Original Article
Macrophages in osteoarthritis: pathophysiology and therapeutics

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Abstract: Osteoarthritis (OA) is the most common cause of disability in worldwide population, which is characterized by cartilage breakdown, synovial fibrosis, osteophyte formation and pain. Synovial inflammation is usually found in both early and late stages in most of the OA patients. Macrophages, the major component of the mononuclear phagocyte system, play a critical role in OA pathogenesis through the induction of inflammatory mediators, growth factors and proteinases. So, drugs that can target macrophages and macrophage-associated inflammatory pathways at an appropriate stage may help to inhibit or slow down the progression of OA. However, despite an emerging role of synovial macrophages in OA pathogenesis, little is known about the biology of synovial tissue macrophages, and attempts to target macrophages therapeutically have had limited success. But the use of selective targets of macrophages may minimize the side effects and support the promising therapeutic strategy in the treatment of OA. More pre-clinical animal models and clinical trials are necessary to evaluate the role of selective targets of macrophages in the prevention and treatment of OA. This review article discusses the association of macrophages in OA development and possible OA therapeutics by targeting macrophages.

Keywords: Osteoarthritis, inflammation, macrophages, cytokines, synovial tissue

Introduction

Macrophages are bone marrow-derived cells that consist of blood monocytes, and tissue macrophages. Macrophages are widely distributed throughout the body, contributing to physiologic homeostasis by responding to internal and external changes within the body throughout the life [1, 2]. As essential effectors of the innate immune system, macrophages play a central role in inflammation and host defense. A major characteristic of macrophages is their ability to recognize, internalize, and destroy harmful endogenous and foreign substances in response to inflammatory signals in a process called phagocytosis [3, 4]. They are highly heterogeneous cells that can rapidly change their function in response to different tissue environments. Activated macrophages may secrete proinflammatory cytokines, which play important role in bone loss in inflammatory bone disease [5, 6].

Osteoarthritis (OA) is the most prevalent form of joint disease that causes disability in adults due to pain and impaired joint function. According to the World Health Organization (WHO), the prevalence of OA is more than 150 million in people worldwide [7, 8]. OA is characterized by pain, stiffness, locomotor restriction, bony enlargement, and sometimes swelling of the specific joints such as knees, hips, hands and spine, all of which can result in impaired function [9-11]. The exact mechanisms involved in the pathogenesis of OA are not well understood yet. The pathogenesis of OA seemed to be the result of the complex interaction between mechanical, cellular and inflammatory fac-

tors. With the understanding of pathogenesis of OA, it is considered to be predominantly mechanical in a so-called "wear and tear" process whose immune system was unlikely to be affected. OA cannot be cured totally. Only conventional therapy for OA is directed toward pain management as pain is an important factor in strategies to manage OA. The available conventional therapeutic strategies include physiotherapy, pharmacological agents and surgery [12-14]. However, these treatments are not always satisfactory as they are not powerful enough to modify the course of the underlying disease and are not able to prevent cartilage degenerative processes. Therefore, there is a large demand for disease-modifying therapies for the treatment of OA [15, 16].

Targeting activated macrophages at an appropriate stage may help to inhibit or to slow the progression of bone loss in patients with OA. In this review, we discuss the pathogenic and protective functions of macrophage in OA.

Functional roles of macrophages

Macrophages are the major component of the mononuclear phagocyte system that arises from yolk sac and fetal liver progenitors during embryonic development. They are present in mammals from midgestation period, and play a central role in host defense as well as in normal physiological processes such as the maintenance of tissues throughout the life [17, 18]. Macrophages can be found in almost all organs in the body, including the liver, spleen, brain, bones, lymph nodes and lungs. They have specific functions in each organ and the surrounding environments influence their properties during differentiation [2, 19]. Macrophages may function as scavengers by their ability to recognize, internalize, and destroy harmful endogenous and foreign substances. They sometimes play a role in host antimicrobial defense, antitumor immune responses and anti-inflammatory responses, tissue repair, and homeostasis while they sometimes promote inflammation and tumor growth [20-22]. Generally, it is considered that embryonic-derived macrophages play a strong role in the maintenance of tissue homeostasis while bone marrow derived macrophages are related to host defense reactions and inflammatory diseases [2].

In response to microenvironmental stimuli, macrophages (both resident and inflammatory macrophages) can be classified on basis of the activation: classically activated macrophages (proinflammatory M1) and alternatively activated macrophages (antiinflammatory M2). M1 macrophages have proinflammatory functions and are responsible for the release of molecules crucial for joint inflammation. M1 macrophages are stimulated by interferon (IFN)-γ or lipopolysaccharide (LPS), granulocyte macrophage colony-stimulating factor or other toll-like receptor (TLR) ligands to produce high levels of proinflammatory cytokines, such as IL-1β, IL-12, tumor necrosis factor-α (TNF-α), and superoxide anions; induce Th1 immune response, and mediate defense of the host from various bacteria, protozoa and viruses. M1 macrophages also mediate antitumour immune responses [23-25]. Conversely, M2 macrophages have an anti-inflammatory function and contribute to tissue repair and resolution of inflammation. M2 macrophages can also regulate wound healing. M2 macrophages are stimulated by interleukin (IL)-4 or IL-13 to produce anti-inflammatory cytokines such as IL-10, IL-1α, transforming growth factor (TGF)-β and arginase-1 (Arg1); induce the activation of the Th2 immune response and antiinflammatory functions (**Figure 1**) [26, 27]. There are some other less-well-defined macrophages, including tumor-associated macrophages (TAM), which suppress antitumor immunity; "immature" monocyte-like (GR1/Ly6C+) or "mature" neutrophil-like (GR1/Ly6G+) myeloid-derived suppressor cells (MDSCs) [28, 29].

Under normal conditions, most macrophages display an M2 phenotype to maintain tissue homeostasis [30]. In inflammation, macrophages are activated and polarized to an M1 phenotype. These M1 macrophages have three major function; antigen presentation, phagocytosis, and immunomodulation through production of nitric oxide and proinflammatory cytokines, which can lead to tissue damage [4, 17]. During the resolution of inflammation, macrophages are predominantly polarized to an M2 phenotype. They can repair damages tissues, clear debris, and restore tissue homeostasis through production of anti-inflammatory cytokines and cytokine antagonists (**Figure 2**). Macrophages use pattern recognition receptors (PRRs), including TLRs, C-type lectin receptors, scavenger receptors, retinoic acid-inducible gene 1 (RIG1)-like helicase receptors
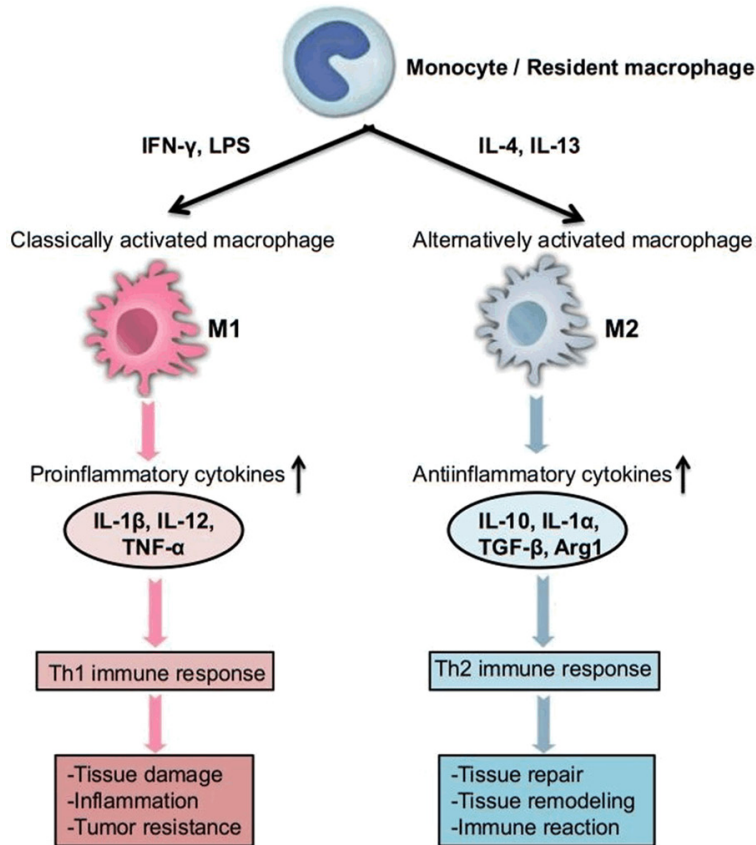
Figure 1. Functional role of macrophages. In response to microenvironmental stimuli, M1 macrophages are stimulated by IFN-γ or LPS to produce high levels of proinflammatory cytokines, induce Th1 immune response and mediate defense of the host. Conversely, M2 macrophages are stimulated by interleukin IL-4 or IL-13 to produce anti-inflammatory cytokines, induce the activation of the Th2 immune response and mediate antiinflammatory functions.

(RLRs) and NOD-like receptors, to recognize, internalize, and destroy harmful foreign substances and dead or dying cells [31-33].

Association of macrophages in osteoarthritis

OA is the most common cause of disability of people worldwide characterized by cartilage breakdown, synovial fibrosis, osteophyte (bony outgrowths at the joint margin) formation and pain. It has been demonstrated that synovial inflammation can be found in both early and late stages in most OA patients [34-36]. Abundant proinflammatory cytokines that are responsible for inflammation and cartilage degradation have been found in the synovium of patients with OA. The accumulation of macrophages in the synovial lining can be recognized is a hallmark of synovitis [37, 38]. It has been implicated that both inflammatory and destruc-

tive responses are dependent largely on macrophages which play a critical role in OA pathogenesis through the induction of inflammatory mediators, growth factors and proteinases [39, 40]. As M1 macrophage is believed to be pro-inflammatory whereas M2 macrophage anti-inflammatory, the balance between M1 and M2 macrophages might be distorted in OA and the degree of imbalance was associated with severe level of OA. The failure of synovial macrophages to transform from M1 to M2 subtypes may contribute to the initiation and progression of OA (**Figure 3**) [41-43]. In the synovium of OA patients, M1 cytokines, including IL-12, IL-1β and TNF-α are increased while M2 cytokine such as IL-1α is reduced. The overproduction of cytokines and growth factors from the inflamed synovium can influence the cartilage degradation through the production of other pro- and anti-inflammatory cytokines, production of matrix metalloproteinases (MMPs), and expression of aggrecanases in the OA synovium. The polarization of macrophages also has an effect on the progression of OA. The increased macrophages found in the synovium and subchondral bone of OA patients can be identified by cell surface markers, including CD163, CD68, CD14, MHC class II genes and F4/80. When patients with OA are examined, an increase in CD14 and CD163 indicates inflammatory phenotypes and OA severity [27, 39, 44]. In OA, macrophage activation can occur due to cartilage damage through the secretion of MMPs (MMP-1, -3 and -9), cytokines and growth factors. Potential mediators including damage-associated molecular patterns (DAMPs) leak into the synovial fluid by damaged cartilage and activate synovial macrophages. Activation of synovial macrophages leads to release of pro-inflammatory cytokines as well as catabolic and anabolic factors, which can induce osteophyte formation [45, 46].
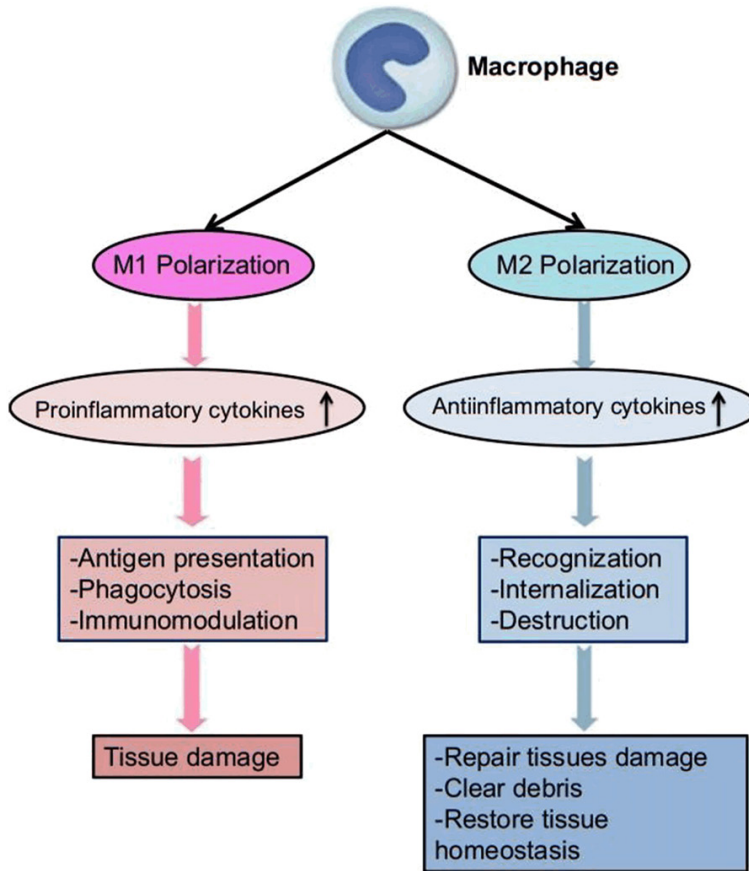
Figure 2. Basic mechanism of macrophages in inflammation. During inflammation, macrophages are activated and polarized to M1 phenotype. These M1 macrophages can lead to tissue damage through production of proinflammatory cytokines. During the resolution of inflammation, macrophages are predominantly polarized to M2 phenotype. They can repair damaged tissues, clear debris, and restore tissue homeostasis through production of antiinflammatory cytokines.

taneously producing a variety of both pro- and anti-inflammatory cytokines as well as the major MMPs and tissue inhibitors of MMPs (TIMPs). They demonstrated that CD14 deficiency is correlated to delayed cartilage degradation in OA. In this study, they depleted synovial macrophages by anti-CD14 conjugated magnetic beads resulting in reduced production of IL-1β, TNF-α and MMPs by synovial fibroblasts and reduced cartilage damage. Moreover, they injected clodronateladen liposomes in destabilized medial meniscus (DMM) OA models in mice, leading to the inhibition of osteophyte formation through the depletion of macrophages. In another mouse model of papain-induced OA treated with triamcinolone acetonide (TA), Siebelt et al. [51] reported that enhanced macrophage infiltration reduced osteophyte formation by increased proportions of CD163/FRβ-positive M2 anti-inflammatory macrophages. Rebalance of the ratio of M1/M2 might be used as a novel therapeutic alternative for OA. Glucocorticoids increase synovial macrophage expression of CD163 and slightly reduce CD68+ macrophages in the lining layer in patients with OA [42]. Utomo et al. [52] added dexamethasone to synovium explants of OA patients and showed that dexamethasone suppressed the pro-inflammatory M1 macrophages and enhanced the anti-inflammatory M2 macrophages. Choi [53] used Tissuegene-C (a cell-mediated gene therapy) for localized delivery of TGF-β1 in OA patients. Their findings suggested that Tissuegene-C induces an anti-inflammatory environment in the joint of OA patients. In a rat MIA model, they found elevated production of IL-10 and other M2 macrophage markers in joints of the Tissuegene-C group as compared to the control group. Tissuegene-C is currently being tested in phase II clinical trials in OA. Targeting cytokines has yielded disappointing results. Chevalier et al. [16] reported that anti-
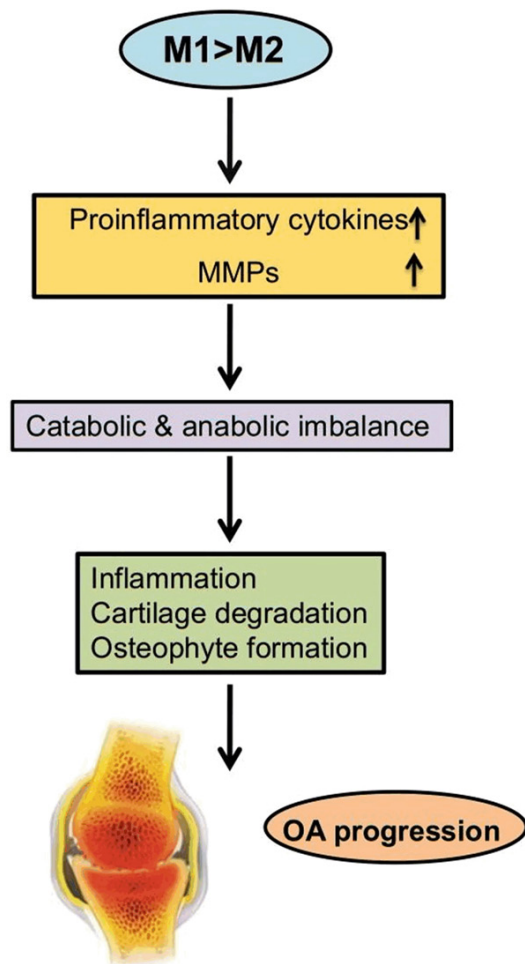
Therapeutic aspects of macrophages in osteoarthritis treatment

OA is one of the most common causes of chronic disability in adults due to the result of deep pathologic changes in the articular tissues. There is no effective treatment strategy of OA [47, 48]. Therapeutic approaches involved in macrophage phenotype modulation are promising. Drugs targeting macrophages and macrophage-associated inflammatory pathways might be a promising therapeutic strategy in the treatment of OA. Direct inhibition of M1 or promotion of M2 polarization may be useful therapeutic approaches in the treatment of OA [49, 50].

Bondeson et al. [38] examined OA cultures of synovial cells that have the advantage of spon-

Figure 3. Pathogenic mechanism of macrophages in OA progression. In OA, macrophage activation leads to release of proinflammatory cytokines as well as MMPs which induce cartilage damage and osteophyte formation through catabolic and anabolic imbalance.

NGF-β therapy in OA patients resulted in substantial pain reduction but was accompanied by serious side effects. However, broadly-acting anti-inflammatory drugs have yielded positive effects. Akasaki et al. [54] found that statins inhibited CCL2 and MMPs, reduced infiltration of CD68+ macrophages and decreased articular cartilage degradation in the OA subintima of ACLT rabbits. Similarly, CCL9 neutralization reduced macrophage and CD4+ T cell infiltration and pro-inflammatory IL-1β expression whereas decreased osteoclast formation and MMP-13 expression in ACLT mice [55]. Furthermore, a study in patients with advanced OA revealed that celecoxib treatment decreased macrophage infiltration and cytokine expression in the synovial membrane [56]. Methotre-

xate (MTX) may exert a positive effect on OA as the folate receptor β (FRβ) on synovial macrophages may serve as an entry route and assessment of FRβ expression by non-invasive macrophage imaging could prove a useful diagnostic tool to identify OA patients eligible for MTX therapy. A pragmatic phase III trial of MTX in OA patients is currently running [44, 57, 58].

Future direction

OA is a multifactorial pathology characterized by inflammation and immune response in the joint. Macrophages are the most common immune cell type present in this inflamed synovial tissue. These are thought to be an important player in promoting the production of inflammatory and degenerative mediators in OA. As macrophages play critical role in the pathogenesis of OA, modulating synovial macrophages might be sufficient to alleviate OA symptoms and prevent progression [59-61]. Despite an emerging role for synovial macrophages in OA pathogenesis, little is known about the biology of synovial tissue macrophages and attempts to target macrophages therapeutically have had limited success. The etiopathogenesis of OA is still object of intense pre-clinical and clinical research [61-63]. Development of targeted therapies for OA is critical for gaining clinical benefit without adverse effects. A better understanding of the role of human synovial macrophages in the regulation of joint homeostasis offers the prospect of new regenerative therapeutic strategies for OA [64, 65].

Conclusion

OA is a slowly progressive disease which includes the involvement of macrophage, leading to macrophage-related inflammation and degradation of the local cartilage. A better understanding regarding the pathogenic mechanisms of OA is necessary for the development of novel and effective therapeutic strategies. Development of OA therapeutic drug will require the use of biologic approaches that may alter the pathologic responses and activities of macrophages. The use of selective targets of macrophages may minimize the side effects and support the promising therapeutic strategy in the treatment of OA. More pre-clinical animal models and clinical trials are necessary to evaluate the role of selective targets of macrophages in the prevention and treatment of OA.

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Disclosure of conflict of interest

None.

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