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Review

The role of macrophages in osteoarthritis and cartilage repair

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SUMMARY

Osteoarthritis (OA) is a family of degenerative diseases affecting multiple joint tissues. Despite the diverse etiology and pathogenesis of OA, increasing evidence suggests that macrophages can play a significant role in modulating joint inflammation, and thus OA severity, via various secreted mediators. Recent advances in next-generation sequencing technologies coupled with proteomic and epigenetic tools have greatly facilitated research to elucidate the embryonic origin of macrophages in various tissues including joint synovium. Furthermore, scientists have now begun to appreciate that macrophage polarization can span beyond the conventionally recognized binary states (i.e., pro-inflammatory M1-like vs anti-inflammatory M2-like) and may encompass a broad spectrum of phenotypes. Although the presence of these cells has been shown in multiple joint tissues, additional mechanistic studies are required to provide a comprehensive understanding of the precise role of these diverse macrophage populations in OA onset and progression. New approaches that can modulate macrophages into desired functional phenotypes may provide novel therapeutic strategies for preventing OA or enhancing cartilage repair and regeneration.

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Introduction

Osteoarthritis (OA) is a disease of the joint organ system characterized by the degradation of articular cartilage, inflammation of the synovium and joint fat pad, as well as alterations in bone structure. Over 27 million people are estimated to suffer from OA in the US, resulting in a tremendous socioeconomic burden[1]. The etiology of OA has been shown to be heterogenous, and may in fact represent a family of diseases rather than one disease[2,3]. As such, several risk factors such as genetic predisposition, obesity, aging, and joint trauma have been identified for OA. Irrespective of this multifaceted nature of OA pathophysiology, it is now accepted that joint inflammation plays a major role in OA onset and progression[2].

Inflammation is classically regulated by a variety of immune cells such as T cells, neutrophils, and macrophages. Macrophages are phagocytic cells that can be found in almost every tissue (including brain, liver, skin, and tissues of the joint organ system). The primary role of macrophages is to maintain tissue homeostasis and protect the host from infection. Although primarily considered to be critical components of innate immunity, macrophages are capable of bridging and instructing the response of the adaptive immune system via various secretory mediators. Based on their interaction with T cells, macrophages have been typically dichotomized into two phenotypes: M1-like macrophages ("classically" activated) are antimicrobial and pro-inflammatory, and are activated in response to stimuli from T helper type 1 (Th1) cells, while M2-like macrophages ("alternatively" activated) are anti-inflammatory and pro-resolving, and are induced by Th2 cells. The dysregulated balance between pro- and anti-inflammatory macrophages may lead to chronic low-grade inflammation and has been suggested to be critical in the development of several musculoskeletal diseases, including OA[3].

Here, we review recent key findings of the origin of tissue macrophages and how these cells modulate anabolic and catabolic responses in OA, with a particular focus on the constituents of the joint organ system. Furthermore, recent advances in next-

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generation sequencing technologies and transcriptomic analysis have greatly facilitated our understanding of phenotypic identity and heterogeneity of the macrophage populations[4,5]. Thus, we also summarize the potential contributions of previously unrecognized macrophage subpopulations to OA pathogenesis. Finally, we present novel therapeutic strategies, particularly biomaterial scaffolds and cell reprogramming, that have the potential to modulate the specificity and protective function of macrophages for mitigating OA progression or enhancing cartilage repair.

Origin and heterogeneity of macrophages

Conventionally, macrophages were thought to derive solely from circulating bone marrow (BM)-derived monocytes[6]. However, the use of single-cell ribonucleic acid (RNA) sequencing and flow cytometry for wide-scale analysis of gene and protein expression has improved identification of macrophage origin and subtypes. New evidence suggests that embryonic development of macrophages in mammals, in fact, occurs in three successive waves: (1) the primitive wave, (2) the transient definitive wave, and (3) the definitive wave (Fig. 1). A summary of main macrophages markers discussed in the current review is listed in Table I.

While not yet precisely described in humans, the primitive wave during mouse development occurs around embryonic day 7.5 (E7.5), during which myeloid progenitors arise in the blood islands of the yolk sac (YS) and differentiate into primitive macrophages without the need of a monocytic intermediate[7,8]. For instance, microglia, a subtype of macrophages found in brain, were shown to be definitively derived from YS precursors[9,10]. However, there is some debate if other tissue-resident macrophages, such as those seen in the epidermis, liver, and lung, are derived from the primitive wave or from migratory erythro-myeloid precursors (EMPs) and lympho-myeloid progenitors (LMPs) in the following transient definitive wave[10,11].

The transient definitive wave occurs between E8.5 and E10.5. During this stage, the fetal liver of the embryo sequentially acquires C-Myb^{+}/CX3CR1^{+} EMPs and LMPs that arise from the hemogenic endothelium of the YS. These precursors do not exhibit the long-term reconstitution potential of hematopoietic stem cells (HSCs)[12].

The final definitive wave occurs in two overlapping stages and relies on the differentiation of HSCs. In the first stage, HSCs arise in the aorta-gonado-mesonephros (AGM) region and migrate to the fetal liver around E10, initiating definitive hematopoiesis. At the second stage, starting around E16.5, fetal liver hematopoiesis

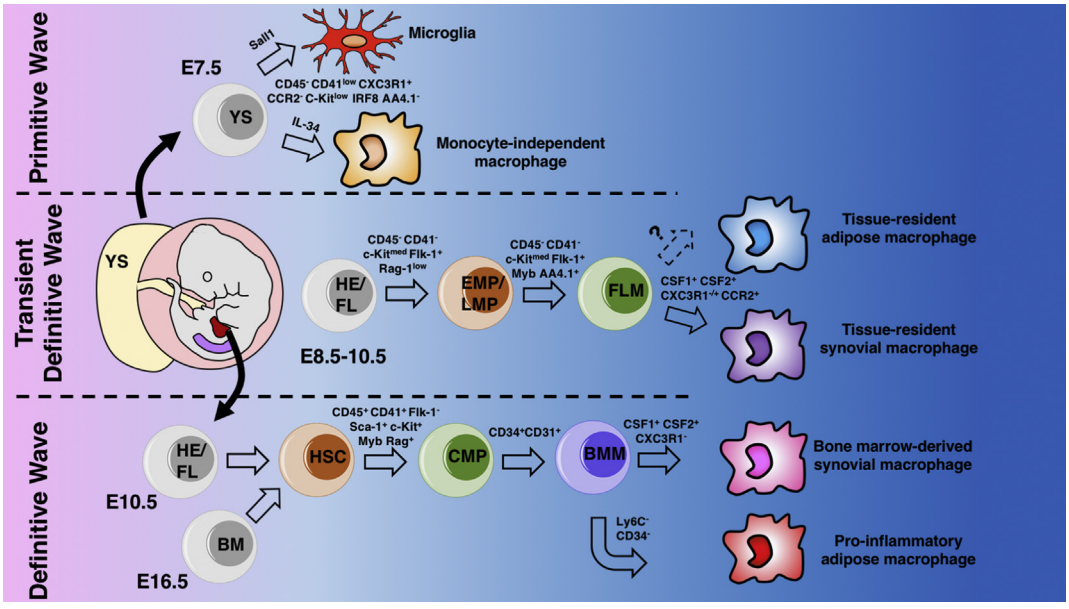

Fig. 1

Schematic representation of the origins of embryonic and adult macrophage lineages in the mouse yolk sac (YS), aorta-gonado-mesonephros region (AGM, purple color in the embryo), and fetal liver (FL, red color in the embryo), ***a. Primitive wave***. Primitive hematopoiesis starts at E7.5 in the blood islands of the yolk sac (YS) which generates erythro-myeloid progenitors (EMPs) and monocyte-independent macrophages that develop prior to the formation of the blood brain barrier. ***b. Transient definitive wave***. Upon establishment of the blood circulation around E8.5, the YS hemogenic endothelium (HE) generates late EMPs and additional progenitors with lymphoid potentials (LMPs) without long-term reconstitution capacity. These YS generated progenitors seed the fetal liver and rapidly produce myeloid progenitors that can generate fetal monocytes which differentiate into macrophages once circulated into tissue. ***c. Definitive wave***. Hematopoietic stem cells (HSCs) emerge from the main HE situated in the aorta gonado mesonephros (AGM) - regions and in the placenta around E10.5. These HSCs continue to seed the fetal liver and produce fetal monocytes until E16.5, at which point hematopoiesis switches completely from the fetal liver to the bone marrow (BM). AGM: aorta-gonado-mesonephros region, BM: bone marrow, BMM: bone marrow monocyte, CMP: common myeloid progenitor, EMPs: erythro-myeloid progenitors, FL: fetal liver, FLM: fetal liver monocytes, HE: hemogenic endothelium, LMPs: lymphoid-myeloid progenitors, YS: yolk sac.

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Marker (*alternative names*)	Role	References
CX3CR1 (Gpr13)	Immune cells migration, expressed during transient definitive wave	7,18
F4/80 (Emr, Adgre1)	Necessary for murine macrophages development, pan macrophages marker	18
CD11b (Itgam)	Common myeloid marker, mediates leukocytes migration	10,15,19
Ly6C (Gr1)	Marker of classical monocytes, potential marker for bone marrow/monocyte-derived macrophages	22
CD14	Macrophage marker in human, co-receptor for TLR4	10,15,19
CD206 (Mrc1)	Expressed on macrophages and immature dendritic cells, play role in phagocytosis, antigen presentation, and resolution of inflammation	43,44
CD11c (Itgax)	Found on dendritic cells, monocytes, and macrophages play a role in phagocytosis, cell migration, and cytokine production	95
CD115 (Csf1r)	Receptor for Colony stimulating factor 1 (Csf-1), a cytokine which controls the production, differentiation, and function of macrophages.	91–93
CD163	Marker for monocytes and macrophages, scavenger receptor	85

Table I Summary of main macrophages markers

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declines and is replaced by BM hematopoiesis, which produces BM-derived monocytes throughout life.

Notably, the heterogeneity of macrophages is related to their origin. For instance, embryonically derived tissue-resident macrophages were shown to be able to self-renew, and their primary role is to maintain tissue homeostasis during adulthood[13]. Upon irradiation, tissue-resident macrophages are less affected than their BM counterparts and are not replenished by circulating monocytes, such as BM-derived macrophages[14,15]. Generally, tissue-resident macrophages and BM-derived macrophages exhibit distinct genetic and functional signatures, with tissue-resident shown to be more expressive of proangiogenic markers. This observation suggests that tissue-resident macrophages may have a phenotype similar to previously described "M2-like" macrophages[16].

Macrophages and OA

OA is a disease that affects most joint tissues, and increasing evidence suggests that specific phenotypes of macrophages may differentially modulate the anabolic or catabolic responses of different cell types with the onset or progression of OA [Fig. 2(A)–(C)].

Synovium

The joint synovium is comprised of a synovial lining layer and a sublining compartment. Macrophages, one of the most abundant immune cells in the synovium, are mainly located in the synovial lining layer along with fibroblasts and play a critical role in maintaining homeostasis of healthy synovial tissues[17]. Both tissue-resident (i.e., embryonically derived) synovial macrophages and non-tissue resident (i.e., BM-derived) synovial macrophages are capable of self-renewal although tissue-resident synovial macrophages can persist independently of BM hematopoiesis for prolonged periods of time.

In the synovium, tissue-resident macrophages have been proposed to originate from both primitive and transient definitive waves during embryonic development, although predominately from the latter wave. In mice, it is reported that embryonically derived synovial macrophages generated from these two waves are positive for F4/80 (a mouse-specific marker of macrophages) but negative for CD11b (i.e., F4/80^{+}/CD11b^{-}). On the contrary, macrophages both generated from the definitive wave, classified as F4/80^{lo}/CD11b^{+}/Ly6C^{+} macrophages, and those generated substantially later from BM hematopoiesis, such as CX3CR1^{+}/Ly6C^{high} macrophages, are relatively short-lived and are categorized as non-tissue resident macrophages. These non-tissue resident macrophages have been shown to differentiate into pro-inflammatory M1-like or anti-inflammatory M2-like phenotypes after homing to tissues, regulating inflammation and healing[18]. Additionally, macrophages that develop after birth generally represent a mixed phenotype (F4/80^{+}/CD11b^{+}), further complicating the distinction between embryonic and BM-derived precursors[10,15,19]. In an attempt to elucidate the origin of these differential macrophage populations, Culemann *et al.* examined the spatiotemporal composition of macrophages in the synovium. The group identified two distinct synovial macrophages subtypes: CX3CR1^{-} interstitial and CX3CR1^{+} lining macrophages. The interstitial macrophages, developed from the two early waves, were proposed to make up a pool of proliferating major histocompatibility complex class II (MHCII^{+}) macrophages that could differentiate into additional subpopulations of interstitial macrophages (resistin-like molecule (RELM)-α^{+}) or lining macrophages. Interestingly, CX3CR1^{+} lining macrophages also displayed a limited response to inflammatory stimulation, conserved their naïve state, and restricted inflammatory progression through a shield of tight junction-like structures[20].

Recent studies have demonstrated that with rheumatoid arthritis (RA), macrophages that infiltrate into the synovium are mainly migratory BM-derived macrophages, rather than tissue-resident macrophages. Supporting this evidence, BM-derived Ly6C^{high} monocytes are recruited and differentiate into macrophages at inflammatory sites in RA[21]. Furthermore, BM-derived macrophages express CD80 and CD86, and secrete high levels of pro-inflammatory mediators such as interleukin (IL)-1β and IL-12, as compared with tissue-resident macrophages. Additionally, BM-derived macrophages are located near blood vessels, in contrast to F4/80^{+} tissue-resident macrophages, implying that BM-derived macrophages are more likely to be newly recruited cells upon injury[15,22]. Interestingly, Wood *et al.* reported two subtypes of macrophages in human OA synovium: inflammatory-like macrophages (iOA) and classical macrophages (cOA) are both present in OA synovium and possess different proliferation abilities. The cOA were characterized by a remodeling phenotype and expression of the *IGFBP5* gene, while iOA were more proliferative and were enriched in *MK167* and *CTRL* genes[23].

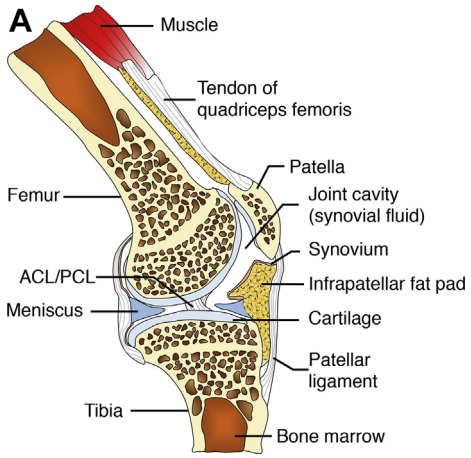

Fig. 2

A. Tissue compartments of a healthy knee joint. ACL: anterior cruciate ligament. PCL: posterior cruciate ligament. B. Tissue compartments of an OA knee joint. ① Pro-inflammatory M1-like macrophages in the synovial lining layer secrete inflammatory cytokines such IL-1, IL-6 and IL-8, as well as cartilage matrix degradation enzymes including MMPs, leading to cartilage degeneration. Although ② anti-inflammatory M2-like macrophages can release reparative mediators such as IL-4 and IL-10 into joint synovial fluid, these anti-inflammatory molecules are often not sufficient to encounter the catabolic inflammatory response, partially due to a high ratio of M1-like to M2-like macrophages[31]. High-fat diet-induced obesity results in adipocyte hypertrophy in the infrapatellar fat pad due to increased lipid storage. ③ Hypertrophic adipocytes secrete IL-6, activating ④ macrophages in both the synovium and joint fat pad to secrete IL-1 and TNF-α, leading to a vicious inflammatory cycle[103]. ⑤ Chondrocytes in OA cartilage also secrete MMPs and disintegrin metalloproteinase with thrombospondin motifs (ADAMTs)[104]. Additionally, ⑥ hypertrophic chondrocytes secrete vascular endothelial growth factor (VEGF) which leads blood vessel invasion into cartilage, an important of step of cartilage and bone remodeling[105]. Bone remodeling in OA can be driven by several factors. For example, ⑦ synovial macrophages, with a potentially M2-like phenotype, secrete transforming growth factor (TGF)-β and BMPs, facilitating osteophyte formation. Furthermore, ⑧ osteomacs, a recently discovered phenotype of macrophages within bone marrow, form a canopy over osteoblasts, supporting the bone matrix deposition function of osteoblasts. However, whether osteomacs directly contribute to ⑨ subchondral bone sclerosis or ⑩ osteophyte formation in OA remains unknown. C. Tissue compartments of an OA knee joint. It has been suggested that loss of muscle integrity and associated functional deficits due to injury or obesity may alter joint loading, leading to onset of OA. Particularly, ⑪ pro-fibrotic macrophages activate collagen-producing myofibroblasts via the TGF-β signaling pathway. While this reparative process is essential in tissue healing, it may lead to tissue fibrosis if not modulated. ⑫ Currently, the origin and phenotypic plasticity of pro-fibrotic macrophages remain unclear, although some evidence suggests that pro-fibrotic macrophages are associated with anti-inflammatory M2-like macrophages. Interestingly, recent studies demonstrate that bone marrow-derived macrophages can also transdifferentiate into myofibroblasts[106]. Additionally, ⑬ pro-fibrotic adipocytes, a potential phenotypic switch of adipocyte progenitors due to obesity, can also significantly contribute to tissue fibrosis in muscle[107]. Ligament injury often leads to abnormal loading forces on cartilage, predisposing the injured joint to OA development. In addition to altered mechanical loading, macrophage-associated inflammation provides an alternative mechanism for OA pathogenesis post-ligament injury. Specifically, ⑭ pro-inflammatory M1-like macrophages infiltrate into the injury site, releasing IL-1 and MMPs into joint synovial fluid. ⑮ These pro-inflammatory molecules can activate chondrocytes to secrete more extracellular matrix (ECM) degradation enzymes including MMPs and ADAMTs, further accelerating cartilage degradation.

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Regardless their origin, it is well recognized that synovial macrophages are an important source of post-joint injury pro-inflammatory signaling molecules, including alarmins (e.g., S100A9)[24,25] and cytokines such as IL-1 and tumor necrosis factor (TNF)-α[26,27]. Moreover, human $CD14^+$ synovial macrophages are reported to activate the production of matrix metalloproteinases (MMPs), destructive matrix-degrading enzymes, from synovial fibroblasts[28,29]. Although some macrophages, particularly the M2-like phenotype, can release anti-inflammatory cytokines including IL-4 and IL-10, these anti-inflammatory molecules are often not sufficient to counter the catabolic inflammatory response, particularly in the presence of a high pro-inflammatory (M1-like) to anti-inflammatory (M2-like) ratio[30]. As a result, these MMPs and inflammatory mediators shift the cytokine profile of joint synovial fluid in favor of inflammation with high levels of IL-1β, IL-6, and IL-8, stimulating chondrocytes to produce more extracellular matrix (ECM)-degradative enzymes and further aggravating cartilage matrix destruction. Macrophages and monocytes have also been detected in the synovial fluid of OA joints. Their presence positively correlates with joint stiffness, pain[32], and reduced quality of life[31,32].

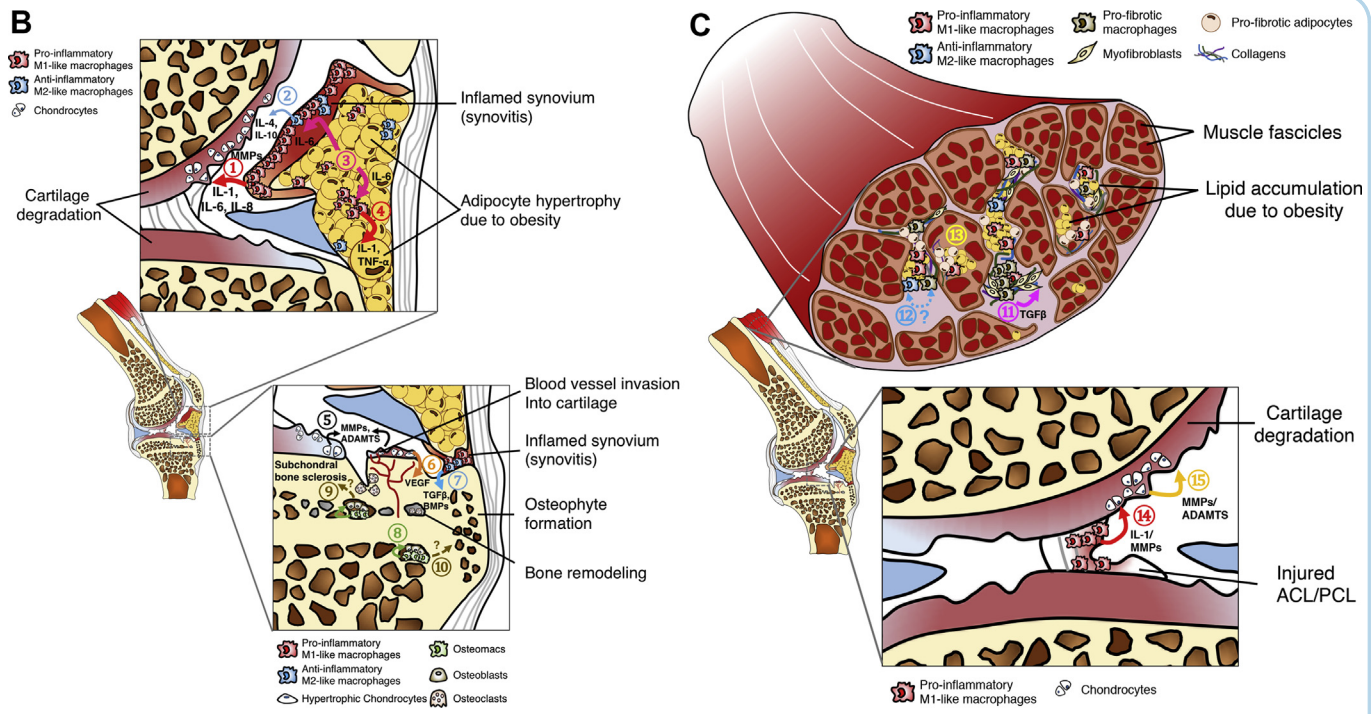

Fig. 2 Continued

Individuals with obesity and metabolic disorders such as hypercholesterolemia exhibit chronic low-grade systemic inflammation. Macrophages, particularly those accumulated in visceral fat, are involved in dysregulated systemic inflammation and may be essential contributors for obesity-associated OA. In obesity-driven OA, macrophage content increased in the synovium both with injury[33–35] and with spontaneous OA[30] in mice. For instance, Sun *et al.* demonstrated an increase in M1-like (NOS2$^+$) macrophages in OA synovium from obese mice[36]. In patients with obesity, increased macrophage content in the synovium has also been described[17].

Infrapatellar fat pad (IPFP)

The infrapatellar fat pad (IPFP), or Hoffa's pad, is an adipose tissue depot found in the knee joint. It is an active endocrine organ within the joint and a potent producer of adipokines, including adiponectin and leptin[37], and has been proposed to have an important role in the pathogenesis of OA[38]. The IPFP also contains macrophages[39,40] with an immune cell profile comparable but not identical to that of the synovium[17] and subcutaneous adipose tissue[41]. The number of macrophages in IPFP was reported to increase during OA and other in joint pathologies[42,43]. Both M1-like (CD11c$^+$) and M2-like (CD206$^+$) macrophage markers have been reported in this tissue[44]. Wei and co-workers suggest that macrophages harvested from the IPFP of diseased joints inhibit chondrogenesis of mesenchymal stem cells (MSCs)[45], supporting the notion that IPFP macrophages play a potentially detrimental role in cartilage regeneration. However, the precise function and origin of macrophages in the IPFP remain to be elucidated.

The IPFP has been shown to contain multipotent cells[46] and to increase in size and develop fibrosis in obese mice[44]. In humans, the IPFP also increases in size with age and OA[47], potentially due to adipocyte hypertrophy[43]. Interestingly, in early OA, high-fat diet induced obese mice do not have increased numbers of M1-like macrophages in IPFP relative to lean mice[44], while the study on obese OA patients have reported either no differences[39] or an increased content of M2-like (CD206$^+$) macrophages in the IPFP as compared to lean patients[43]. Thus, IPFP is a potent reservoir of macrophages, whose roles in OA development remain unknown.

Bone

During OA development, the subchondral bone compartment undergoes active remodeling, a process that is partially influenced by macrophages. Although some studies implied that bone microdamage might be the first sign of OA-related bone remodeling, followed by increased bone thickening and ultimately resulting in bone sclerosis, the sequence of cartilage degradation and subchondral bone alteration remains controversial and may likely depend on the subtypes of OA[48]. During OA progression, CD68$^+$ cells have been shown to localize in sclerotic regions of subchondral bone[49]. Increased subchondral bone turnover is also accompanied by abnormal osteophyte formation, one of the hallmarks of OA that is positively correlated with immobility and increased pain. Synovial lining macrophages have been shown to mediate formation of osteophytes in OA. The depletion of synovial macrophages by clodronate-laden liposomes (which lead to macrophage apoptosis when being phagocytized) significantly reduced the onset of osteophyte formation[50], and it is believed that transforming growth factor (TGF)-β produced by synovial macrophages plays a major role in osteophyte formation[51]. The effect of macrophage depletion on OA development will be discussed in detail in a later section of this review.

Bone remodeling is tightly regulated by osteoblasts originating from the mesenchymal lineage and osteoclasts derived from myeloid lineage. Recent studies show that in addition to resident tartrate-resistant acid phosphatase ($TRAP^+$) osteoclasts, two other monocyte-derived cell populations may be involved: 1) osteal macrophages (osteomacs) and 2) BM-derived macrophages. BM-derived macrophages are located in the BM stroma and are important in regulating hematopoiesis throughout adulthood[52]. Osteomacs, reported to be $F4/80^+/CD115^+/Mac3^+/CD169^+/TRAP^-$ cells, reside within periosteal and endosteal compartments of the bone and may play an anabolic role through stimulating osteoblasts both to increase the mineralization process as well as to promote bone healing and remodeling following fracture[53]. However, whether osteomacs respond to mechanical stimuli such as joint loading, as well as whether they directly contribute to subchondral bone sclerosis and osteophyte formation in OA development, requires further investigation.

Ligament

Ligaments are essential components for joint formation as they connect two joint bones together and provide joint stability. Hence, ligament injuries are among the risk factors for developing OA. For instance, around 50% of patients with anterior cruciate ligament (ACL) injuries show evidence of OA within 10–20 years post-trauma[54]. Immediately post-ligament injury, the malaligned joint results in abnormal loading forces on cartilage, leading to chondrocyte apoptosis, a potential mechanism for initiating the onset of OA. In addition to mechanical factors, several lines of evidence suggest that macrophages infiltrate into the injury site and regulate inflammation and healing of the ligament by secreting various mediators, providing an alternative mechanism that is driven by cytokines in modulating OA pathogenesis.

Muir *et al.* reported that ACL diseases and rupture significantly increased the number of pro-inflammatory macrophages expressing degradative enzymes including TRAP and cathepsin K (a potent collagenolytic protease) within torn canine ligament tissue, compared to dogs with intact ACL[55,56]. For humans, infiltration of $TRAP^+$ macrophages, but not cathepsin K^+ macrophages, was observed in the injured ACL tissue of patients[57], implying distinct differences between dogs and humans in proteolytic activities of injured ligaments. Nevertheless, the infiltration of macrophages plays an important role in the acute inflammatory phase post-injury (about 24–28 h), by removing tissue debris and remodeling matrix via secreted proteases and mannose receptor-mediated phagocytic routes[58,59]. However, macrophages may also decrease the mechanical properties of the injured ligament due to excess matrix degradation if the resolution of inflammation is not regulated. Importantly, ligamentous injury may result in macrophage infiltration of other surrounding joint tissues such as the meniscus[60], leading to a local pro-inflammatory response, particularly in the vascularized zone[61].

Tendon

Tendon is a fibrous, connective tissue bridging muscles to bone. Tendons are composed mainly of collagen fibrils, and their main function is to respond to mechanical forces, providing joint flexion and stability. Similar to ligaments, the instability of tendons due to injury is among the risk factors for OA onset and contributors to OA progression, but the direct influence of inflammatory tendon changes on OA remains a largely open area for investigation. The presence of M1-like macrophages in conjunction with loading may facilitate tendon repair compared to injured tendon without loading[62]. However, in $IKK\beta CA^{Scx}$ mice (a cre-mediated mouse model in which IKKβ is constitutively active in scleraxis (*Scx*) positive tendon fibroblasts), an overabundance of pro-inflammatory macrophages in tendon induced by increased NF-κB activity negatively affected tendon healing, the surrounding attachment site, and bone integrity[63]. Therefore, determining the time-course and balance of pro- and anti-inflammatory macrophages is likely critical in maintaining homeostasis and joint integrity. When macrophages are modulated using extracellular exosome vesicles to encourage a lower ratio of M1-like to M2-like macrophages, healing is improved in the Achilles tendon[64]. While some pro-inflammatory mediators potentially driven by M1-like macrophages have been identified as detrimental to tendon healing, adipose-derived mesenchymal stromal cells can modulate the tendon inflammatory response *in vitro*[65]. In obesity/type II diabetes, a higher incidence of M1-like macrophages has been reported, coupled with elevated and prolonged expression of M2-like markers, resulting in increased extracellular matrix deposition and stiffer tendons[66]. Together with altered muscle integrity, stiffer tendons could contribute to alterations in joint loading that may antagonize OA with obesity. While the ideal ratio of pro- and anti-inflammatory macrophages for tendon repair remains unknown, it appears that a certain threshold of M1-like macrophages may be beneficial. Nevertheless, chronic exposure to overabundant M1-like macrophages may be deleterious for tendon repair. Identifying threshold for unique macrophage subtypes in tendon injuries may offer therapeutic opportunities for tendon repair.

Muscle

Muscle integrity, particularly strength and structure, plays a role in the onset and progression of OA. It is plausible that altered muscle integrity and associated functional deficits would alter joint loading, leading to joint degeneration. Macrophages are one of the most active immune cells in skeletal muscle and, together with satellite cells, are involved in maintaining muscle homeostasis[67]. For example, macrophages protect against muscle atrophy by releasing insulin-like growth factor 1 in repair scenarios[68]. The tissue-resident macrophage population in muscle has been described as $CD11b^+/F4/80^+/CD11c^-/Ly6C^-/CX3CR1^{-}$[69], a phenotype which activates the innate immune response to injury. Interestingly, both $Ly6C^+$ and $Ly6C^-$ monocytes migrate to muscle post-muscle injury[70]. It is postulated that incomplete or maladaptive repair may induce muscle weakness in a low-level systemic inflammatory environment, and macrophages are implicated as one of the key cell types in this process[71].

OA patients with obesity consistently demonstrate muscle weakness[72]. It appears that human patients with composition-based obesity and sarcopenic obesity, but not sarcopenia or muscle wasting alone, are positively associated with knee OA[73]. In muscle of lean athletes, lipid and adipose stores can be found within and between muscle fibers, presumably to provide a readily-available fuel source[74]. With obesity, pro-inflammatory M1-like macrophages are present in the quadriceps muscle of mice[75], rats[76,77], and humans[75,78]. Other inflammatory alterations in skeletal muscle with obesity are reviewed in detail by Wu and colleagues[79].

Nutrient overload results in the development of additional lipid stores within and between muscle fibers[71], and intracellular lipid accumulation is associated with metabolic disturbance and insulin resistance[80]. Macrophages and T-cells can migrate and infiltrate local fat and/or lipid stores within the muscle[79] and alter the inflammatory status of the lipid and muscle. In fact, T-cells and macrophages are located predominately in intramuscular and perimuscular adipose tissue in skeletal muscle of individuals with obesity, supporting the notion that intramuscular adipose depots can become active inflammatory sites within this tissue[78]. There is

known lipid-muscle cross-talk *in vitro*, where inflammation from obese adipocytes can induce muscle atrophy[81]. Furthermore, our group demonstrated that high-fat diet induced obese mice receiving muscle-target gene therapy for follistatin, an activin binding protein, showed increased muscle mass coupled with both decreased OA severity and numbers of M1-like ($CD11b^+/CD11c^+$) macrophages in visceral fat compared to wild type obese mice[82].

While the direct relationship among muscle inflammation, muscle macrophage populations, and OA is yet to be established, the ability of muscle macrophages to directly and indirectly influence the homeostasis of joint organ system is an interesting therapeutic concept for future studies.

Modulation of macrophages and their associated phenotypes as immunotherapies for OA and cartilage repair

Macrophage depletion as a therapeutic intervention

As macrophages play a crucial role in orchestrating other cells in initiation of OA immunopathogenesis, numerous studies have investigated whether cartilage health and joint integrity would benefit from ablation of macrophages. For example, the removal of synovial lining macrophages by intra-articular injection of clodronate-laden liposomes significantly decreased gene expression of MMP-3 and MMP-9 in the synovium (but not in cartilage), along with reducing TGF-β-mediated osteophyte formation in a collagenase-induced mouse model[50,83,84]. Additionally, to understand the effect of macrophage depletion on ligament healing, Chamberlain and co-workers systemically depleted macrophages via intravenous injection of clodronate-laden liposomes 2 days prior to injury induction in medial collateral ligament (MCL) in rats. The authors observed that the MCL of the rats receiving macrophage depletion showed deteriorated mechanical strength along with decreased number of M1-like ($CD68^+$) and M2-like ($CD163^+$) macrophages compared to the MCL of the rats without treatment[85].

Interestingly, systemic macrophage depletion in mice using clodronate-laden liposomes (4 h prior and then daily post-injury for 4 days) appeared to be beneficial for the healing process of injured Achilles tendon by enhancing its ultimate tensile strength and Young's modulus[86]. Similarly, using tendon as a graft, weekly systemic macrophage depletion by liposomal clodronate (up to 42 days) was found to improve the healing and mechanical strength at the tendon–bone interface in a model of ACL reconstruction in rats[87]. The disparities in the results of these studies imply that to achieve a beneficial effect of macrophage depletion on wound healing, depletion strategies, particularly routes (systemic vs local) and frequency (short-term vs long-term), could be tissue-dependent. The reasons for the varying effectiveness of tissue-specific depletion approaches may be associated with the phenotypic identities of macrophages within a given joint issue. Moreover, the degree and duration of vascularity of the tissues during the healing process may influence tissue-specific macrophage depletion outcomes, as the vessel channels serve as the entry sites of monocytes/macrophages.

These earlier findings have led to the hypothesis that depletion of macrophages could mitigate OA in obesity. In our recent study, macrophage Fas-induced apoptosis (MaFIA) mice – a transgenic mouse model that allows conditional depletion of macrophages expressing colony stimulating factor 1 receptor (CSF1R) upon administration of the small molecule AP20187 – were placed on a high-fat diet and underwent destabilization of the medial meniscus (DMM) surgery to induce knee OA[88]. $CSF1R^+$ macrophages were systemically depleted 2 weeks prior to DMM surgery in obese MaFIA mice. Surprisingly, systemic depletion of $CSF1R^+$ macrophages did not attenuate the severity of OA in obese mice; instead, it induced inflammation and led to a massive infiltration of $CD3^+$ T cells and neutrophils, but not B cells, into the injured joints. Furthermore, ablation of synovial macrophages by liposomal clodronate led to oxidized low-density lipoproteins (ox-LDL)-induced catabolic processes, including increased pro-inflammatory mediators as well as increased infiltration of monocytes and neutrophils into the joint synovium[89,90]. Taken together, these findings indicate that macrophages modulate the homeostasis of immune cells with diet-induced obesity in part by regulating levels of ox-LDL in the joint synovial fluid.

While it is well-recognized that the CSF1/CSFR1 signaling axis is required for maintenance of the BM-derived macrophage populations in adult mice[91,92], recent studies have demonstrated that $CSF1R^+/Kit^-/CD45^+$ cells can be detected in the YS at 20–25 somite pairs, and later in the head regions of the embryo from E9.5, and the fetal liver from E10.5 onwards[7,93]. These findings suggest that a subset of $CSF1R^+$ macrophages is developed during the embryonic stage and independent of BM. In addition, using *Csf1r*^{creER}*R26-tdTomato* and *Csf1rGFP* mice, Culemann *et al.* observed that tissue-resident interstitial macrophages in embryonic joint synovium express CSF1R and Ki67 (proliferation marker). These results suggest that in a MaFIA mouse model, both tissue-resident and BM-derived macrophages can be targeted by AP20187 as long as these macrophages express CSF1R. Nevertheless, there is still the possibility that certain subpopulations of macrophages may lose their ability to express CSF1R at later stages of development or in adults[94], in which case the treatment of AP20187 would not eliminate $CSF1R^-$ macrophages in MaFIA mice.

Despite the fact that several animal models of broad macrophage depletion, including MaFIA and diphtheria toxin receptor (DTR) transgenic mice, have greatly expanded our knowledge on macrophage function in OA, these techniques cannot precisely target specific phenotypes of macrophages without affecting other myeloid lineages such as dendritic cells and neutrophils. For example, CD11c, a conventional marker for pro-inflammatory macrophages, is also expressed by dendritic cells, and some subsets of neutrophils. Furthermore, depletion of $CD11c^+$ macrophages has been shown to lead to distinct neutrophilia responses in three commonly used DTR-based depletion mouse models[95]. Thus, the choice of transgenic mouse lines for the purpose of macrophage depletion must be considered in experimental settings where other myeloid cells may be involved, and caution should be exercised in interpreting the results of these models.

Biomaterial-based and cell engineering-based modulation of macrophages

Biomaterial scaffolds are widely employed to promote cartilage repair. However, scaffold-induced foreign body reaction can lead to innate immune responses from host myeloid cells including neutrophils and macrophages. While the prolonged presence of pro-inflammatory M1-like macrophages is detrimental for tissue repair, it is generally accepted that anti-inflammatory M2-like macrophages facilitate tissue regeneration. Thus, several studies aim to harness the healing capacities of M2-like macrophages by designing scaffolds that can modulate macrophages toward a reparative phenotype. For example, synthetic scaffolds (such as those made of polyethylene glycol, PEG) can elicit a chronic inflammatory response including neutrophil infiltration and the loss of M2-like macrophage markers, while biological scaffolds (such as those derived from extracellular matrix, ECM) induce high expression of the surface marker CD206 on macrophages[96]. This study, although conducted in a mouse model of volumetric muscle loss injury, may provide important insights into the choice of scaffold for cartilage regeneration. While biological scaffolds can

promote anti-inflammatory responses, they are known to lack mechanical strength as compared to synthetic ones. One commonly used strategy to improve mechanical properties of a biological scaffold is through cross-linking; however, it is reported that a biological scaffold cross-linked by carbodiimide switches from an M2-like macrophage dominant profile to M1-like macrophage dominant[97]. Indeed, a recent study shows that macrophage polarization in response to the stiffness of biological scaffolds is dependent on the cross-linking agent, suggesting that both chemical and physical properties of scaffolds are essential in fine-tuning biomaterials to promote a pro-regenerative macrophage phenotype[98].

While many researchers focus on modulating macrophage-induced inflammation through the use of biomaterial scaffolds, some seek to use macrophages themselves as a means of drug delivery or therapy. Macrophages possess an intrinsic homing ability, allowing them to migrate to injury or inflammatory sites including arthritic joints. Visser *et al.* developed a therapy using the transport of nanoparticle-encapsulated drugs inside autologous M1-like macrophages to induce transient phagosome maturation arrest[99]. Recently, by using clustered regularly interspaced short palindromic repeats (CRISPR)-Cas9 genome editing, our group created a cell-autonomous system (i.e., "SMART" cells) in which mouse induced pluripotent stem cell (miPSC)-derived chondrocytes can modulate inflammation in an auto-regulated manner both *in vitro* and in an inflammatory arthritis mouse model. Specifically, once these "SMART" chondrocytes sense specifically targeted inflammatory cytokines (e.g., IL-1 or TNF-α), they release a corresponding biologic drug (e.g., IL-1Ra and or soluble TNFR1) to attenuate inflammatory signals[100,101]. Similarly, self-regulating "SMART" macrophages can be engineered in a manner that not only homes these cells to inflammatory/injury sites, but also enables cytokine-activated feedback-controlled capabilities for effectively targeted therapeutic drug delivery for joint diseases[102].

Conclusions and future perspectives

Accumulating evidence suggests that macrophages do not have only two polarized states (i.e., M1-like vs M2-like), but rather should be categorized as a large family of cells with broad and potentially plastic phenotypes that are capable of defending hosts against pathogens, maintaining tissue homeostasis, and/or providing pro- or anti-inflammatory signals that are origin- and tissue microenvironment-dependent. However, whether tissue-resident macrophages in different joint compartments arise from the same or distinct waves during embryonic development remains to be elucidated. Furthermore, although many studies have implied that tissue-resident macrophages appear to be pro-healing while circulating BM-derived monocytes/macrophages tend to be pro-inflammatory in tissue injury, whether such an immune activation holds true in OA pathogenesis requires further investigation.

Although the origin and development of tissue-resident and BM-derived macrophages have been investigated in the synovium, macrophage lineage development in other joint tissues remains poorly understood, a significant gap we wish to address, as elucidating the heterogeneity of macrophage populations in these tissues may provide key insights into OA pathogenesis. Moreover, we believe that the exploration of approaches for modulating macrophages, either via biomaterials or engineered cells, could be an important future research direction for developing novel therapeutics for OA.

Author contributions

All authors contributed substantially to drafting the article or revising it critically for important intellectual content.

Conflict of interest

All authors declare no conflict of interest. No benefits in any form have been received or will be received from a commercial party related directly or indirectly to the subject of this article.

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