Innovations

Macrophages: polarisation, regulation and role in periodontal inflammation-a mini review

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Received: 17 April 2022 **Accepted:** 18 May 2022 **Published:** 15 June 2022

Abstract:

Periodontitis is a multifactorial chronic inflammatory condition of the supporting tissues of the periodontium with progressive attachment loss and bone destruction.The pathogenesis involves a complex interplay between periodontal pathogens and the host immune-inflammatory responses.Macrophages are immune cells involved in the detection, phagocytosis and destruction of bacteria and other harmful organisms.Various stimuli instigate distinct functional characteristics inducing an M1 (or classic) and M2 (or alternative) phenotype, which mirror the Th1/Th2 dichotomy.Macrophage polarization plays a prime role in maintaining periodontal homeostasis. Knowledge on the regulation and homeostasis between various components of the immune system and their influence on periodontal health/ diseaseis essential to understand the complex nature of periodontitis and for therapeutic management.This review discusses in detail the distinct subsets of macrophages, their role in maintaining periodontal health / disease and the role of microRNAs in macrophage polarization. Additionally, details on the future perspective of therapeutic modalities to abate inflammation associated with macrophage polarization and the various areas of research have been reviewed.

Keywords: *1.Macrophage polarization, 2.Periodontitis, 3.microRNA, 4.Periodontal inflammation.*

Introduction:

Periodontitis is a multifactorial chronic inflammatory condition of the supporting tissues of the teeth with progressive attachment loss and bone destruction.[1] The pathogenesis involves a complex interplay between periodontal pathogens and the host immunity, under the influence of environmental and genetic factors. Regulation of immune-inflammatory mechanisms governs patient susceptibility to this disease .[2]

Macrophages are cells involved in the detection, phagocytosis and destruction of bacteria and other harmful organisms. Further, they are involved in antigen presentation to T cells and initiate inflammation by releasing

cytokines that activate other cells. Macrophages recognise pathogens via Toll-like receptors (TLRs) that bind specifically to different pathogen components and undergo diverse forms of activation in response to cytokine and microbial signals. Various stimuli instigate distinct functional characteristics inducing an M1 (or classic) and M2 (or alternative) phenotype, which mirror the Th1/Th2 dichotomy.[3,4,5]M1-polarized macrophages mediate resistance to intracellular pathogens, tissue destruction, and antitumor resistance.[6]In contrast, M2-polarized cells are generally oriented to tissue remodelling and repair, resistance to parasites, immunoregulation, and tumour promotion.[7]Macrophage polarization has been demonstrated to play a prime role in maintaining periodontal homeostasis . Hence the knowledge of how the regulation and homeostasis between various components of the immune system influences the periodontal health/ diseaseis significant for understanding the complex nature of periodontitis.

This paper discusses in detail the distinct subsets of macrophages, their role in maintaining periodontal health / disease and the role of microRNAs in macrophage polarisation. Additionally, details on the future perspective of therapeutic modalities to abate inflammation associated with macrophage polarization and the various areas of research have been reviewed .

Macrophage biology:

The term macrophage is derived from a Greek word meaning “big eaters”.[8] Macrophages exhibit a unique feature of phenotypic heterogeneity. This heterogeneity corresponds to its specific function in a specific environment. This is referred to as **“polarization”.**[9]Colony forming unit-granulocyte, monocyte (GM-CFU) is the precursor for monoblasts and myeloblasts.Monoblast gives rise to monocytes, myeloid dendritic cells and osteoclast. These are distinct and act as irreversibly differentiated sublineages. The growth and differentiation of macrophages depends on lineage-determining cytokines, such as macrophage colony- stimulating factors (M-CSF) and interactions with stroma in haematopoietic organs. Once distributed to the bloodstream, monocytes enter all the tissues of the body and this cell migration is controlled by adhesion molecules such as integrins, Ig-superfamily molecules (such as CD31),selectins and epidermal growth factor -7-transaminase spanning type receptors(EGF-M7).

The surface and secretory products of adjacent cells, extracellular matrix and bacterial end products act as signals that are responsible for tissue-specific phenotypes of macrophages. Cytokines, chemokines and growth factors which are often bound to local proteoglycans influence the expression of macrophage genes. Tissue resident macrophages do not proliferate but there is an active mRNA and protein synthesis occurring within them. These undergo local activation and enhance the recruitment of monocytes and precursors from bone marrow pools resulting in the accumulation of more macrophages with enhanced turnover and an altered phenotype.[9]

Monocytes and macrophages migrate to the sites of inflammation or injury to eliminate the primary inflammatory signals and bring about wound healing and tissue repair.[10,11] This process is mainly initiated by pathogen-associated molecular patterns (PAMPs) and damage-associated molecular patterns (DAMPs). In addition, activation of tissue-resident memory T cells by antigens can trigger the recruitment of macrophages via secretion of various inflammatory cytokines and chemokines.[12]Chemokines are directly involved in monocyte migration and activation through the endothelium.[13] Monocyte chemoattractant protein-1 (MCP-1), is a potent chemoattractant factor for monocytes, involved in the initiation of inflammation.[14,15] It triggers chemotaxis and migration of monocytes by interacting with the membrane CC chemokine receptor 2 (CCR2) on monocytes. Along with IL-8 or CXC ligand-8(CXCL-8), MCP-1 has been shown to trigger the firm adhesion of monocytes to vascular endothelium under blood flow conditions.[11-14]Cytokines produced by macrophages, like TNF-α and IL-1β induce endothelial expression of adhesion molecules ligands that mediate influx of monocytes into the tissues.

Macrophages types and classification:

Macrophages are classified into 3 groups[16]-Naïve macrophages (which differentiates as M1 and M2), M1 (pro-inflammatory) and M2(anti-inflammatory) macrophages.

Naïve macrophages (Mϕ):

Naïve macrophage and monocytes are widely distributed throughout the body via the blood circulation.[17]Mϕ is present in various organs and tissues and are given different name at different sites.eg microglial cells in brain; Kupffer cells in liver etc.[18] They are involved in clearing of senescent/apoptotic cells via phagocytosis, and participate in wound healing and tissue repair. The local cytokines in the specific microenvironment induce differentiation of these Mϕ to M1 & M2 phenotypes. M1 acts as a proinflammatory which mediates the production of proinflammatory cytokines ,while M2 is anti-inflammatory cytokine mediator that ingest pathogens and paves way for resolution of inflammation.

Classically activated macrophages (M1):

They are activated by toll-like receptor (TLR) ligands such as LPS or interferon-γ(IFN-γ).[19]IFN γ is produced by Th1 cells, CD8+ lymphocytes, Natural killer cells, professional Antigen presenting cells and B cells. Macrophage can be activated by PAMP, such as LPS, through Pattern Recognition Receptors (PRR), such as TLRs and Nucleotide Oligomerization Domain (NOD) receptors. This activation is referred to as innate activation and is characterized by release of proinflammatory cytokines, such as IL-1β, IL-6 and TNFα, and higher expression of costimulatory molecules.

Innate activation usually fails to fully develop into an M1 profile as typical TLR ligation, induces expression of low levels of p40 and hence it is insufficient to trigger production of the IL-12. M1 macrophages are characterised by high antigen presentation and expression of pro-inflammatory cytokines such as IL-12,IL-23 and TNF-α [20] that are associated with inflammatory, microbial and tumoricidal activities.[21]

Functionally, M1 cells are characterised by enhanced endocytic functions and enhanced ability to degrade intracellular pathogens.[22] This increased microbial activity is mediated by restriction of iron and other micronutrients for microbes , acidification of phagosomes, synthesis of reactive oxygen species and release of nitric oxide from L-arginine by iNOS activity. [23]

Alternatively activated macrophages (M2):

M2 cells play a key role in the resolution and tissue repair phase. The Type 2 helper T cells (Th2), mast cells and basophils secrete IL-4 and IL-13 which induces alternate activation of macrophages[24]. These cytokines upregulate the expression of mannose receptor and MHC class-II molecules on the macrophages inducing endocytosis and antigen presentation and also consequently inducing the expression of selective chemokines such as CCL2,CCL-17 and upregulation of intracellular enzymes like arginase that are implicated in cell recruitment and granuloma formation.[25]

These alternatively activated M2 macrophages can be further subdivided into M2a, M2b, M2c, and M2d. IL-4/IL-13 and down regulation of jumonji domain-containing-3 (Jmjd3) and interferon regulatory factor (IRF)-4 induce M2a or alternatively activated macrophages, while M2b or type 2 activated macrophages macrophages are induced by immunoglobulin complexes with TLR agonists, [24,25] M2c macrophages induction is by IL-10, transforming growth factor β (TGF-β), or glucocorticoids and the M2d phenotype (till now described only in mice) is induced by adenosine in pro-inflammatory M1 macrophages via activation of the adenosine 2A receptor (A2AR). M2 cells synthesise polyamine and proline that stimulate cell growth, collagen formation, and tissue repair. [26]

M2a macrophages express high levels of the chemokines CCL13/MCP-4, CCL8/MCP-2, and CCL26/eotaxin-3, which coordinate the recruitment of eosinophils, basophils and some polarized Th2 cells, through the activity on CCR3 and are involved in proangiogenic networks. M2b cells efficiently sustain antibody production, the majority of which are of the IgG1 isotype, consistently with a type II IgG class switch. [27]M2b are also clearly distinct form M2a cells in terms of the expression of the sphingosine kinase 1 (SPHK1) enzyme, which catalyzes the production of sphingosine-1 phosphate from sphingosine.[28]SPHK1 is also highly expressed in M1 cells but downregulated in M2a cells. [29] M2c are usually regarded as deactivated macrophages in that their common hallmark is the down regulation of proinflammatory cytokines, the increased debris scavenging activity, and the carryover of a prohealing functional program. [30]

Depending on the activation stimulus M2 diversifies into various subsets. [31,12]

Macrophage plasticity and polarization:

Macrophages undergo phenotype switching referred to as "macrophage polarization", a process by which they convert to one specific phenotype and function according to the stimuli and signalling specific to that tissue microenvironment. [32-36] In 1986, Mosmann, Coffman and collaborators reported that murine T helper (Th) lymphocytes could be divided into Th1 and Th2 cells, based on their corresponding cytokine production profile. They further demonstrated that cytokines carry cross-regulatory properties such that they coordinate two fundamentally opposite immune responses called type 1 and 2 immune responses. Macrophages that secrete IL-2 and IL-10 are of Th-1 type and those which secrete IL-4 and IL-13 comprises the Th-2 type.[35,36] In 2002 the extended classification proposed that M1 polarization is classical activation, that involves stimulation by pathogen-derived LPS alone or in combination with IFNγ. While M2 polarization is by alternative pathway linked to anti parasite and tissue repair programming.[37]

M1 macrophages produce and secrete higher levels of pro-inflammatory cytokines TNF-α, IL-1, IL-1β, IL-6, IL-12, IL-23 and low levels of IL-10. Functionally they participate in the removal of pathogens during infections through activation of NADPH oxidase systems and subsequent generation of ROS. Therefore M1 macrophages have robust anti-microbial and anti-tumoricidal activity and mediate ROS induced tissue damage and impair tissue regeneration and wound healing.[36-38],[13]To protect against such tissue damage ,the chronic inflammatory response is mediated by anti-inflammatory actions of M2 polarized by IL-10,IL-4,IL-13 via activation of STAT6 through IL-4Rα. The polarization towards M2 is mediated also by decreased levels of IL-12 and increased levels of IL-10 and TGF-β. Functionally M2 have potent phagocytic capacity, scavenging debris and apoptotic cells ,promote tissue repair and wound healing and possess pro-angiogenic and pro-fibrotic properties. Therefore M2 cells take part in dampening of inflammation, orchestrate the promotion of tissue remodelling, angiogenesis, immune regulation, tumor formation and progression. [39-44]The preferential production of IL-12 or IL-10 set the basis for the M1/M2 polarization paradigm in response to the factors that dominate the inflammatory scene.[45]

Macrophage activation can be either proinflammatory or anti-inflammatory, contributing to tissue destruction or regeneration and wound healing. Switching between M1 and M2 phenotype is based on the specific microenvironment, and many key transcription factors are involved in macrophage polarization, like signal transducer and activator of transcription (STATs), interferon-regulatory factor (IRFs), nuclear factor (NF)-κB, activator protein (AP) 1, peroxisome proliferator- activated receptor (PPAR)-γ and cAMP-responsive element-binding protein (CREB), which interact with each other and regulate macrophages to a specific phenotype in the various inflammatory diseases. When tissues are challenged by pathogens, inflammatory monocytes in circulation are recruited and differentiate into macrophages, which keep a homeostatic status with the resident macrophages in the affected tissues.

Generally, macrophages are deliberated to be polarized toward an M1 phenotype in the early stage of bacterial infection. PAMPs in the bacteria are recognized by pathogen recognition receptors (PRRs) on the macrophages, with subsequent activation and production of a large amount of pro-inflammatory mediators including TNF-α, IL-1, and NO which kill the invading organisms and further activate the adaptive immunity. If macrophage-mediated inflammatory responses are not efficiently controlled, a cytokine storm is formed thereby contributing to the pathogenesis of disease. In order to counteract the excessive inflammatory responses, macrophages undergo apoptosis or polarize to M2 phenotype to protect the host from excessive injury and facilitate wound healing. If the infection persists i.e chronic state, the host enters a LPS-tolerant state, and the macrophages are polarized to M2 phenotype.

Signalling pathways in macrophage polarization:

Switching between M1 and M2 phenotype is based on the specific microenvironment, and many key transcription factors are involved in macrophage polarization, like signal transducer and activator of transcription (STATs), interferon-regulatory factor (IRFs), nuclear factor (NF)-κB, activator protein (AP) 1, peroxisome proliferator-

activated receptor (PPAR)-γ and cAMP-responsive element-binding protein (CREB), which interact with each other and regulate macrophages to a specific phenotype in the various inflammatory diseases.[46]

Several mechanisms are involved in macrophage polarization and there occurs a feedback regulation between M1 and M2 signal pathways. The driver stimuli include IL-4, IL-10, glucocorticoids with TGF-β, glucocorticoids alone, LPS, LPS and IFN-γ, and IFN-γ alone. Different signalling pathways direct the macrophages differentially to act as two separate strains. They include the activation of STAT1 mediated by IFN-γ receptor, increase in IRF5, NF-κB, as well as AP1 expression mediated by TLR4, enhanced AP1 expression mediated by cytokine receptor, activation of STAT6 and increased IRF4 mediated by IL-4 receptor, increased level of PPARγ mediated by fatty acid receptor, and enhanced expression in CREB by TLR4. The feedback regulation between M1 and M2 are implemented by STAT1-STAT6, IRF5-IRF4, NF-κB-PPARγ, AP1-CREB, and AP1-PPARγ, and they play essential roles in the initiation, development, and cessation of inflammatory diseases.[46]

Mirnas in macrophage differentiation and polarisation:

MicroRNAs (miRNAs) are small single-stranded, evolutionary conserved, non-coding RNA molecules containing 18-24 nucleotides[47]. miRNAs induce gene silencing by modulating gene regulation at the post-transcriptional level through binding to the 3'-untranslated region (3'UTR) of target mRNA specially in immune cells including monocytes and macrophages[47]. They play important roles in many aspects of macrophages biology and thereby affect many biological and pathological conditions, like monocyte differentiation and development, macrophage polarization, infection, inflammatory activation, cell survival and proliferation, and phagocytosis[47,48].

Differentiation of monocytes to macrophages is inhibited by miRNA-24, miRNA-30b, miRNA-142-3p, and miRNA-199a-5p. Several miRNAs have been revealed to be highly expressed in polarized macrophages. miR-21 has been shown to play both pro- and anti-inflammatory roles, its anti-inflammatory roles are more prominent. MiR-155 and miR-142-3p inhibit macrophage proliferation compared to let-7a.miR-155 has both pro- and anti-apoptotic roles, whereas miR-21 and let-7e negatively regulate macrophage apoptosis [47-49].

miRNAs regulating m1 polarization:

miR-9, miR-127, miR-155 and miR-125b have been shown to promote classical activation of macrophages (M1) and pro-inflammatory responses.

miRNA-9 : Increased expression of miR-9 suppresses PPARδ activity, thereby preventing the Bcl-6-mediated anti-inflammatory effects. Thus, miR-9 may act to keep macrophages in the M1 polarized state.[50]

miR-127 : Inhibition of miR-127 suppressed the expression of M1 signature genes and promoted transcription of M2 marker genes . Elevated miR-127 inhibited the expression of Bcl6, which in turn suppressed the phosphatase Dusp1 which led to increased phosphorylation of JNK, which promoted the inflammatory response and M1 phenotype.[51]

Mir-155: Stimulation of macrophages with TLR2, TLR3, TLR4 and TLR9 ligands remarkably upregulated the expression of miR-155. It promoted M1 polarisation by activating NF-κBsignaling, induction of the JNK pathway or by inhibiting STAT6 activation.[52]

miR-125b: Positively regulates M1-phenotype in macrophages . Over expression of miR-125b in macrophages was found to enhance responses of macrophages to M1 inducer IFN-γ through targeting of IRF4. Thus it enhances M1 activation and pro-inflammatory responses in macrophages.[52]

miRNAs regulating m2 polarization:

miR-124, miR-223, miR-34a, let-7c, miR-132, miR-146a and miR-125a-5p have been identified thus far to promote anti-inflammatory responses and M2 polarization in macrophages.

miRNA-124 : Promotes cholinergic agonist-elicited anti-inflammatory effect, also it targets STAT3 and TNF-α converting enzyme (TACE) to reduce IL-6 and TNF-α release, respectively thus suppressed the expression of M1 markers (i.e., CD86, iNOS, TNF) [53]

miRNA-223 : Highly expressed in myeloid cells of the bone marrow and over expression in RAW 264.7 macrophages inhibited LPS-stimulated release of IL-6 and IL-1β by targeting STAT3.[54]

miRNA-34a: It blocked pro-inflammatory responses in LPS-stimulated macrophages and the levels were reduced in LPS-treated RAW 264.7 macrophages. Transfection of miR-34a mimics diminished proinflammatory responses, evidenced by lower levels of M1 cytokines TNF-α and IL-6. Mechanistically, miR-34a targets Notch1, essential for LPS-mediated production of proinflammatory cytokines in macrophages.[55,56]

Let-7c : Promotes M2 phenotype by targeting p21-activated kinase 1 (PAK1), a serine/threonine kinase that is upregulated in M1 polarized macrophages. let-7c is highly expressed in M2 macrophages compared to M1 macrophages and overexpression of let-7c reduces the of M1 genes (i.e., IL-12 and iNOS), and increases the levels of M2 marker (i.e., FR-β) in M1 macrophages via targeting C/EBP-δ.[57]

miR-146a: Transfection of peritoneal macrophages with miR-146a reduced the expression of M1- associated proteins (i.e., iNOS), and increased the expression of M2-associated genes after LPS treatment. It possibly targets NF-κBsignaling mediators such as IRAK1 and TRAF6, thereby preventing pro-inflammatory responses .[58]

miR-125a-5p: Kruppel-like factor 4 (KLF4) is targeted thereby inhibiting M1 polarization and promoting M2 polarization in bone marrow macrophages.[59]

Role of macrophage polarization in periodontitis:

Macrophage polarisation is not permanent; rather, various triggers such as pathogens, injured, dying, and dead cells, and the tissue microenvironment stimulate macrophage plasticity. Regulation of these cells is crucial because these cells are involved in the removal of pathogens and debris, biodegradation, tissue regeneration and vascularisation, and extracellular matrix reorganisation after tissue damage[60], they can tip the balance between chronic inflammation and wound healing, either directly or by modulating the function of other cell types.[61-63]

Mossmann and Coffman first reported on the variability within the helper T-cell compartment in 1989. They compared the functioning and production of lymphokines by two types of cloned helper T cells (Th) and coined the terms Th1 and Th2.[5] Consequently, Abramson and colleagues proposed that inflammation primarily initiated by Th2 cells, may transform macrophages into a unique activation state, as opposed to IFN-induced activation, which suppressed respiratory burst and enhanced MHC-II expression.[6]The concept of alternatively activated macrophages (AAM, also known as M2) was first reported in 1992, with the finding of up-regulated macrophage mannose receptor (MRC1) as a chosen marker of IL-4/IL-13-activated macrophages, which included increased expression of MHC-II.[7] Mosser and Edwards further evaluated the entire spectrum of macrophage activation and acknowledged that M1 and M2 were two terminals of the spectrum.[8]

Recent studies have demonstrated various phenotypic indicators to investigate the alterations in macrophages in periodontitis in vivo and in vitro. Yu et al.,2016 discovered that mice periodontal gingiva had a higher M1/M2 ratio than bone marrow-derived macrophages, indicating a phenotypic flip from M2 to M1 in periodontitis. Using flow cytometry Lam et al demonstrated elevated M1/M2 ratio in gingiva with periodontitis. Similar findings were reported by J. Yang et al. Influence of p.gingivalis in altering the programming of the M1 macrophage resulting in a hyperinflammatory environment and minimizing the ability for T cell immunomodulatory influx into the lesions was described by Huang et al.,whose results were similar to the study by Yubo Hou.et al. The authors indicated that gingipain promotes M1 macrophage polarization in order to benefit P. gingivalis infection through the C5a pathway.Zhou et al in 2018, correlated M1/M2 ratio with levels of IFN-**γ**,IL-6 and clinical parameters. They observed that the M1/M2 ratio was positively correlated with clinical probing depth (PD), and both positively

correlated with IFN-γ and IL-6 levels. Further PD was negatively correlated with IL-4.These results were in agreement with Ting Yu et al, they demonstrated a 14- fold increase in M1 cells and four-fold more M2 cells with an enhanced M1/M2 ratio in the periodontium of periodontitis patients.M1-type markers were significantly upregulated at the mRNA level, whereas M2-type markers were down regulated at both the mRNA and protein levels in BMMs after LPS stimulation. These studies reveal that Periodontal inflammation is associated with enhanced M1 and M2 phenotypes in which a phenotypic switch of M2 to M1 might be a critical mechanism in mediating periodontal tissue damage, including alveolar bone loss.[64]

Changes in macrophage differentiation, polarisation, re-polarization, and activation within the local environment play a critical role in the pathogenesis of many autoimmune and inflammatory illnesses. The aetiology of various inflammatory disorders, including periodontitis, atherosclerosis, and obesity[65,66], is linked to an imbalance in the M1/M2 phenotype. The mechanisms that control macrophage deactivation are critical for maintaining homeostasis and controlling the immunological response. The functional phenotype of macrophages can be influenced by both innate and adaptive signals, which can have potentially harmful repercussions if not properly managed.

Future scope in diagnostics and therapeutic strategies:

As discussed previously, macrophages exhibit plasticity in cellular functions during various diseases and pathological processes, making them ideal but difficult targets for therapeutic interventions focusing on cellular activities and signalling cascades relevant to macrophage polarisation.

Some existing chemicals, such as lupeol, celetrol, emodin, salidroside, and others, have garnered considerable attention since studies have shown that they have a lot of regulatory effects on macrophage polarisation. The precise mechanisms, signalling routes, and target genes involved in its pharmacological activities, however, remain unknown.

Broussonin E, a completely unique phenolic compound derived from the barks of Broussonetiakazinoki, enhanced M2 macrophage polarisation by reducing phosphorylation of ERK and p38 MAPK109 in an animal study conducted by Shao-Peng Huang et al.[61] Furthermore, it promotes M2 macrophage polarisation by enhancing the JAK2-STAT3 signalling pathway. However, there are still some differences in the therapeutic activities of those chemicals that have yet to be discovered. Clearly, increased in-depth analysis of macrophage polarisation and related therapeutic chemicals is required in order to identify the most straightforward ways for focusing on macrophages for disease treatment.

Nonetheless, techniques that induce tissue macrophages to re-polarize from proinflammatory M1 to anti-inflammatory M2 phenotypes could be useful in the treatment of periodontitis as well as other inflammatory disorders such as diabetes, atherosclerosis, obesity, and others. However, because macrophage polarisation is a broad term that encompasses a variety of pathways, the particular targets for therapeutic benefit are currently being investigated.

Targeting macrophage polarisation for therapeutic purposes is still in its infancy. M1 polarisation has been reported in human gingival tissue with experimental gingivitis or drug-induced gingival hyperplasia[67], suggesting that M1 polarisation may be a crucial mechanism driving periodontal inflammation and its repercussions. In 2016, Ting Yu et al. proposed that the phenotypic switch to M1 could be explained by a proinflammatory state in the circulation, such as an increased IL-6/IL-10 ratio in the serum, which would attract more proinflammatory monocytes to infection sites, or by a phenotypic switch of tissue macrophages from M2 to M1 at local sites. As a result, elements that operate as switches in changing macrophage polarisation towards M1 or M2 might aid therapeutic and diagnostic development in the field of periodontics.

Targeting miRNAs with altering oligonucleotides and compounds could be a useful therapeutic intervention technique for avoiding macrophage-mediated immunological disorders. The development of carriers that

transport miRNAs to macrophages in a macrophage-specific manner could substantially increase the range of possibilities for miRNA-based therapeutics. Unfortunately, the miRNA-specific network is still understudied, making medication development challenging, however it's not impossible within the near future.

By modulating the above influencing factors, macrophages can be turned into a desired phenotype for the treatment of diseases. In the future, macrophage polarization phenotypes can be used to treat various inflammatory diseases by drug intervention targeting the phenotypic switch, as well as other signaling pathways.

Impediment for research :

Over the last decade, the importance of macrophage polarisation in normal physiology and disease has grown significantly. Despite the fact that technology has played a critical role in understanding macrophage polarisation, such as improved cell separation approaches and single-cell and deep sequencing, many questions about macrophage polarisation and their role in health and disease remain unanswered, obstructing macrophage polarisation research progress.[65] Firstly, cell surface markers that identify macrophage morphologies during inflammatory disorders need to be confirmed. For example, several recent research have indicated that detecting TAM through the expression of CD68 is ineffective since it is also expressed by other stromal populations; thus, findings made from the TAM CD68 marker should be re-evaluated.[65]Second, because it is sometimes difficult to obtain fresh human macrophages, most human research are undertaken using cell lines, which are inadequate and impede the transition to clinical trials .Finally, there are significant changes between mouse and human macrophages. In vitro polarised human macrophages, for example, do not express Arg1, a mouse M2 macrophage marker. As a result, if the distinctions between mice and human cannot be completely examined, preclinical development of medicines supported by animal models will be challenging.[65,66]

Macrophage functions does not limit to only “kill and repair.” In fact this dualistic nature of macrophages is yet to be validated[58,59]and there are certain misconceptions on macrophage polarization. For instance, ornithine is produced by both M1 and M2 macrophages through Arg1(present in both M1 and M2) however at different concentrations, and this contributes to the difference in their function as well. Arginine1 more frequently is associated with M2 phenotype and through the synthesis of ornithine contributes to proline production, an essential component of collagen. In the absence of Arg1 in M2, there is increased fibrosis. However, the metabolic fate of ornithine in M2 is yet to be studied extensively as it is exported as a waste product rather than as a healing signal.[60,61]

Similarly, the relationship between IL-10 and macrophage polarisation is another commonly misunderstood concept.IL-10 is essential to reduce inflammation and regulate polarisation,it is secreted by both the phenotypes. However, M2 produces more IL-10 than M1. This is attributed to the fact that IL-10 increases IL-4Ra chain on cell surfaces making the macrophages more sensitive to IL-4 and IL-13.In a similar manner IL-4Ra is sensitive to STAT-3 activating receptor, thus increasing IL-4 and IL-13 expression and these in turn increases the sensitivity to M2 phenotype switching.[62,63]

Conclusion:

It is evident that macrophage polarization play a very vital role in maintaining periodontal tissue homeostasis. It is a double edged sword where an imbalance in the various subsets of these cells will result in either destruction or resolution of the disease state. The mechanism of activation and deactivation of these subsets is very complicated and more evidences are required to conclude at an particular attribute to its pathophysiology. A better understanding on how the balance between these subsets could be established to maintain the periodontal homeostasis can pave way for a better and simple way to various diagnostic and therapeutic modalities in management of periodontal disease in the near future.

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List of tables

Table 1. Cytokine, Chemokines and Cell Surface Markers of M1 Macrophages

CYTOKINES	CHEMOKINES	CELL SURFACE MARKE
IL-1β IL-15 IL-18 TNF-α IL-2 IL-3	CCL15/HCC-2 CCL2-/MIP-3α CXCL13/BCA-1 CXCL9/Mig, CXCL10/IP-10 CXCL11/I-TAC	CD 80 CD 86

Table 2. Subsets of M2 Macrophages

	M2A	M2B	M2C
INDUCTION	IL-4 IL-13	IMMUNE COMPLEXES TLR AGONIST IL-1 RECEPTOR LIGNAD	GLUCOCORTICOIDS IL-10
EXPRESSION	UPREGULATION OF CD206	IL-10 1L-1β IL-6 TNF-α	STILL UNDER RESEARCH

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Figure 1. Signalling pathways associated with macrophage polarization