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The interactions of macrophages, lymphocytes, and mesenchymal stem cells during bone regeneration

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Bone regeneration and repair are crucial to ambulation and quality of life. Factors such as poor general health, serious medical comorbidities, chronic inflammation, and ageing can lead to delayed healing and nonunion of fractures, and persistent bone defects. Bioengineering strategies to heal bone often involve grafting of autologous bone marrow aspirate concentrate (BMAC) or mesenchymal stem cells (MSCs) with biocompatible scaffolds. While BMAC shows promise, variability in its efficacy exists due to discrepancies in MSC concentration and robustness, and immune cell composition. Understanding the mechanisms by which macrophages and lymphocytes – the main cellular components in BMAC – interact with MSCs could suggest novel strategies to enhance bone healing. Macrophages are polarized into pro-inflammatory (M1) or anti-inflammatory (M2) phenotypes, and influence cell metabolism and tissue regeneration via the secretion of cytokines and other factors. T cells, especially helper T1 (Th1) and Th17, promote inflammation and osteoclastogenesis, whereas Th2 and regulatory T (Treg) cells have anti-inflammatory pro-reconstructive effects, thereby supporting osteogenesis. Crosstalk among macrophages, T cells, and MSCs affects the bone microenvironment and regulates the local immune response. Manipulating the proportion and interactions of these cells presents an opportunity to alter the local regenerative capacity of bone, which potentially could enhance clinical outcomes.

Article focus

- Investigating the interactions of macrophages, lymphocytes, and mesenchymal stem cells (MSCs) in bone regeneration.
- Exploring how the immune cells influence the osteogenic potential of MSCs and bone healing.
- Evaluating potential strategies for optimizing cell-based therapies for bone regeneration by utilizing specific interactions among immune cell interactions with MSCs.

Key messages

- Macrophages and lymphocytes play critical roles in bone regeneration, with different subtypes promoting either bone formation or resorption. Their interaction with MSCs is crucial for effective bone healing.
- The balance between pro-inflammatory and anti-inflammatory immune cells significantly influences the osteogenic potential of MSCs, with M2 macrophages, helper T2 (Th2) and regulatory T (Treg) cells being particularly supportive of bone formation.
- Enhancing the regenerative capacity of bone marrow aspirate concentrate (BMAC) by modulating immune cell composition and interactions offers a promising means to improve clinical outcomes in bone repair and regeneration.

Strengths and limitations

- This study provides a comprehensive overview of the crosstalk between immune cells and MSCs, highlighting the potential for targeted immunomodulation to promote bone regeneration.

- The manuscript identifies specific immune cell subtypes that can be targeted to improve the efficacy of BMAC-based therapies for bone healing, and provides practical insights for clinical applications.
- The variability in outcomes due to differences in MSC concentration and immune cell composition in BMAC highlights the challenge of achieving consistent results, necessitating further research to standardize therapeutic approaches.

Introduction

Compromised bone healing often affects a patient's function, potential for ambulation, and quality of life. Factors that could lead to undesirable clinical outcomes include: poor overall health; serious medical comorbidities such as diabetes and chronic renal disease; obesity; medications; and ageing.[1,2] Chronic inflammation is present in many of these scenarios,[3] and is associated with several commonly observed conditions including corticosteroid-induced osteonecrosis, fracture nonunions,[4-6] and persistent bone defects.

Recently, bioengineering strategies have been developed for augmenting bone regeneration and repair. One approach includes grafting of reparative cells and a biocompatible scaffold. The most common source of cellular components is autologous bone marrow aspirate concentrate (BMAC), which fulfills the principle of "minimal manipulation" mandated by the USA Food and Drug Administration (FDA).[7] Another strategy is the use of stem cell therapy, which is permissible by regulatory bodies in some countries despite these cells undergoing more than "minimal manipulation".

BMAC is an autologous, safe, and reliable source of cells that has demonstrated a solid foundation with sufficient biological basis for bone regeneration. Some applications of BMAC include the enhancement of healing of osteonecrotic lesions during core decompression[8,9] and healing of long bone fractures in pre-clinical animal studies, as well as in clinical trials.[10]

However, the outcomes of BMAC use are not uniformly positive. In a study by Cuomo et al,[11] neither bone marrow aspirate (BMA) nor mesenchymal stem cell (MSC)-enriched BMA mixed with demineralized bone matrix resulted in reliable healing of a 6 mm critical-sized bone defect in the rat femur. The authors suggested that the number of MSCs, the presence of an enhanced osteoinductive signal (e.g. bone morphogenic protein-2 (BMP-2)), or the variability of the carrier are among many contributing factors to deficiency of bone formation in this model.

Clinical grafting of progenitor cells for fracture nonunion is safe and effective. In one study, the radiological outcome evaluated by preoperative and four-month postoperative CT scans, for the treatment of nonunions, was dependent on the percentage of progenitor cells present.[12] BMAC contains a mean MSC concentration of 0.001% detected by flow cytometry; thus, the quality of the BMAC is an important factor when considering cell-based therapy.[13] Furthermore, a recent meta-analysis of the use of BMAC for the treatment of small non-critical size fracture nonunions reported a healing rate of only 71% to 77%. Although there are reports that MSCs alone can promote bone formation,[14,15] bone union was achieved in only 4% to 59% of cases using MSCs alone without immune cells.[16] These healing rates in small defects suggest that substantially worse results are anticipated for larger critical-size bone defects. These results also highlight the importance of immune cells as a source of osteoinductive paracrine signals. Not only are immune cells involved, but the presence of endocrine/metabolic pathways and environmental factors also influence the process of bone formation.

Current clinical and pre-clinical reports together advocate for further opportunities for the optimization of BMAC as a cell-based therapy. The strategy could encompass specific targeting of the deficiencies of BMAC including the low progenitor cell number, suboptimal immune cell composition, or the variability of the delivery biomaterial. The purpose of this review was to summarize the roles of major immune cell components, including macrophages and lymphocytes (T cells) in BMAC, in terms of their effects on osteogenesis, and to identify the knowledge gap and potential opportunities to enhance osteogenesis by a minimal manipulative cytotherapeutic approach.

Major cellular components of the bone marrow

MSCs have self-renewal and multipotent differentiation capabilities, and are able to differentiate into various cell types such as osteoblasts, adipocytes, chondrocytes, myotubes, fibroblasts, and more. Currently, MSCs find broad applications in cell-based therapies owing to their immunomodulatory properties and regenerative potential. In the bone marrow, MSCs constitute a very small fraction, ranging from 0.001% to 0.01% of nucleated cells, while macrophages (granulocytic lineage) account for 40% to 55%, and T cells account for up to 25%.[17] However, in addition to the potential differentiation into osteoblasts, MSCs influence macrophages and T cells by secreting paracrine factors and extracellular vesicles (EVs), thereby contributing to bone formation. The relevance of macrophages and T cells interacting with MSCs in facilitating bone formation and remodelling is briefly outlined below. Nonetheless, other immune cells also play important roles in regulating bone generation, including neutrophils and mast cells in tandem with MSCs, macrophages, and T cells; these interactions will also be briefly discussed.

Macrophages

Macrophages are members of the monocyte/macrophage/foreign body giant cell/osteoclast/dendritic cell lineage. Macrophages are present in most tissues, where they play crucial immunomodulatory roles by recognizing, engulfing, and degrading cellular debris and pathogens. In the non-stromal cell population of the bone marrow, 50% of cells are white blood cells (WBCs), i.e. monocytes/macrophages, polymorphonuclear leucocytes, mast cells, and their precursors; 25% are in the erythropoietic lineage; and the balance, about 25%, are in the T cells lineage.[18] Macrophages present antigens to T cells and induce the expression of co-stimulatory molecules on antigen-presenting cells. Macrophages can alter their polarization phenotype in response to local environmental cues.[19] Activated macrophages are typically classified into two general phenotypes: the pro-inflammatory (M1) and anti-inflammatory (M2) macrophage phenotypes.[20,21] M1 macrophages are induced by inflammatory cytokines such as tumour necrosis factor-alpha (TNF-α) and interferon-gamma (IFN-γ) from helper T1 (Th1) cells, as well as inflammatory

stimuli like bacterial lipopolysaccharide (LPS).[22] M1 macrophages release high levels of pro-inflammatory cytokines such as interleukin-1-beta (IL-1β), IL-6, TNF-α, and IFN-γ.[23] Conversely, M2 macrophages are induced by Th2 cytokines such as IL-4 and IL-13, and they secrete anti-inflammatory cytokines like IL-10 and transforming growth factor-beta (TGF-β).

Macrophages can differentiate into osteoclasts involved in bone resorption and metabolism.[24] Furthermore, it has been reported that M1 macrophages are more prone to differentiate into osteoclasts rather than naïve or M2 macrophages.[25] Osteoclasts are particularly involved in bone resorption and are promoted in inflammatory environments. Inflammatory cytokines such as IL-1 and TNF-α secreted by M1 macrophages enhance the production of receptor activator of nuclear factor kappa-B ligand (RANKL), which promotes osteoclast formation and activity. Furthermore, inflammatory cytokines IFN-γ and TNF-α induce apoptosis in bone marrow mesenchymal stem cells (BMMSCs), inhibit collagen and proteoglycan synthesis, and thereby impede bone formation.[26,27] Consequently, during inflammation, the bone resorptive activity of osteoclasts can outpace the bone-forming ability of osteoblasts, greatly contributing to inflammatory bone loss. Therefore, the balance between M1 and M2 phenotypes plays a crucial role in various microenvironments including bone.[28] This suggests a potential for immunomodulation at target sites by adjusting this macrophage balance, for the purpose of bone regeneration.

T cells

T cells comprise approximately 25% of the non-stromal cells in the bone marrow. They originate from haematopoietic stem cells in the bone marrow and mature in the thymus. T cells are further divided into αβ and γδ T cells; αβ T cells consist of CD4+ Th cells and CD8+ cytotoxic T cells. CD4+ Th cells are the most extensively studied subset. CD4+ Th cells interact with other immune cells via surface receptors and modulate activation states by secreting cytokines.[29] CD8+ cytotoxic T cells play a crucial role in eliminating intracellular pathogens and emerging neoplasms.

CD4+ Th cells can be subdivided into different subsets based on their cytokine expression profiles, such as Th1, Th2, Th17, and regulatory T (Treg) cells. Th1 cells are polarized by IL-12, produce IFN-γ and TNF-α, induce cell-mediated immune responses, and regulate the activation of M1 pro-inflammatory macrophages and inflammatory reactions.[30,31] Th2 cells, stimulated by IL-2 and IL-4, secrete IL-4, IL-5, IL-10, and IL-13 and regulate immune responses such as activation of B lymphocytes, eosinophils, and M2 macrophages.[32,33] Th17 cells are stimulated by cytokines like IL-6 and IL-23, secrete IL-17 and IL-22, and participate in inflammatory and autoimmune responses.[34,35] Treg cells, induced by cytokines such as TGF-β, IL-2, and IL-10, are involved in suppressing autoimmune responses and inflammation.

Generally, cytokines produced by Th1 and Th17 cells exhibit pro-inflammatory properties, while those from Th2 and Treg cells demonstrate anti-inflammatory effects. The inflammatory microenvironment has secondary effects on both osteoclasts and osteoblasts; thus, the balance between Th1/Th2 cells and Th17/Treg cells has great relevance to the homeostatic equilibrium between bone resorption and formation.

T cells are believed to play important roles in coordinating metabolism and assist in the process of tissue regeneration. For example, conditioned medium from human CD4+ T cells has been shown to statistically significantly upregulate the expression of Runt-related transcription factor 2 (Runx2), osteocalcin, alkaline phosphatase (ALP), and bone sialoprotein in allogenic MSCs, enhancing mineralization of bone in culture of MSCs.[36] Therefore, it is important to delineate the roles of macrophages and T cells, and their crosstalk with stromal cells from BMA, in order to exploit their unique characteristics and enlist them as supporting factors for bone regeneration.

Neutrophils

Neutrophils are a subset of granulocytes derived from the haematopoietic stem cell lineages and are part of the innate immune system; neutrophils exhibit chemotaxis, phagocytosis, and bactericidal activity.[37,38] Neutrophils are the first inflammatory cells to migrate to the injury site in response to chemotactic stimuli from resident macrophages, and participate in the clearance of bacteria, dead cells, and debris.[39] Additionally, neutrophils secrete inflammatory and chemotactic mediators such as IL-6 and CCL2, which recruit monocytes/macrophages.[40,41] These recruited monocytes/macrophages influence MSC migration and osteogenic differentiation.[42] However, excessive and continued neutrophil-induced inflammation may contribute to impaired fracture healing, by heralding a state of chronic inflammation.[3,43]

Mast cells

Mast cells (MCs), derived from the haematopoietic stem cell lineage, are tissue-resident immune cells well known for promoting allergic reactions.[44] Like neutrophils, MCs are part of the innate immune system and are capable of phagocytosis. They regulate vascular permeability and blood flow to initiate the rapid recruitment of effector cells such as neutrophils, eosinophils, and natural killer cells.[45] MCs store and newly synthesize mediators, including cytokines and enzymes, which can be rapidly released in response to stimuli in acute inflammation or allergic reactions.[46] These mediators include histamine, IL-6, and TNF-α, which promote osteoclast formation, and IL-1 and TNF-α, which inhibit osteoblast activity, thereby promoting bone resorption and inhibiting bone formation. Conversely, MCs can promote bone formation through TGF-β and potentially reduce osteoclast formation and bone resorption via IL-12. MCs also enhance MSC proliferation and migration.[47] However, the effects on bone metabolism remain unclear, as studies using MC-deficient mice have shown contradictory results depending on the mouse model used.

Crosstalk between major cell types

MSCs and macrophages

MSCs and macrophages mutually influence each other, promoting osteogenesis. Studies using human buffy coats showed that factors secreted by pro-inflammatory macrophages statistically significantly increased MSC adhesion and migration, whereas factors from anti-inflammatory macrophages enhanced MSC osteogenic activity and cell migration.[48] However, research utilizing human inflammatory synovium revealed that only the conditioned medium from

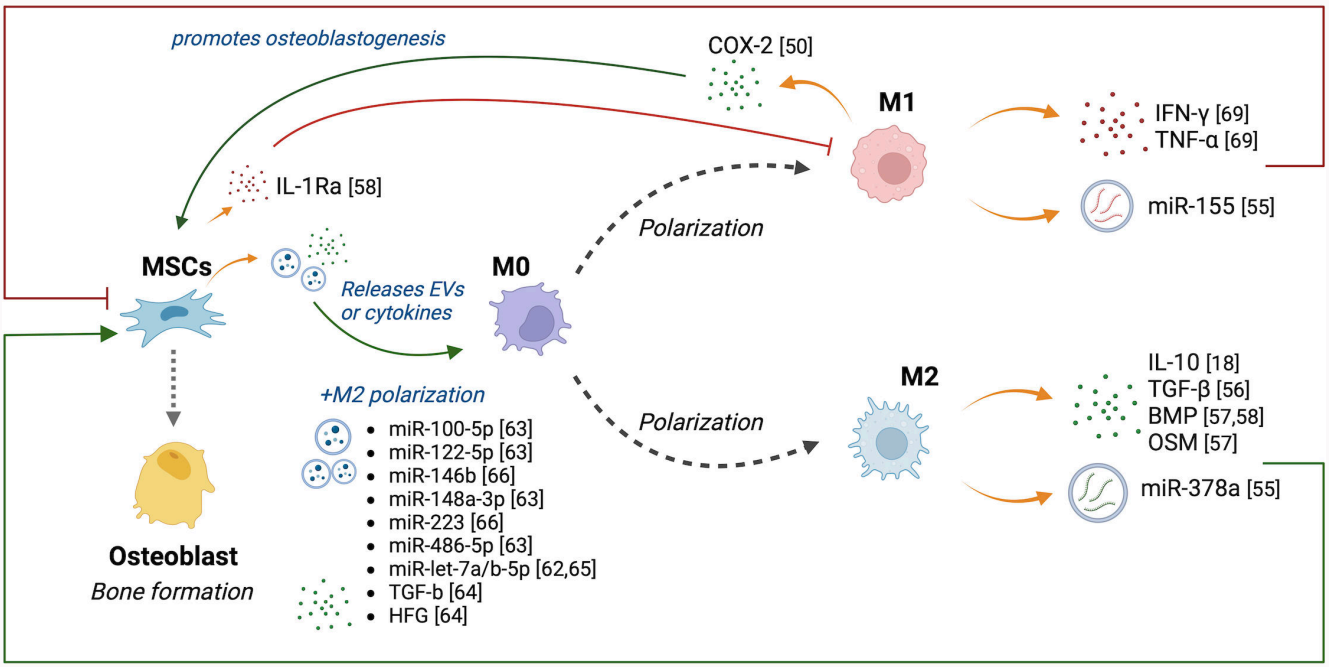

Fig. 1
Crosstalk between mesenchymal stem cells (MSCs) and macrophages. MSCs promote macrophage polarization into the anti-inflammatory M2 phenotype by the cytokines and extracellular vesicles (EVs) described. The cytokine interleukin-1 receptor antagonist (IL-1Ra) from MSCs inhibits polarization of macrophages into the pro-inflammatory M1 phenotype. Cytokines and EVs secreted by M2 macrophages promote osteogenesis of MSCs, while cytokines and EVs secreted by M1 macrophages inhibit MSC osteogenesis. BMP, bone morphogenic protein; COX-2, cyclooxygenase-2; HFG, hepatocyte growth factor; IFN-γ, interferon-gamma; miR, microRNA; OSM, oncostatin M; TGF-β, transforming growth factor beta; TNF-α, tumour necrosis factor-alpha.

anti-inflammatory macrophages enhanced MSC migration, with no statistically significant impact observed with pro-inflammatory macrophage-conditioned medium.[49]

In studies involving direct co-culture of MSCs and macrophages, it has been reported that the initial inflammatory phase regulated by M1 macrophages promotes osteogenesis by MSCs via the COX-2-PGE2 pathway.[50] Macrophages derived from human monocytic leukaemia THP-1 cell line secrete IL-23 in the inflammatory environment, which activates the signal transducer and activator of transcription 3 (STAT3) and β-catenin pathways, thereby enhancing expression of markers of bone formation and osteogenic differentiation by MSCs.[51] Moreover, bone formation was enhanced by promoting the differentiation of inflammatory M1 macrophages into anti-inflammatory M2 macrophages 72 hours after the initial inflammatory phase,[52,53] emphasizing the importance of M1 macrophages initially and the early inflammatory environment in bone formation.[42] Furthermore, recent studies have revealed that EVs, containing proteins and microRNAs and other molecules, are endocytosed by target cells, where they exert their functional influence.[54] Enrichment of miR-155 in the EVs of M1 macrophages decreased osteogenic differentiation of MSCs, while treatment of MSCs with miR-378a, enriched in the EVs of M2 macrophages, increased MSC osteoinductive gene expression.[55] Additionally, polarized M2 macrophages release TGF-β, promoting osteogenesis by MSCs;[56] BMP-2 secreted by M2 macrophages also enhances bone differentiation,[57,58] suggesting that macrophages in the M2 polarized phenotype may have a greater impact on MSC osteogenesis compared to M1 macrophages. MSCs have also been shown to possess anti-inflammatory properties and immunomodulatory functions. MSCs were shown to regulate macrophage polarization, phagocytosis, and metabolism.[59] In one study, naïve macrophages cultured with MSCs promoted the secretion of the proinflammatory cytokines TNF-α and IL-12. In contrast, M1 macrophages cultured with MSCs shifted towards an M2 macrophage phenotype.[60] One of the MSC-derived EVs, miR-181c, has been found to inhibit the expression of Toll-like receptor 4 (TL4) and reduce the expression of inflammatory factors such as TNF-α and IL-1β.[61] Furthermore, MSC-derived EVs promoted polarization towards the M2 phenotype, leading to enhanced expression of anti-inflammatory cytokines.[62,63] Additionally, it has been observed that the polarization effect of MSC-derived EVs towards the M2 phenotype is more pronounced when MSCs are pre-treated with pro-inflammatory substances or cytokines, such as LPS or elevated reactive oxygen species (ROS).[64–66] MSC-derived EVs regulate macrophage polarization toward anti-inflammatory M2 macrophage subtypes, especially when inflammatory cytokines are present. In summary, the interaction between MSCs and macrophages influences the process of osteogenesis (Figure 1). The presence of macrophages was generally shown to enhance osteogenesis of MSCs alone, with M2 macrophages showing a greater beneficial effect on bone formation compared to the M1 phenotype.

MSCs and T cells

The absence of T cells in mice has been identified as a determinant for decreased differentiation and proliferation of MSCs, emphasizing the substantial crosstalk between T cells and MSCs.[67] Focusing on the relationship between MSCs and

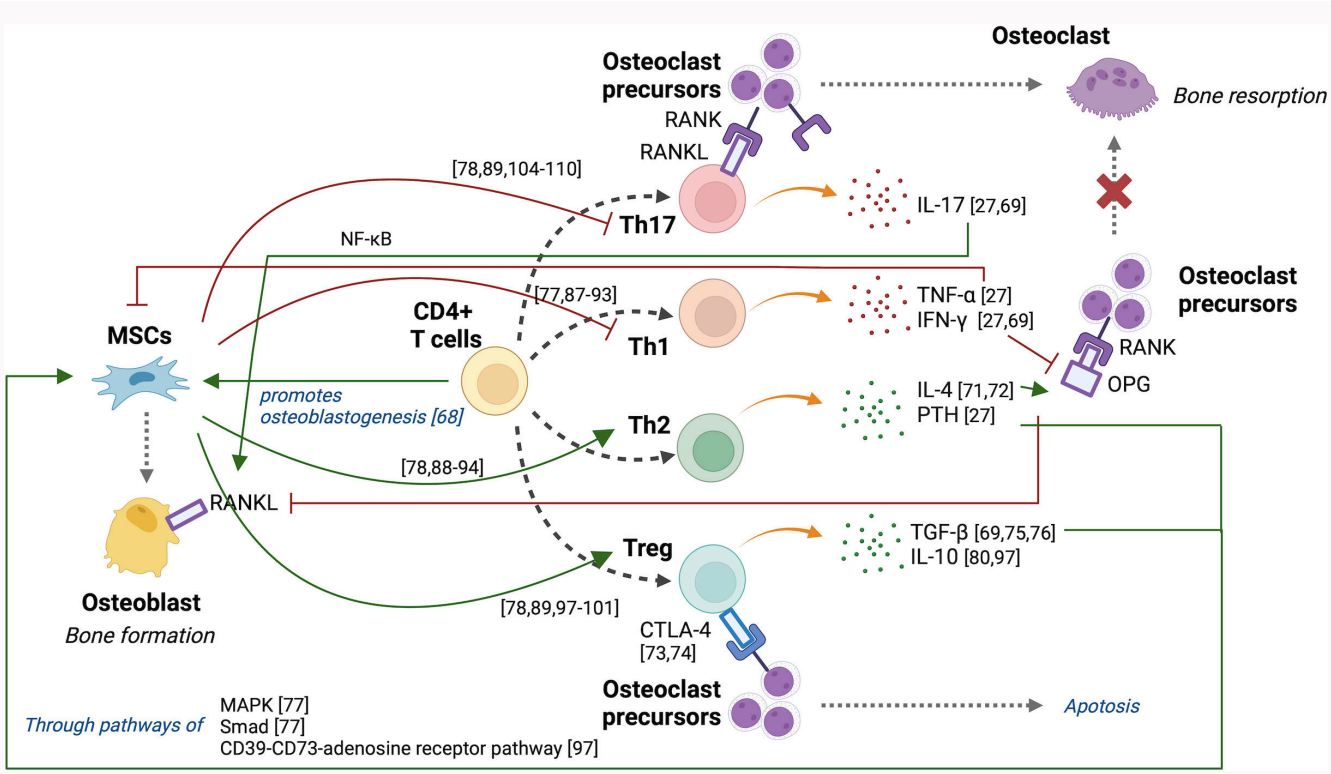

Fig. 2

Crosstalk between mesenchymal stem cells (MSCs) and T cells. MSCs promote differentiation of CD4+ T cells into anti-inflammatory Th2 and regulatory T (Treg) cells, and inhibit differentiation of CD4+ T cells into inflammatory Th1 and Th17 cells. Th2 and Treg cells promote osteogenesis of MSCs, while Th1 cells inhibits MSC osteogenesis. Treg cells induce apoptosis of osteoclast precursors, while Th17 cells promote osteoclastogenesis by receptor activator of nuclear factor-kappa B ligand (RANKL) and cytokines. Th2 cytokines increase osteoprotegerin (OPG) and inhibit differentiation into osteoclasts, while Th1 cytokines decrease OPG and promote differentiation into osteoclasts. CTLA-4, cytotoxic T-lymphocyte associated protein 4; IFN-γ, interferon-gamma; MAPK, mitogen-activated protein kinase; NF-κB, nuclear factor-kappa B; PTH, parathyroid hormone; TGF-β, transforming growth factor beta; Th, T helper; TNF-α, tumour necrosis factor-alpha.

cytokine-releasing CD4+ Th cells, the impact of T cells on MSCs is summarized below.

Activated T cells promoted the secretion of BMP-2 by MSCs, leading to enhanced bone formation.[68] Additionally, conditioned media from human CD4+ T cells, but not CD8+ T cells, promoted bone formation in allogenic MSCs.[36] CD4+ T cells consist of subsets with inflammatory Th1, Th17, and anti-inflammatory Th2, Treg characteristics. High levels of Th1 cytokines, such as IFN-γ and TNF-α, were correlated with decreased new bone formation.[27] Furthermore, Th1 cells, which promote inflammation, inhibit osteoprotegerin (OPG) expression via IFN-γ production, leading to an increase in the RANKL/OPG ratio and promotion of osteoclast formation.[69] Conversely, Th2 cytokines, such as IL-4 and IL-13, suppress RANKL expression by osteoblasts, enhance OPG expression, and decrease the overall RANKL/OPG ratio. These results suggest that Th2 cytokines decrease osteoclast formation and promote osteoblast activity.[70–72]

Treg cells can inhibit osteoclast formation through direct contact with high expression of cytotoxic T-lymphocyte-associated antigen 4 (CTLA-4) by Treg cells and cytokine-dependent mechanisms.[73,74] Treg cells promote the proliferation and differentiation of osteoblasts by activating intracellular effectors such as mitogen-activated protein kinases (MAPKs) and Smad-related proteins, which induce differentiation of MSCs to osteoblasts through the secretion of TGF-β.[69,75,76] Furthermore, treatment of MSCs with dihydroepiandosterone (DHEA) in a mouse model increased the proportion of Tregs, and resulted in increased osteoblastogenesis and osteogenesis.[77] Additionally, Tregs have been shown to enhance the immunomodulatory properties of MSCs through the secretion of anti-inflammatory factors such as IL-10.[78–80]

Unlike Treg cells, Th17 cells can promote osteoclast formation through both direct and indirect mechanisms. Th17 cells directly express RANKL on their surface, stimulating the proliferation and differentiation of osteoclast precursors.[81,82] IL-17 secreted by Th17 cells indirectly induced the expression of macrophage colony-stimulating factor (M-CSF) and RANKL on the surface of MSCs, promoting osteoclast formation.[81,83,84] Furthermore, many cytokines produced by Th17 cells induced the production of inflammatory factors, enhanced the expression of NF-κB, and further promoted RANKL expression. IL-17 is also associated with migration and motility of MSCs.[4] Additionally, a study using mouse bone marrow MSCs found that IL-17 enhances MSCs' immunosuppressive function by increasing the expression of inducible nitric oxide synthase (iNOS) and subsequent production of nitric oxide (NO).[85] Similarly, Th1 cells have also been found to enhance the immunomodulatory functions of MSCs through the secretion of proinflammatory cytokines.[86,87]

In summary, anti-inflammatory Th2 and Treg cells are involved in bone formation, while pro-inflammatory Th1 and

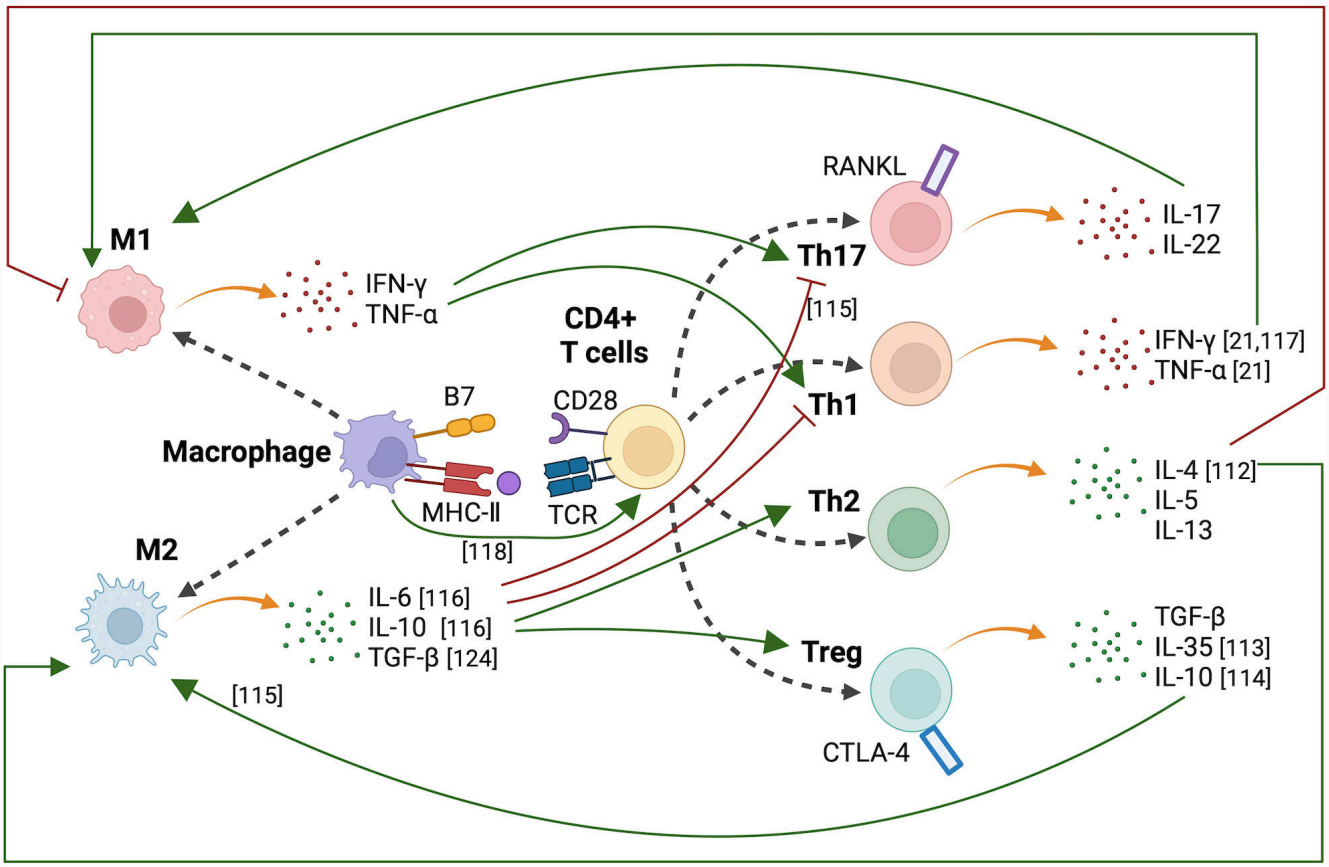

Fig. 3
Crosstalk between macrophages and T cells. Macrophages activate CD4+ T cells; M1 macrophages promote differentiation of CD4+ T cells into inflammatory Th1 and Th17 cells. Th1 and Th17 cells promote polarization of macrophages into M1 macrophages. M2 macrophages inhibit the differentiation of CD4+ T cells into inflammatory Th1 and Th17 cells, and promote the differentiation of CD4+ T cells into anti-inflammatory Th2 and regulatory T (Treg) cells. Th2 and Treg cells promote the polarization of macrophages into M2 macrophages. CTLA-4, cytotoxic T-lymphocyte associated protein 4; IFN-γ, interferon-gamma; MHC, major histocompatibility complex; RANKL, receptor activator of nuclear factor-kappa B ligand; TCR, T cell receptor; TGF-β, transforming growth factor beta; Th, T helper; TNF-α, tumour necrosis factor-alpha.

Th17 cells are implicated in osteoclast formation, thereby promoting bone resorption.

Regarding the impact of MSCs on T cells, co-culture of murine T cells and MSCs led to a decrease in the levels of TNF-alpha and IFN-gamma, suggesting that MSCs exert anti-inflammatory effects.[88] One of the key mechanisms through which MSCs attenuate the immune response is through modulation of the Th1/Th2 cell and Th17/Treg cell balance.[78,89] MSCs are involved in shifting the Th1/Th2 balance towards Th2 cells, demonstrating their anti-inflammatory effects.[90–94]

Treg cells have potent and well-established anti-inflammatory effects.[95–97] Numerous studies have indicated that MSCs are involved in the proliferation and differentiation of Tregs, via different pathways including the Notch signalling pathway, the Fas/Fas ligand signalling pathway, and the mTOR signalling pathway.[78,98–101]

Th17 cells are pro-inflammatory cells that exert their effects through the secretion of pro-inflammatory cytokines including IL-17.[102,103] Multiple studies suggest that MSCs are involved in the inhibition of Th17 cells and their ability to secrete IL-17. MSC-dependent suppression of Th17 cells involves many cells and pathways including the IL-10 signalling pathway, the prostaglandin E2 (PGE2) signalling pathway, the CCL2 signalling pathway, and the PD-1/PDL1 signalling pathway.[104–110] These pathways have been shown to be context-dependent. For example, PGE2 signalling by MSCs can stimulate or downregulate Th17 cells under different conditions, depending on cell maturity and the local microenvironment.[105,110,111]

In summary, MSCs generally promote the differentiation of T cells to anti-inflammatory Th2 and Treg cell phenotype, particularly in inflammatory environments, leading to anti-inflammatory effects (Figure 2). Conversely, Th2 and Treg cells enhance osteoblastogenesis and bone formation. Therefore, it is suggested that the interactions between MSCs and T cells are mostly skewed towards the anti-inflammatory side where MSCs, Th2, and Treg are promoting one another toward resolving the acute inflammatory response, decreasing the secretion of pro-inflammatory cytokines, and increasing the secretion of anti-inflammatory cytokines, thus enhancing bone regeneration.

Macrophages and T cells

When murine macrophages were co-cultured with Th1 cells to simulate inflammatory bowel disease (IBD), M1 polarization of macrophages was found to be promoted through STAT3 signalling.[21] It was suggested that Th1 cells may play a causative role in the immune response and pathology in IBD patients, and perhaps other inflammatory disorders.

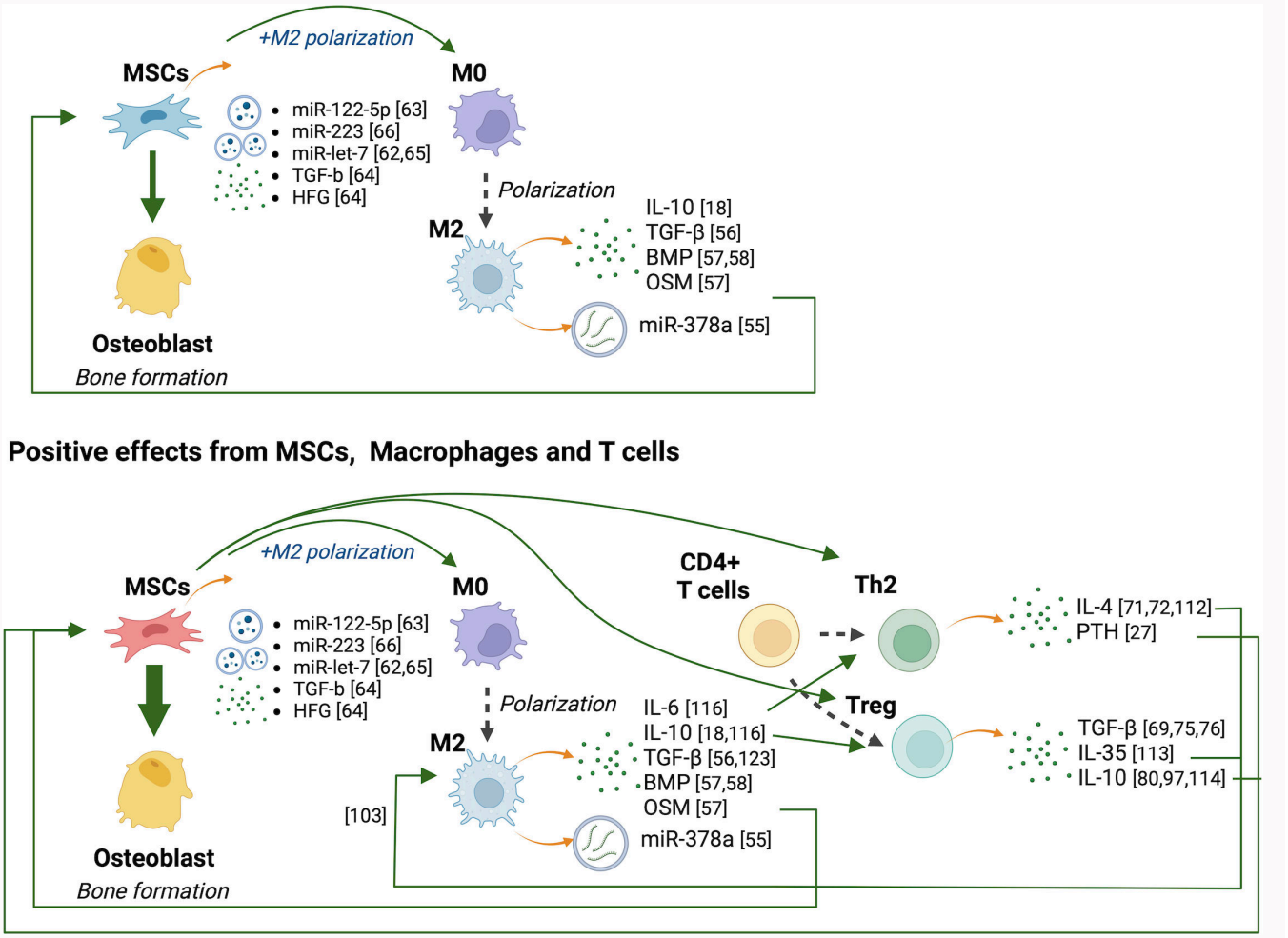

Fig. 4

Upper panel: positive effects from the interactions between MSCs and macrophages. MSCs differentiate M0 into M2, and anti-inflammatory M2 macrophages promote osteogenesis of MSCs. Lower panel: positive effects from MSCs, macrophages, and CD4+ T cells. MSCs polarize M0 into M2 macrophages. MSCs and M2 macrophages differentiate CD4+ T cells into anti-inflammatory T helper (Th2) and regulatory T (Treg) cells. Th2 and Treg cells polarize M0 into M2 macrophages. M2 macrophages, Th2 cells, and Treg cells promote MSC osteogenesis. BMP, bone morphogenic protein; HFG, hepatocyte growth factor; miR, microRNA; MSCs, mesenchymal stem cells; M0, naïve macrophages; OSM, oncostatin M; PTH, parathyroid hormone; TGF-β, transforming growth factor beta.

The polarization pathway is dependent on the helper T cells' phenotypes. Unlike Th1 cells, Th2 cells produce IL-4 to activate the M2 macrophage phenotype while simultaneously suppressing polarization of the M1 macrophage phenotype (Figure 3).[112] Similarly, αβ T cells inhibit the inflammatory response by promoting M2 macrophage proliferation. One study found that knocking out αβ T cells led to increased polarization toward the M1 state, and decreased polarization toward the M2 state. This is a role that is also shared by Treg cells, which secrete cytokines to induce a shift towards M2 polarization, consistent with their anti-inflammatory function.[67,113,114] This induction is associated with increased IL-10 production, and decreased major histocompatibility complex (MHC)-class II molecule expression. Subsequently, there is a decrease in MHC-related co-stimulation, which appears to control inflammatory processes such as Th17 cell expansion and promote immune tolerance.[115] In tumours, Treg cells induce the release of IL-10 and IL-6 by macrophages, which subsequently promote tumour cell survival and Treg function.[116] There is also modulation of the macrophage signalling pathway via microRNAs. Wu et al[117] found that the release of IFN-γ by T cells primed the activation of macrophages, which was associated with a decrease in levels of miR-3473b. Indeed, restoration of miR-3473b levels reversed macrophage activation, suggesting a regulatory role of the microRNA in this inflammatory pathway.[117] Understanding the downstream effects of these T cell/macrophage interactions, as well as potential modulators, can facilitate the identification of novel targets for immunotherapies and bone metabolism.

T cell activation requires antigen presentation via major MHC molecules. This is accomplished by different immune cells, including macrophages. Macrophages can present antigens via MHC class I or class II proteins. Macrophages also function as phagocytic cells and will digest foreign molecules and present subsequent antigens to activate T cells.[118] Depending on the co-stimulatory molecules, these interactions can lead to variable T cell fates. The B7-1 ligand on macrophages can activate or inhibit T cell proliferation by binding to the CD28 receptor or cytotoxic T-lymphocyte associated protein 4 (CTLA-4) receptor on the T cell surface, respectively.[119,120] One study found that the B7-CD28 interaction was synergistically acted upon by IL-12 secretion

by macrophages, leading to enhanced T cell activation. These results suggest that IL-12 serves as a soluble signalling component in the T cell regulation pathway seen in macrophages.[121] Another study found that administration of anti-B7 antibodies to macrophages diminished T cell responses, further underscoring the role of macrophages in early T cell activation.[122]

Cytokines released by macrophages also serve as a mechanism for regulating naïve T cell differentiation. Macrophages can secrete IL-12 and IFN-γ to induce Th1 proliferation. Acting in a cyclic manner, these Th1 effector cells will subsequently produce IFN-γ and TNF to upregulate macrophage phagocytosis. Another study found that synovial macrophages release IL-2 to induce Th1 differentiation. Th2 differentiation is induced by IL-4 secretion by macrophages, ultimately enhancing the anti-parasitic response. Egan et al[123] showed that synovial macrophages release IL-1B, IL-6, and IL-23 to induce Th17 differentiation. Macrophages can also release TGF-β, which polarizes Treg cells and generates a phenotype characterized by immunosuppression.[124] The delivery of TGF-β by M2 macrophages was found to induce the expression of CTLA-4 and other Treg-associated molecules on naïve CD4 T cells.[125] MSCs secrete TGF-β, which can induce macrophages to secrete IL-10 and CCL-18. These factors subsequently stimulate Treg growth.[126] Together, these findings suggest the potential of utilizing macrophages as a mechanism for controlling inflammation following surgery or as a therapy to target the immune disorders. For example, tumour-associated macrophages have been shown to suppress T cell proliferation through expression of programmed cell death ligand 1 (PD-L1) and secretion of IL-10, an anti-inflammatory cytokine. The immunosuppressive effect is also achieved by recruiting Treg cell migration to carcinomas via CCL22 signalling.[127] However, this tumour-induced T cell proliferation can be reversed to target tumour growth. Macrophage-induced T cell stimulation in tumours was restored via inhibition of B7-H4 expression on tumour macrophages, highlighting a mechanism for how the macrophage–T cell axis can be regulated in various microenvironments.[128] Given the key role that macrophages and T cells play in the immune and healing responses, manipulating immune cell subpopulations to optimize recovery in the clinical context should be explored. Future research should investigate how impacting the sub-composition of the immune microenvironment can improve the efficacy of immunotherapeutics and ultimately enhance clinical outcomes.

In summary, the results from crosstalk among various cell types (Figure 4) suggest opportunities to manipulate BMAC in terms of the composition or the proportions of macrophages, T cells, and MSCs so that bone regeneration can be optimized. MSCs have immunomodulatory effects. When they are co-cultured with macrophages, the macrophages would preferentially differentiate into the M2 phenotype, and these differentiated M2 macrophages promote osteoblast differentiation and bone formation. Additionally, when CD4+ T cells are added, MSCs and M2 macrophages will promote CD4+ T cells to differentiate into Th2 or Treg cells, the anti-inflammatory phenotypes. The increase in Th2 and Treg cells would further promote the polarization of macrophages into M2. Additionally, promoting anti-inflammatory M2 macrophages and Th2/Treg cells, rather than pro-inflammatory M1, Th1, and Th17 cells, reduces osteoclast formation. In other words, Th2 cells, Treg cells, and M2 macrophages are believed to not only enhance bone formation by MSCs, but also promote bone formation by inhibiting bone resorption. Therefore, the simultaneous presence of macrophages, CD4+ T cells, and MSCs is important for successful coordinated bone repair and regeneration. Understanding the optimal ratios of these cells could further enhance the bone-forming effects of BMAC, offering promising prospects for future treatments.

However, in some patients, secretion of inflammatory cytokines due to senescense-associated secretory phenotypes (SASP) from senescent cells in ageing,[129] decreased oestrogen levels in postmenopausal osteoporosis,[130] sustained hyperglycaemia in diabetes,[131,132] and chronic inflammatory conditions like rheumatoid arthritis may alter the functions of these cells.[26]

Conclusion

In the context of osteogenesis, co-culture of MSCs with macrophages has been demonstrated to enhance bone formation.[50,57,133] This may be attributed to the potential of M2 EVs to promote MSC-associated osteogenesis and the capacity of MSCs to differentiate from an M1 to M2 phenotype. Elevated levels of inflammatory cytokines, including M1 cytokines such as IFN-γ and TNF-α, are associated not only with a deficiency in new bone formation but also with promoting osteoclast formation, thereby enhancing bone resorption.[27] Co-culturing MSCs with T cells decreases the levels of TNF-α and IFN-γ in the co-culture medium, suggesting an anti-inflammatory effect of the MSCs,[88] which could influence bone formation. Furthermore, activated CD4+ T cells produce soluble factors that contribute to osteoblastic differentiation of human MSCs.[84] In macrophages and T cells, Th1 and Th17 promote the pro-inflammatory M1 phenotype,[21] whereas Th2 and Treg cells promote the anti-inflammatory M2 phenotype.[114,115] Additionally, M1 macrophages induce the proliferation of Th1 and Th17 cells, whereas M2 induces proliferation of Th2 and Treg cells. These findings suggest the potential for promoting bone formation by co-culturing MSCs, macrophages, and T cells together, highlighting the importance of understanding the roles of these cells. These findings have major implications for future therapies for immunomodulation of bone to enhance fracture healing and repair bone defects.

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Data sharing

The data that support the findings for this study are available to other researchers from the corresponding author upon reasonable request.