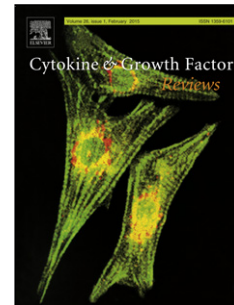


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Authors: Jinwei Xie, Zeyu Huang, Xijie Yu, Li Zhou, Fuxing Pei



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## The macrophage in knee osteoarthritis: evidence and clinical implications

Jinwei Xie<sup>1</sup> MD. PhD., Zeyu Huang<sup>2</sup> MD. PhD., Xijie Yu<sup>3</sup> MD. PhD, Li Zhou<sup>4</sup>, MD, Fuxing Pei<sup>2</sup> MD. PhD.

1. Department of orthopaedics surgery, Laboratory of Endocrinology and Metabolism, National Clinical Research Center for Geriatrics, West China Hospital of Sichuan University, Chengdu, Sichuan Province, China

2. Department of Orthopaedics surgery, West China Hospital, Sichuan University, Chengdu, Sichuan Province, China

3. Laboratory of Endocrinology and Metabolism, National Key Laboratory of biotherapy, West China Hospital, Sichuan University, Chengdu, Sichuan Province, China

4. Core Facility of West China Hospital, Sichuan University, Chengdu, Sichuan Province, China

### Corresponding author:

Fuxing Pei, Department of Orthopaedics surgery, West China Hospital, Sichuan University, 37# Guoxue Road, Wuhou District, Chengdu, Sichuan Province, 610041, China

Email: peifuxinghuaxi@126.com

Fax: +86-028-8542343

Tel: +8618980601380

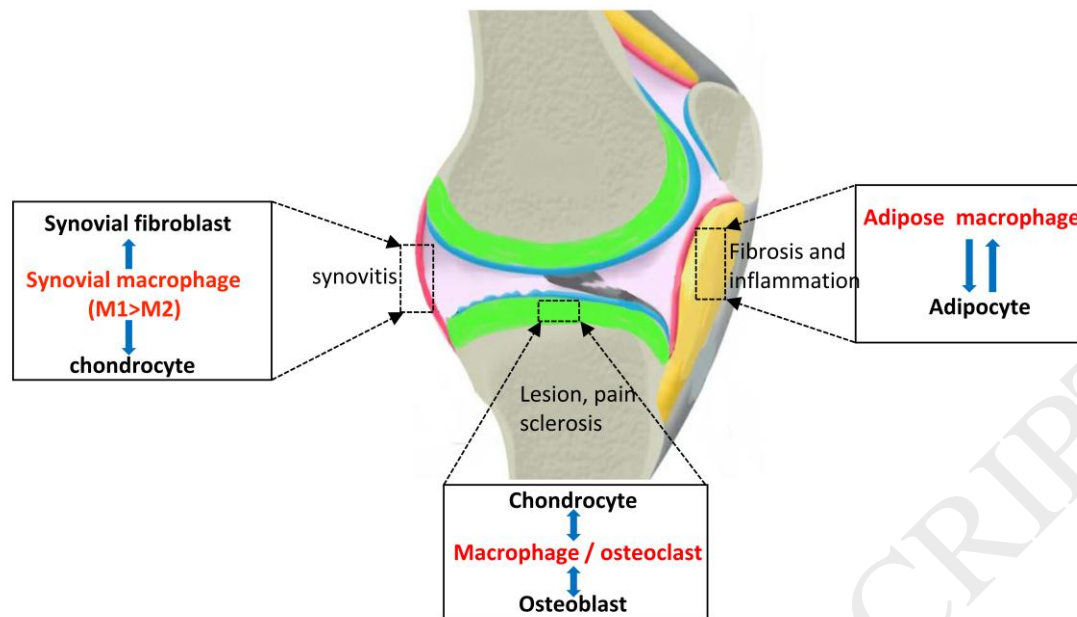
Xijie Yu, Laboratory of Endocrinology and Metabolism, National Key Laboratory of biotherapy, West China Hospital, Sichuan University, 37# Guoxue Road, Wuhou District, Chengdu, Sichuan Province, 610041, China

Email: xijieyu@hotmail.com

Fax: +86-028-8542343

Tel: +8618980602004

### Graphical abstract



### Highlights

- OA is associated with low-grade inflammation state.
- The failure of synovial macrophages to transition from M1 to M2 may contribute to the onset and progression of OA.
- Direct inhibition of M1 or promotion of M2 polarization may be useful therapeutic targets.
- Adipose tissue macrophages and osteoclasts were involved in obesity-induced OA and subchondral bone remodeling.

### Abstract:

Osteoarthritis (OA) is the most common form of arthritic disease, leading to disability and impaired quality of life and no curative treatments exist. Increasing evidence indicates that low-grade inflammation plays a pivotal role in the onset and progression of OA. In this review, we summarize emerging findings on the pathological roles of synovial macrophages, adipose tissue macrophages, and osteoclasts in OA and their potential clinical implications from cell biology to preclinical and preliminary clinical

trials. The failure of synovial macrophages to transition from pro-inflammatory M1 to anti-inflammatory M2 subtypes may contribute to the initiation and maintenance of synovitis in OA. M1 macrophages promote the inflammatory microenvironment and progression of OA through interactions with synovial fibroblasts and chondrocytes, thus increasing the secretion of matrix metalloproteinases. Direct inhibition of M1 or promotion of M2 polarization may be useful therapeutic interventions. Adipose tissue macrophages present in the infrapatella fat pad (IPFP) were involved in the progression of obesity-induced OA, which contributed to changes in the integrity of the IPFP. Furthermore, macrophages and osteoclasts in the subchondral bone were involved in bone remodeling and pain through uncoupled osteoclast/osteoblast activity and increased nociceptive signaling. Growing evidence has indicated an important role for macrophage-mediated low-grade inflammation in OA. Fully understanding the link between macrophages and other cells in joints will provide new insights into OA disease modification.

**Keywords:** Osteoarthritis; inflammation; immunity; macrophage

## **1. Introduction**

Osteoarthritis (OA) is a degenerative joint disease characterized by the loss of cartilage, changes in subchondral bone, formation of osteophytes, and inflammation of the synovium. It usually involves the knee, hip, and distal interphalangeal joints. According to the global burden of disease 2010 study, hip and knee OA was ranked as the 11<sup>th</sup> highest contributor to global disability and 38<sup>th</sup> highest in disability adjusted life years (DALYs). The global age-standardized prevalence of knee OA was 3.8% [1], which has doubled since the mid-20<sup>th</sup> century [2]. As a whole joint disease, OA is caused by the interaction of systemic susceptibility factors and local mechanical factors. Although the mechanism of OA initiation is unclear, some conditions are recognized risk factors, such as sex, obesity, aging, and joint trauma [3, 4].

OA results in pain and disability, which has serious effects on patients' productivity and quality of life. Although the treatment methods have been continuously improved in recent years, there is still a lack of Disease-Modifying Osteoarthritis Drugs (DMOADs). Current treatments focus on pain alleviation, and it is necessary for the most advanced patients to restore joint function by joint replacement surgery, which results in huge medical expenditure. Although OA has long been considered as a non-inflammatory condition, research is beginning to define it as a low-grade inflammatory state.

In this review, we will examine the pathophysiological basis of inflammation and tissue damage repair processes with respect to the immune cells and cytokines. We next discuss the relationship of inflammation within OA. Finally, we discuss the potential therapeutic targets of different tissue-specific macrophages in OA progression including synovial macrophages, adipose tissue macrophages, and osteoclasts. Therefore, this review will give an overview of some important recent findings regarding the role of macrophage-mediated inflammation in the pathogenesis of OA, and the potential for macrophages to be used as therapeutic targets for the development of DMOADs.

## **2. Inflammation and tissue damage repair**

Tissue injury triggers a series of overlapping events: hemostasis, an inflammatory phase, cell proliferation, and a resolution phase. The process is precisely regulated. All of the phases of wound healing depend on macrophages [5, 6]. They can accelerate the repair processes through "auto-debridement", whereby cell debris, dead cells, and necrotic tissues are devoured by the cells.

As part of the innate immune system, macrophages play an important role in the defensive, inflammatory, and resolution phases of wound healing. Macrophages present in adults are derived from two different sources [7-9]: the extraembryonic yolk sac and monocytes. The yolk sac produces a subset of erythro-myeloid progenitor cells that subsequently colonize developing

fetal organs to become tissue resident macrophages. In steady state conditions, the resident macrophages are the sentinels maintaining organ homeostasis, but upon infection or injury, they may become stimulated to mount an appropriate immune response [7-8]. Another source of macrophages is the blood circulating monocytes, which are derived from hematopoietic stem cells [9]. Upon injury, monocytes are recruited into the wound where they develop into mature macrophages. Macrophage replenishment is due to the self-renewal of tissue resident macrophages, with a secondary contribution from blood monocytes; but little is known about the signals that allow the build-up and replenishment of the macrophage pool by self-renewal [10, 11].

Depending on their origin, function, and the signature molecules produced in the regeneration process, macrophages can be classically divided into three distinct phenotypes (Figure 1): unstimulated macrophages (M0), pro-inflammatory (M1) and anti-inflammatory/ resolving macrophages (M2) [12]. However, it has recently become clear that such typecasting is limiting and inadequate to describe macrophage heterogeneity and functional exchangeability. The M1 and M2 subtypes can be generated in vitro by exposing M0 macrophages to interferon (IFN)- $\gamma$  / lipopolysaccharide (LPS) or interleukin (IL)-4 / IL-13, respectively. According to the distinct molecular phenotype, there are at least two different types of monocyte [13, 14]. The first type is inflammatory monocytes, characterized as CX3R1<sup>low</sup>, CCR2<sup>high</sup>, Ly6C<sup>high</sup> (in mice), and CD14<sup>high</sup>CD16<sup>low</sup> (in humans); the second type is characterized as CX3R1<sup>high</sup>, CCR2<sup>low</sup>, Ly6C<sup>low</sup> (in mice), and CD14<sup>low</sup>CD16<sup>high</sup> (in humans). These findings raised the question of whether different macrophage subsets are derived from different monocytes, although some researchers identified a reprogramming of macrophages. [47]

In the inflammatory phase, M1 macrophages are recruited and produce high levels of pro-inflammatory cytokines and chemokines such as tumor necrosis factor (TNF)- $\alpha$ , IL-1, and IL-6 [12, 15]. Pattern recognition receptors (PRR), such as Toll like receptors (TLRs), and NOD-like receptors (NLRs) recognize pathogen-associated molecular patterns (PAMPs) and damage-associated

molecular patterns (DAMPs), thereby activating downstream inflammatory signaling pathways, such as nuclear factor (NF)- $\kappa$ B signaling, [16] inducing a massive release of pro-inflammatory cytokines and chemokines. In the resolving phase, M2 macrophages are recruited and secrete large amounts of  $\omega$ -3 fatty acids, which can be converted into pro-resolving molecules to stop inflammation and promote tissue regeneration [17]. Moreover, other growth factors such as vascular endothelial growth factor (VEGF), transforming growth factor (TGF)- $\beta$ , and arginine are secreted to promote tissue regeneration. Of which, arginine can be converted into ornithine increasing the synthesis of matrix components such as collagen and polyamine proteoglycan [12]. Importantly, however, the dysfunction of M2 macrophages can also lead to both persistent tissue damage and fibrosis [18]. The precise transition from the inflammatory phase into the wound repair process has important physiological implications for resolving the inflammation and tissue regeneration.

### **3. OA is associated with an abnormal resolution of inflammation**

For a long time, OA was considered a “wear and tear” disease. However, increasing evidence suggests that inflammation is present in OA and has brought to light the possibility that inflammation and the immune system could be active players in the development and progression of OA [19-23]. Histopathological studies confirmed that immune cell infiltration was extremely common in OA histological specimens, although the degree was not as pronounced as rheumatoid arthritis [19]. Additionally, biomarker studies of OA have shown that the level of inflammatory cytokines, such as IL-1 $\beta$  and TNF- $\alpha$  in peripheral blood or joint fluid were significantly higher compared with healthy controls [20, 21]. Advanced imaging technology has further confirmed the role of low-grade inflammation in the pathophysiological process of OA. Aryal and colleagues [22] used MRI to continuously observe 422 patients with Kellgren-Lawrence 2–3 grade OA for 12 months and found the extent of synovial inflammation in the knee joint could predict the prognosis of OA. Roemer *et al.* [23] also found that diffuse synovitis was positively associated with cartilage destruction after a follow-up study of 514 normal knee joints for 30 months. These findings collectively indicate that OA is a persistent chronic inflammatory

state.

The local healing response after joint damage determines the individual susceptibility of OA. Although the acute inflammatory response after injury can promote tissue regeneration, persistent chronic inflammation is harmful [8]. Upon joint injury, platelets activated by thrombin in the synovium can secrete micro-vesicles containing IL-1 $\beta$  [24], which can promote the production of matrix metalloproteinases (MMPs) [25]. The damaged cartilage can be further degraded by the MMPs as a result of automatic debridement leading to the release of fragments of cartilage such as fibronectin [26], ultra-low molecular weight hyaluronan [27, 28], and proteoglycan fragments [29], which can activate the innate immune system, thereby inducing local inflammatory responses [30, 31].

However, according to the literature, the local inflammatory response induced by automatic debridement often enters a vicious cycle (Figure 2). Previous studies [32, 33] found that the severity and duration of local inflammatory responses were significantly lower in the tibia plateau fracture model of super-healer (MRL/MpJ) mice than wild-type, as was the incidence and severity of post-traumatic OA in these super-healer mice. These findings suggest that the failure to transition from the inflammatory phase to the tissue repair phase is the key to the persistence of OA.

#### **4. Macrophages and OA**

Macrophages are diffusely scattered in many tissues such as adipose tissue, bone marrow, mucosa, and bone. Each type of macrophage, determined by its location, has a specific name and function. The mesoderm is the precursor for mesenchymal tissues that comprise the appendicular skeleton, synovium, cartilage, tendons, ligaments, joint capsule and their associated lymphatics and vasculature. The tissue resident macrophages, as well as other stromal cells including fibroblasts, endothelial cells, chondrocytes and osteoblasts, form the joint and function as an integrated unit [34]. In the sections below, we focus exclusively on synovial macrophages,



adipose tissue macrophages, and osteoclasts (Figure 3).

#### **4.1 Synovial macrophages**

##### **4.1.1 Synovial macrophages and OA**

The synovium is divided into two compartments, the outer layer (sub-intima) and the inner layer (intima). The intima is mainly composed of two cell types, specialized macrophages (macrophage-like synoviocytes) and fibroblast-like synoviocytes, which are important in maintaining the internal joint homeostasis. The disintegration of chondrocytes or extracellular matrix act as DAMPs and can activate synovial macrophages in response to joint injury or aging stress [35]. Activated macrophages abundantly express folate receptors, which can be targeted and traced using radioactive-labeled folic acid by advanced imaging [36-39]. Pisco and colleagues [36] used SPECT/CT to trace  $^{111}\text{InCl}_3$ -labeled folic acid in a rat OA model of monoiodoacetate (MIA) and anterior cruciate ligament transection (ACLT). Their results showed that the MIA model had high initial macrophage activation, with a peak after 2 weeks and that disappeared after 8 weeks. While the ACLT model showed less activation but was still active 12 weeks after induction. de Visser [37] used another folate receptor radiotracer, and found  $^{111}\text{In}^{\text{cm}09}$  signaling increased by 28.4% in the high fat diet-induced OA model. Meanwhile, Kraus [38] observed the signal distribution of  $^{99\text{m}}\text{Tc}$ -EC20-labeled folate receptor in OA patients by SPECT/CT. It was found that the signal intensity of  $^{99\text{m}}\text{Tc}$ -EC20 in the affected knee joint was significantly correlated with the levels of CD14 and CD163 in body fluids (blood and joint fluid), and with joint space narrowing [39]. These findings directly demonstrated the important role of activated synovial macrophages in the pathophysiology of OA. However, the distribution of macrophage subsets was not clearly defined in these studies.

The temporal and spatial distribution of macrophage M1 and M2 subgroups plays a vital role in the precise regulation of inflammation and tissue regeneration. The M1 subgroup is mainly involved in the initiation of inflammation, while the M2 subgroup is mainly involved in the resolution of inflammation. The study of super-healer mice (MRL/MpJ) found that the activation

of M1 cells in synovial tissue after trauma only lasted for a few weeks, while it lasted for two months or more in wild-type mice [33]. Is there any possibility to eliminate inflammation and delay the progress of OA by depleting macrophages? Wu *et al.* [40] investigated the impact of short-term, systemic depletion of macrophages on experimental OA progression with the use of CSF-1R-GFR<sup>+</sup> macrophage Fas-induced apoptosis (MaFIA)-transgenic mice. A small molecule AP20187 was injected into the articular cavity to conditionally deplete all macrophage subsets after OA induction. The results showed that macrophage-depleted mice had significantly fewer M1 and M2 macrophages in the surgically manipulated joints relative to controls and exhibited decreased osteophyte formation immediately following depletion (one week). However, macrophage depletion did not attenuate the severity of OA, instead, it induced systemic inflammation and a markedly increased amount of pro-inflammatory cytokines (nine weeks). The data suggests that inflammation was a double-edged sword in the process of OA. The low-grade inflammation may not be related to the total number of activated macrophages, but to the failure of macrophages to differentiate from M1 to M2. This may be the key to induce the inflammation in OA to become chronic low-grade inflammation. In fact, recent studies [41], as well as our unpublished observations in humans, suggested that M1 macrophages aggregate predominantly in the human and mouse OA synovium as evidenced by immunohistochemistry and specific molecular markers of M1 and M2 macrophages. This further illustrates the pathological role of a failure to transition from M1 to M2 macrophage phenotypes in OA.

How do activated synovial macrophages induce or aggravate OA? Bondeson *et al.* [42] cultured synovial cells from the digested OA synovia of patients undergoing knee replacements, depleted of CD14<sup>+</sup> synovial macrophages by magnetic flow cytometry. The authors found CD14<sup>+</sup> depleted cultures no longer produced significant amounts of macrophage-derived cytokines, such as IL-1 and TNF- $\alpha$ ; furthermore, the other cytokines, produced chiefly by synovial fibroblast, such as IL-6, IL-8, MMP-3 and ADAMTs4 were significantly reduced. The data indicated that synovial macrophage-fibroblast crosstalk played a priming role in the process of synovial inflammation. Blom AB [43] depleted synovial macrophages by inducing apoptosis with the use of clodronate

liposomes prior to inducing OA using a collagenase-induced instability model. The results showed less cartilage degeneration on day 7 and 14 following the induction of OA in macrophage-depleted joints. Meanwhile, the authors also observed a strong decrease in MMP-3 and MMP-9 expression in synovia but not in cartilage tissues in macrophage-depleted joints. Another study [44] by the same team found that the depletion of synovial macrophages by clodronate liposomes resulted in a significant reduction in osteophyte formation, 84% and 66%, 7 and 14 days after OA induction, respectively. In addition, the production of growth factors, such as TGF- $\beta$ , BMP-2 and BMP-4, were reduced in the superficial synovium, while the periosteum did not differ from controls. These results suggest that synovial macrophages are a pivotal cell mediating osteophyte formation and other OA-related pathologies by inducing fibroblast-like synoviocytes to produce MMPs, cytokines and other growth factors, especially in the early stages of OA development.

Another possible mechanism by which macrophages may influence the development of OA is the paracrine effects on the synthesis and catabolism of chondrocytes. Samavedi S [45] co-cultured macrophages with chondrocytes and found that the production of MMP-1, MMP-3, MMP-9, MMP-13, IL-1 $\beta$ , TNF- $\alpha$ , IL-6, and IL-8 by chondrocytes was significantly increased. The mechanism may be that the R-spondin-2 (Rspo2) protein secreted by the M1 subgroup activates the  $\beta$ -catenin pathway in the chondrocytes [41]. In a study by Utomo L [46], human OA cartilage explants were cultured with macrophage-conditioned media (MCM) from M1 (IFN- $\gamma$  + TNF- $\alpha$ ), or M2 (IL-4 or IL-10) macrophages derived from human monocytes. M1 (IFN- $\gamma$  + TNF- $\alpha$ ) MCM affected OA cartilage by the up-regulation of IL- $\beta$ , MMP-13 and ADAMTs-5, while inhibiting Col2A1 and Aggrecan. Furthermore, M2 (IL-4 or IL-10) macrophages did not inhibit the effect of M1 macrophage or IFN- $\gamma$ /TNF- $\alpha$  induced inflammation on the cartilage. However, the mechanism was not clear. The authors surmised that the best therapeutic target was to directly suppress pro-inflammatory macrophages or stimulate anti-inflammatory macrophages.

#### **4.1.2 Clinical implications of synovial macrophages**

Macrophages can exhibit strong plasticity depending on their microenvironment. They can be M1 subtypes producing pro-inflammatory factors and initiating inflammation, or M2 subtypes producing anti-inflammatory factors and growth factors to resolve inflammation and promote tissues regeneration. The current view is that M1 and M2 may be just two extreme types of macrophage, and there may be a third phenotype of macrophage. Transcriptional profiling has advanced the understanding of the plasticity of macrophages suggesting a complex cellular reprogramming in response to stress signals [47]. The pathways involved in reprogramming were reviewed by other authors [48], including JNK, PI3K / Akt, Notch, JAK/STAT, TGF- $\beta$ , TLR / NF- $\kappa$ B, and microRNA.

With the proper stimulation, promoting the reprogramming of M1 to M2 macrophages, may alleviate the progression of OA. Topoluk *et al* [49] cultured OA chondrocytes, macrophages (M1/M2) and placental mesenchymal stem cells. They showed that placental mesenchymal stem cells reduced the M1/M2 ratio, and increased chondrocyte activity and production of glycosaminoglycan, which may be related to prostaglandin (PG) E2 [50]. A recent study by Dai *et al* [51] found that squid type II collagen can activate the M2 subtype through immunomodulation, thereby increasing the secretion of TGF- $\beta$ 1/3 and promoting cartilage repair. A phase II clinical study of the first gene and cell therapy for OA, INVOSSA™ (TissueGene-C), has been completed and showed that INVOSSA™ improved pain, daily activities, sport functions and cartilage structure in patients with OA [52-54]. Furthermore, the study showed that INVOSSA™ may induce an anti-inflammatory environment, especially M2 macrophage differentiation, which contributes to the reduction of pain and cartilage regeneration [55]. The U.S clinical trial phase III of this drug is currently recruiting patients, which is expected to be the first DMOADs in use [56]. Therefore, synovial macrophages may be a bonafide therapeutic target, by inhibiting M1 or promoting M2 phenotypes.

## **4.2 Adipose tissue macrophages in the infra-patella fat pad**

### **4.2.1 Adipose tissue macrophages and OA**

The infra-patella fat pad (IPFP) or Hoffa fat pad was first described by Albert Hoffa in 1904. It is situated in the knee underneath the patella, between the patella tendon, femoral condyle, and tibia plateau, where it completely fills the potential spaces between these structures. It is located close to the synovial layers and cartilage surfaces. As an extra-synovial tissue, the IPFP does not directly interact with the cartilage. Based on its location in close contact with the synovium, the idea is emerging that the IPFP and synovium may be considered as a morpho-function unit [57]. The IPFP is composed of a fibrous scaffold, on which constitutional fat tissue is embedded [58].

The IPFP has been implicated in knee OA. Patients with IPFP signal intensity alterations and/or greater effusion-synovitis volume in the absence of radiographic OA may be at higher risk for accelerated OA. The reason for this may be local inflammation [59]. Furthermore, the signal intensity alterations within the IPFP could predict the requirement for a knee replacement within 5 years [60] and obesity and high body mass index are associated with a higher incidence risk of OA. In addition to the loading forces, the adipose tissue, including the IPFP, contributed to the initiation and progression of OA [61]. Masaki *et al* [62] showed no correlation between the IPFP volume change and obesity with the use of magnetic resonance imaging T1p mapping, which was consistent with other studies [63]; however, the volume change in IPFP was associated with cartilage degeneration. These data indicated the quality of the IPFP, not the quantity, contributed to the progression of OA.

The IPFP contains many cells such as adipocytes, fibroblasts, macrophages, leukocytes, and other cells involved in inflammation. Compared with the synovium of end stage knee OA, macrophages and T cells, followed by mast cells, were the most predominant immune cells in the IPFP [64]. The resident adipose tissue macrophages (ATM) in normal adipose tissues (AT) are similar to alternatively activated M2 macrophages that are important to maintain homeostasis in adipose tissue. Resident ATM are distributed between adipocytes and along vascular structures in AT, with a molecular pattern of F4/80<sup>+</sup> CD64<sup>+</sup> CD206<sup>+</sup> CD301<sup>+</sup> CD11c<sup>-</sup>. These cells can regulate adipocyte lipid metabolism, as well as resolving inflammation [65]. In an obese setting, the population of

ATM expands from 10% of all cells in lean AT to more than 50% in obese mice [66, 67]. The increased number of ATMs in obesity is due to the recruitment of macrophages from monocyte trafficking and local proliferation of resident macrophages [68-70]. These cells undergo a polarization shift toward a pro-inflammatory phenotype. Indeed, a specific ATM phenotype, called “metabolically-activated macrophages” has been introduced [71].

According to the results of Harasymowicz NS *et al* [72], adipocytes from the IPFP of obese patients were significantly larger and the synovium displayed marked fibrosis, increased macrophage infiltration, and higher levels of TLR4. Furthermore, there were increased numbers of CD45<sup>+</sup>/CD14<sup>+</sup> total macrophages and CD14<sup>+</sup>/CD206<sup>+</sup> M2 macrophages in the IPFP of obese patients compared with lean patients. By contrast, de Jong AJ *et al* [73] found no association between high BMI and immune cell numbers in the IPFP, although CD206 was the most abundantly expressed surface marker on macrophages (81%). These data raise the question of how the adipose tissue macrophages affect joint health.

The crosstalk between the immune system and metabolic regulation, “immunometabolism”, is now well described. Metabolically activated macrophages were proposed to develop in response to the altered metabolic white adipose tissue environment in the obese setting, particularly to high insulin levels, free fatty acids, and high glucose concentration. Furthermore, these factors correlated with the initiation and progression of OA [74, 75]. Little is known about the role of local adipose tissue in OA, although some studies found that the IPFP should be considered as an active tissue and an important source of cytokines, chemokines, and adipokines [76, 77]. In a high-fat diet-induced mouse obesity model, Barboza E *et al.* [78], at 20 weeks, found early OA including osteophytes, cartilage tidemark duplication, and IPFP fibrosis. However, a high-fat diet did not increase IPFP inflammation, macrophage infiltration, or adipose tissue macrophage M1 polarization as observed in epididymal fat. Conversely, the enrichment of M2 macrophages and IL-13 expression were found, and may be associated with IPFP fibrosis. Moreover, the different expression of type I, III, VI collagen between lean and obese patients with OA may be another factor contributing to the fibrosis of the IPFP [79]. Moreover, as a functional unit, the crosstalk

between the synovium and the IPFP needs to be better explored [80, 81].

Pain is the most common symptom reported by knee OA patients. In concert with the synovium, the IPFP is a very sensitive structure that contains part of the terminal sensory innervation for the knee, especially the substance P nerves. Local inflammatory mediators, such as TNF- $\alpha$  and IL-6, could promote the maintenance of joint pain by acting as receptors on dorsal root ganglia, which may be exacerbated in obesity [82]. Additionally, bidirectional communication between brain macrophages (microglia) and neurons is well established. Interestingly, emerging evidence suggests that macrophage-neuron crosstalk also occurs outside the central nervous system. Norepinephrine (NE) was shown to be secreted by extrinsic sympathetic neurons and triggered an adrenergic signaling pathway, promoting tissue protective macrophage activation in the intestine [83]. Other neuropeptides, in addition to NE, are involved in the process of ATM-neuron-adipocyte crosstalk, which was reviewed in detail elsewhere [84]. However, it is not known if crosstalk exists in the IPFP in the setting of OA.

#### **4.2.2 Clinical implications of adipose tissue macrophages**

The different phenotype of macrophages in the synovium and the IPFP indicates different therapeutic targets, especially in the early stage of OA, where M2 ATM are predominant in the IPFP, but M1 synovial macrophages are more numerous in the synovium [79]. The pathway or cytokines involved in IPFP fibrosis could be a new target for early OA. However, whether this condition would continue in the middle or end stage of OA is unknown. The new studies into the immunometabolism have shed light on new roles of ATM in OA.

### **4.3 Macrophages in subchondral bone / osteoclasts**

#### **4.3.1 Osteoclasts and OA**

The subchondral bone is a compact and highly vascularized layer of cortical bone that lies immediately below the articular cartilage. In the subchondral bone, rapid bone loss after traumatic injuries and bone sclerosis at the end stage are well recognized hallmarks of OA. There

is a large body of evidence supporting that enhanced subchondral bone turnover plays an active and pivotal role in the onset and progression of OA [85-87]. However, the sequence of cartilage degeneration and subchondral bone changes has been under debate. Histomorphometry analysis of cartilage and subchondral bone in the tibial plateaus from advanced OA patients suggested bone changes were secondary to cartilage degeneration [89]. Microstructural analysis implied concurrent changes in the cartilage and bone [90], while analysis of the biomechanical properties of subchondral bone indicated bone changes may instead precede cartilage damage [91]. Most of these studies were performed in humans and focused on late and advanced OA; however, little is known about the early dysregulation of cartilage and bone turnover. Furthermore, the paradox of how abnormal resorption can eventually lead to bone sclerosis still needs to be resolved. A recent study by Chen Y *et al.* [92] identified a drastic loss of rod-like trabeculae and thickening of plate-like trabeculae in all regions of the tibial plateau, underneath both severely damaged and still intact cartilage with the use of novel microstructural analysis techniques. Thus, it is readily understandable that early characteristics of OA would be detectable in the bone tissue component.

Subchondral bone remodeling is a biological process involving bone marrow resident cells (including osteoblasts, osteocytes, and osteoclasts) and inflammatory cells derived from hematopoietic stem cells. As with other bone diseases, including osteoporosis, the bidirectional crosstalk between bone-resident cells and inflammatory cells plays a decisive role in the bone or subchondral bone remodeling, which was reviewed in detail [88]. The following section will focus on the interaction between osteoclasts and chondrocytes in OA.

The spatiotemporal uncoupling of osteoclastic bone resorption and osteoblastic bone formation contributes to bone loss during OA development. Macrophages (CD68<sup>+</sup>) and osteoclasts (CD68<sup>+</sup>/TRAP<sup>+</sup>) were significantly increased in human knee OA, especially in subchondral osteosclerotic areas, compared with non-sclerotic areas [93, 94]. The different phenotypes of resident macrophages (CD68<sup>+</sup>) and osteoclasts (CD68<sup>+</sup>/TRAP<sup>+</sup>) may influence the subchondral bone



remodeling. It has been shown that both M1 and M2 macrophages are able to promote alkaline phosphatase (ALP) activity and matrix mineralization by secretion of oncostatin M [95, 96]. Furthermore, OA osteoblasts have a different phenotype compared with healthy human osteoblasts. OA osteoblasts present different ratios of important bone factors, such as receptor activator of nuclear factor kappa-B ligand (RANKL), osteoprotegerin (OPG) and different responses to parathyroid hormone (PTH) [97], all of which are pro-bone-resorptive molecules. Furthermore, osteoclasts can be recruited during the progression of spontaneous equine carpal post-traumatic OA in response to RANKL expressed by cartilage, leading to calcified cartilage microcracks and focal subchondral bone loss [98]. Conversely, osteoclastic activity also plays a significant role in pain generation in OA. The serum biomarker of subchondral osteoclast activity, TRAcP5b and cathepsin K, can be used as a prediction of OA pain [99]. First, osteoclasts promote the expansion of microchannels from subchondral marrow spaces into the articular cartilage, resulting in the exposure of subchondral nerves to pro-inflammatory and allogenic cytokines from synovial fluid [101, 102]. Second, they create acidic conditions at the osteo-chondral junction that activate the acid-sensing receptors of sensory neurons [103, 104]. During remodeling, osteoclasts secrete NETRIN1, which binds to its receptor, deleted in colorectal cancer (DCC), to induce sensory nerve axonal growth in subchondral bone [105]. Furthermore, the production of PGD2 by osteoclasts or macrophages in response to an elevation in nerve growth factor (NGF) levels leads to an increase of nociceptive signaling in OA joints [100].

#### **4.3.2 Clinical implications of osteoclasts in OA**

Changes in the microarchitecture and mineralization of subchondral bone are prominent features of OA, indicating that a treatment that could reduce bone remodeling, such as bisphosphonate or strontium ranelate, might be useful for the treatment of knee OA. Bisphosphonates are a class of drugs commonly prescribed for fracture prevention due to their inhibitory effects on osteoclast-mediated bone resorption, which accompanies osteoporosis and other bone conditions. In knee OA, the first positive report of the use of bisphosphonates to alter the course of OA was a phase II clinical trial that evaluated risedronate versus placebo in individuals with mild to moderate OA

of the medial compartment of the knee [106]. Further investigations were motivated by these data, while conflicting findings were presented [107-109]. A recent meta-analysis involving 7 randomized controlled trials found that bisphosphonates neither provide symptomatic relief nor defer radiographic progression in knee OA [110]. While the authors also concluded that bisphosphonate treatment in a specific subset of OA patients who also have bone marrow lesions and active bone remodeling within subchondral bone plates might be effective for the reduction of knee pain, and the ongoing study may provide an answer [110-112]. Therefore, the patient subgroups for who these therapies are most appropriate have yet to be fully defined but would likely include, at a minimum, those with high bone turnover.

## **5. Conclusions and future perspectives**

Current evidence indicates that macrophages are a pivotal cell in the onset and progression of OA, though different tissue specific macrophages may have different effects on OA. Synovial macrophages form a bridge between the synovium, cartilage and the IPFP. The interaction and crosstalk between these tissues contributes to the hemostasis of joint health. Macrophages in subchondral bone or osteoclasts are important for subchondral bone remodeling in specific OA subtypes. Although some new therapeutic strategies have been implicated for OA modification, robust efficacy is lacking. Future studies should focus on the following topics. First, the mechanism of polarization and potential therapeutic targets for the reprogramming of synovial macrophages need to be uncovered. Conversely, direct inhibition of the pro-inflammatory effect of synovial macrophages may be alternative targets. Second, the mechanism of crosstalk between synovial macrophages, fibroblasts, and chondrocytes should be further studied. Finally, understanding the link between osteoclasts, osteoblasts, chondrocytes, and resident inflammatory cells will likely uncover disease mechanisms and additional therapeutic targets.

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**Conflict of interests**

All authors disclose any financial and personal relationships with other people or organizations that could inappropriately influence this work.

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**Jinwei Xie** completed his PhD in Orthopaedics surgery in 2018 at the Sichuan University. Now he is undertaking his post-doctoral work at the Department of orthopaedics surgery, Laboratory of Endocrinology and Metabolism, National Clinical Research Center for Geriatrics, West China Hospital of Sichuan University. His research focused on the mechanism of OA, especially the role of innate immunity and cellular senescence in OA.



**Prof. Xijin Yu** obtained his Phd in 1998 at the Sichuan University, then he undertook post-doctoral work and served as assistant professor at the Peking University and Indiana University from 1999 to 2007. During 2007-2009, he was a scientist at Institute of Human Genes and Health, Maine, USA. His current position is Director and Professor of Laboratory of Endocrinology and Metabolism, National Key Laboratory of biotherapy, in West China Hospital of Sichuan University. His group focus on the pathophysiology of metabolic osteopathy, rare bone disease, and tumor bone metastasis.





Figure 1

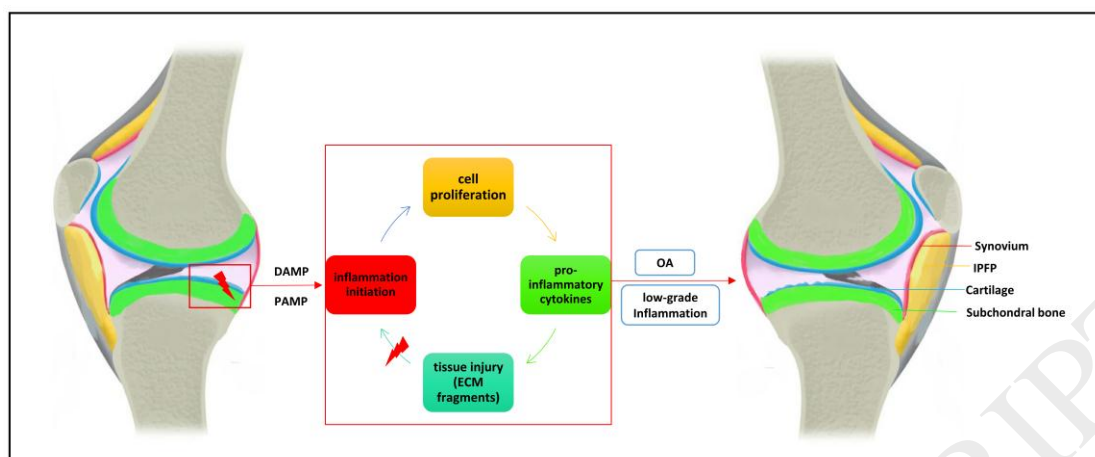


Figure 2

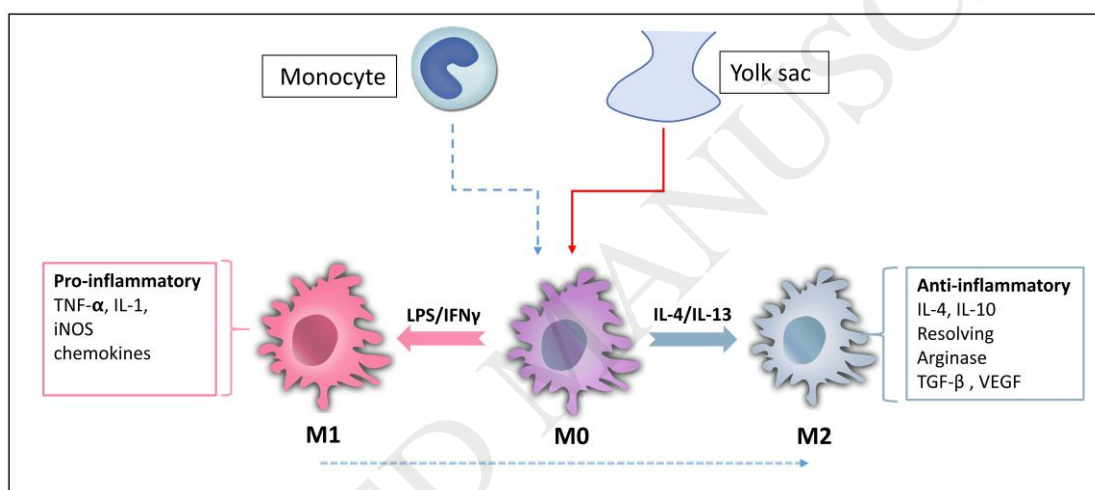


Figure 3

