanced stages.[37,40]

Numerous studies over the past few decades have set out to understand how TAMs can aid cancer progression. Mantovani et al. showed that they have the potential to carry out antitumour responses in vitro, but in the tumour they execute progression over resolution.[41] These findings indicate that interaction between tumour cells and TAMs can dictate macrophage function, and they emphasise the functional plasticity of the macrophage. This idea can be supported by observing differences in macrophage function with respect to location in the tumour cells. TAMs in head and neck SCC are present in the supporting connective tissue known as the tumour stroma, and in between the malignant epithelial cells. The macrophages in immediate proximity to the malignant cells have been shown to possess fewer lysosomes and to have a defective phagosome-lysosomal apparatus (required to present tumour antigens to T cells) compared with those in the tumour stroma. The tumour cells therefore communicate with the TAMs to suppress their ability to present tumour antigens, evading recognition by T cells and enabling survival of the tumour cells. This shows that the interaction between tumour cells and TAMs is important with regards to TAM function.[42]

There is a dynamic interaction between oral SCC and TAM-derived factors that enable survival, growth, and invasion of tumour. TAMs can promote growth and survival of a tumour through their production of growth factors that include epidermal growth factor (EGF), fibroblast growth factor-1 (FGF-1), and platelet-derived growth factor-1 (PDGF-1), which can sustain activation of NFκB in tumour cells.[43] Angiogenic factors such as VEGF and chemotactic factors such as IL-8 are produced by the TAMs and oral SCC, and act on vascular endothelial cells to induce formation of new blood vessels to enable sustained growth and potential metastasis of the tumour cells.[44] IL-8 can also induce the production of MMPs which break down the extracellular matrix to aid invasion of the tumour.[45,46] In addition to assisting with progression in oral SCC, TAMs can be used by the tumour cells to suppress antitumour responses. Oral SCC and TAMs can produce immunosuppressive cytokines such as IL-10,[47] which can suppress the adaptive immune responses needed for efficient destruction of the tumour cell by inhibiting production

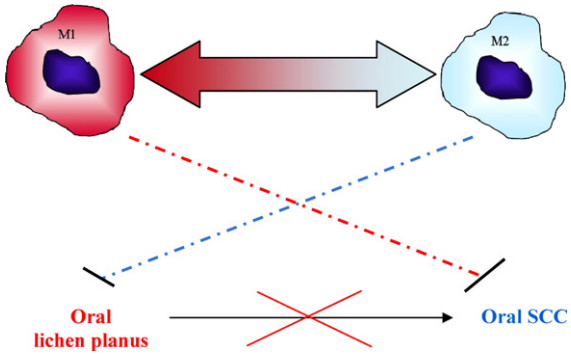

Fig. 3. Macrophages as potential therapeutic targets. M2 macrophages have the regulatory capacity to dampen down (– · –|) chronic inflammation in oral lichen planus and prevent potential malignant transformation, and M1 macrophages can create the antitumoural environment required to resolve (– · –|) oral squamous cell carcinoma (SCC).

of pro-inflammatory cytokines such as TNF-α, IL-1β, and IL-12 (inhibits the cell-mediated response required to kill the transformed cells) by downregulating major histocompatibility complex (MHC) I and II molecules (suppresses presentation of tumour antigen, so avoiding recognition), and by upregulating inhibitory co-stimulatory molecules such as B7-H4, which prevent the activation of adaptive immune cells that are required for an efficient antitumour response.[48–51] In addition, IL-10 and TGF-β can induce the formation of regulatory T cells, which further inhibit the adaptive antitumour response at the tumour site.[52] The presence of TAMs is therefore very important for progression in oral SCC, and their importance in this type of cancer has been shown by the relation between the number of TAMs and five-year survival; a high density results in a poor prognosis.[8,39]

Macrophages as future therapeutic targets

Macrophages clearly have an important role in driving inflammatory disease and cancer in the oral cavity, and the diverse spectrum of activities they display in response to particular factors in their microenvironment suggest that they could be potential therapeutic targets for a number of these conditions. If the macrophage phenotype could be manipulated from M1 to M2 and *vice versa*, there could be the potential to correct a range of oral inflammatory disorders such as lichen planus, recurrent aphthous stomatitis, and leukoplakia, and enable resolution of oral cancers such as SCC (Fig. 3). This could be achieved by the manipulation of macrophage plasticity, activation states, tolerance, or apoptosis, and a few studies have started investigations using other cancer models with some promising results. Targeting macrophages in the context of oral disease is therefore a prospective area for future research and development in cell-based therapeutic interventions that are aimed at redirecting macrophage effector functions, and could benefit patients with inflammatory disease and cancer.[53]

Conflict of interest

There are no conflicts of interest.

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