Article:
El-Jawhari, JJ, Jones, E orcid.org/0000-0001-9365-2283 and Giannoudis, PV (2016) The roles of immune cells in bone healing; what we know, do not know and future perspectives. Injury, 47 (11). pp. 2399-2406. ISSN 0020-1383

https://doi.org/10.1016/j.injury.2016.10.008

Injury, Int. J. Care Injured 47 (2016) 2399–2406

Contents lists available at ScienceDirect

Injury

journal homepage: www.elsevier.com/locate/injury

Review

The roles of immune cells in bone healing; what we know, do not know and future perspectives

Jehan J. El-Jawhari [a,b,c], Elena Jones [a,b], Peter V. Giannoudis [a,b,*]

[a] Leeds Institute of Rheumatic and Musculoskeletal Medicine, St. James Hospital, University of Leeds, UK
[b] NIHR Biomedical Research Unit, Chapel Allerton Hospital, University of Leeds, UK
[c] Clinical Pathology Department, Faculty of Medicine, Mansoura University, Egypt

ARTICLE INFO

Keywords:
Bone healing
Bone fracture
Immune cells
MSCs

ABSTRACT

Key events occurring during the bone healing include well-orchestrated and complex interactions between immune cells, multipotential stromal cells (MSCs), osteoblasts and osteoclasts. Through three overlapping phases of this physiological process, innate and adaptive immune cells, cytokines and chemokines have a significant role to play. The aim of the escalating immune response is to achieve an osseous healing in the shortest time and with the least complications facilitating the restoration of function. The uninterrupted progression of these biological events in conjunction with a favourable mechanical environment (stable fracture fixation) remains the hallmark of successful fracture healing. When failure occurs, either the biological environment or the mechanical one could have been disrupted. Not infrequently both may be compromised. Consequently, regenerative treatments involving the use of bone autograft, allograft or synthetic matrices supplemented with MSCs are increasingly used. A better understanding of the bone biology and osteoimmunology can help to improve these evolving cell-therapy based strategies. Herein, an up to date status of the role of immune cells during the different phases of bone healing is presented. Additionally, the known and yet to know events about immune cell interactions with MSCs and osteoblasts and osteoclasts and the therapeutic implications are being discussed.

Contents

* Corresponding author at: Academic Department of Trauma and Orthopaedic Surgery/Honorary Orthopaedic and Trauma Consultant, Leeds General Infirmary, School of Medicine, University of Leeds, UK.
E-mail address: P.Giannoudis@leeds.ac.uk (P.V. Giannoudis).

http://dx.doi.org/10.1016/j.injury.2016.10.008
0020–1383/

Introduction

The interaction between bone cells, inflammatory mediators and constituents of the immune system involved in bone repair, continue to be of great scientific interest to researchers and clinicians [1–12]. Investigation of the critical role of immune cells during the bone healing is ongoing. Depletion of T- and B-lymphocytes is associated with impairment in bone mineralisation and maturation of osteoblasts with delayed repair and remodelling phases and delayed healing as demonstrated in experimental models [13,14]. Additionally, Cho et al. demonstrated that resident macrophages (osteal) are significantly involved in parathyroid hormone-dependent bone healing [15]. Although there are no experimental models for NK cell depletion in factures, an important role of NK cells during bone repair has been implied when a high level of interferon-gamma (IFN-γ) was detected in the diaphyseal regions of fractured femur in mice lacking T- and B-lymphocytes [16]. Conversely, as shown in immune-compromised animal model, bone marrow (BM) transplantation greatly enhanced the process of bone healing [17]. In addition to experimental findings, immune-compromised HIV patients can have delayed or non-union of fractures [18]. Thus, both animal and human studies confirmed the critical importance of innate and adaptive immune cells.

While the outer layer of cortical bone carries the weight bearing function, inner cancellous bone contains BM, a niche for different cell types including bone progenitor cells and multipotential stromal cells (MSCs). MSCs are classically identified as cells with the adherence capacity, which also express surface molecules CD90, CD73, CD105, but not hematopoietic lineage markers and are able to differentiate into bone, fat and cartilage cells [19]. Beside inflammatory cells and MSCs, two types of bone resident cells, osteoclasts and osteoblasts also play critical roles during the process of bone healing. Osteoclasts are large multinucleated cells are differentiated from monocyte lineage cells and have a bone degradation activity [20]. In contrast, the function of osteoblasts is the bone formation and they are derived from MSC-differentiated bone progenitor cells. Each of the immune cells has both distinctive and common functions with each other or MSCs during the phases of bone healing (Fig. 1). In this study, we review the vital role of the immune cells and their interactions with bone cells and MSCs (Fig. 2) and how this would affect the outcome of fracture healing.

Inflammatory phase

An early event of the injury of bone is the interruption of blood supply and platelet aggregation with the release of platelet-derived pro-inflammatory cytokines, Interlukin-6 (IL-6), Interlukin-1 (IL-1) and tumour necrosis factor-alpha (TNF-α). These cytokines stimulate the homing of lymphocytes and monocyte/macrophages into the fracture site. As shown in

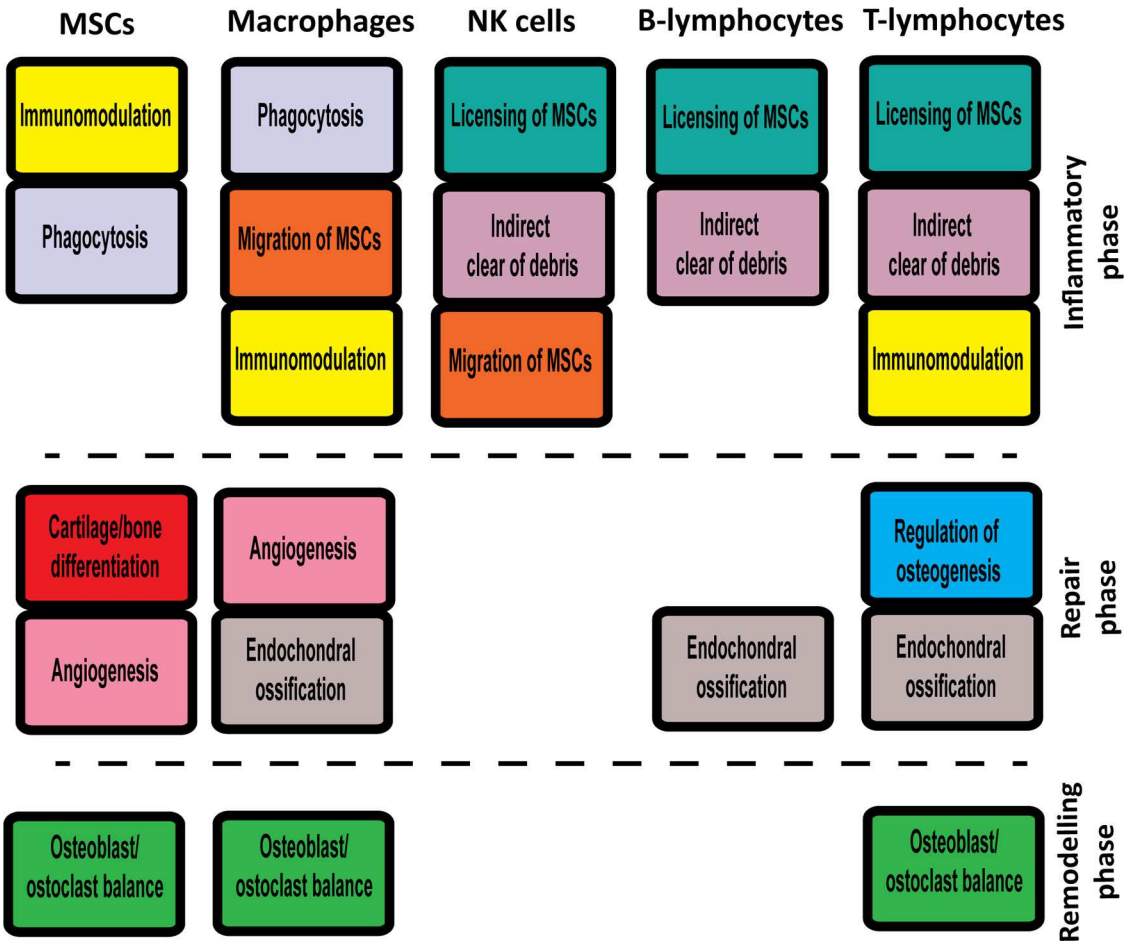

Fig. 1. The various roles of immune cells and MSCs during the phases of fracture healing. Early during inflammatory phase, both macrophages and MSCs can display phagocytic functions. NK cells, T- and B-lymphocytes are contributed into osteoclastogenesis to clear cell debris. The effects of macrophages and NK cells can facilitate the migration of MSCs. The licensing of MSCs can be mediated by cytokines released from NK cells, T- and B-lymphocytes. Then, licensed MSCs together with programmed macrophages and T reg lymphocytes have late immunosuppressive effects to end the inflammatory phase. During the repair phase, MSCs carry the differentiation functions as well as angiogenesis helped by macrophages. Also, T-lymphocytes are involved in regulation of MSC osteogenicity. The conversion of soft cartilaginous callus into hard callus is controlled by macrophages, T- and B-lymphocytes. In the final remodelling phase, osteoblast and osteoclast balance is regulated by MSCs and macrophages and probably T-lymphocytes (IL-17 and TNF-α effects).

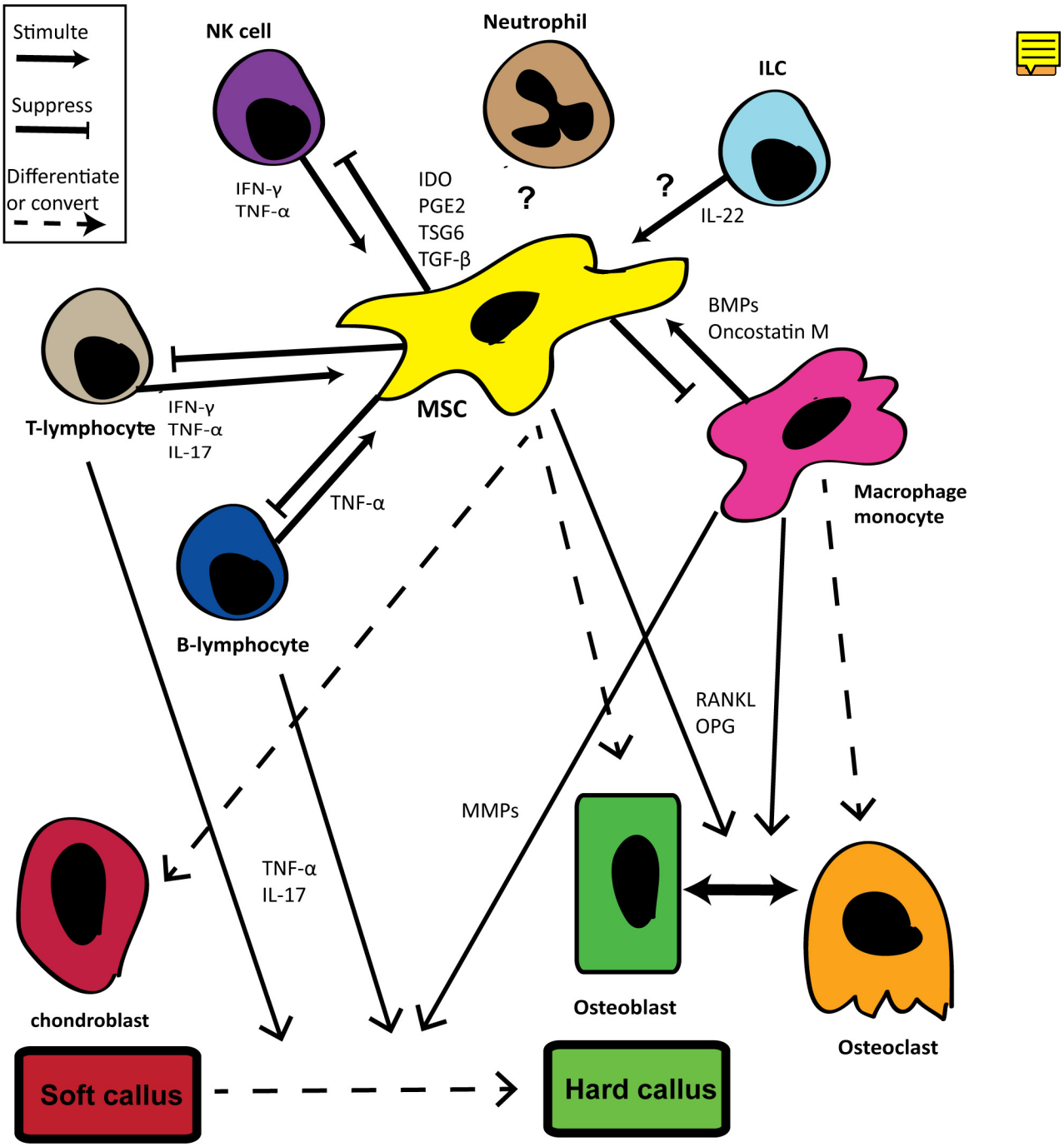

Fig. 2. The key cellular interactions and soluble mediators during fracture healing. Immune cells, MSCs and osteoblasts and osteoclasts interact together during bone healing. While NK cells, T- and B-lymphocytes can be involved in activation of immunosuppressive functions of MSCs via TNF-α, IL-17 and IFN-γ, other cells like macrophages stimulate the osteogenic differentiation of MSCs via BMPs and Oncostatin M. ILCs might have a role in stimulation of osteogenic capacity of MSCs via IL-22. However, only licensed MSCs are able to suppress the functions of both innate and adaptive immune cells via multiple mechanisms such as IDO, PGE2, TSG6 and TGF-β. Also, MSCs can differentiate into osteoblasts and chondroblasts, which form the soft callus. Immune cells; macrophages, T- and B-lymphocytes participate to promote the conversion of soft callus into hard callus via action of MMPs, TNF-α and IL-17. The balance of the activity of osteoblast and osteoclasts are mainly regulated by macrophages and MSCs with involvement of OPG and RANKL.

animal models, T- and B-lymphocytes are recruited at the fracture site after 3 days of injury and then reduced in numbers with the start of cartilaginous callus formation [14,21]. This phase also involves formation of haematoma, which traps inflammatory cells that further produce pro-inflammatory inflammatory cytokines and growth factors. This haematoma is crucial and its removal causes a defective bone healing [22,23]. The main cellular events taking place during inflammatory phase are presented below.

Clearing of damaged areas

Initially, neutrophils arrive to the fracture site as detected in a rat model of fracture [24]. Neutrophils have an anti-septic effect and clear the damaged cells and debris [25,26]. Other cells that help to erode the damaged edges of bones are osteoclasts. Receptor activator of nuclear factor kappa-B ligand (RANKL) that is produced by activated T-lymphocytes and NK cells can induce the differentiation of the osteoclasts from monocytes [27–29]. A role

of B-lymphocytes during inflammatory phase of bone healing has been also linked to osteoclast formation [30]; but this role seem to be tightly controlled as B-lymphocytes can also suppress the osteoclast generation and enhance the apoptosis of osteoclasts [31]. In addition to immune cells and osteoclasts, recruited MSCs into fracture site could be involved in the clearing of dead tissues at the fracture site by phagocytosis of apoptotic cells [32]. This is associated with enhancement of MSC osteogenesis and secretion of interleukin (IL)-8, monocyte chemoattractant protein-1, and RANTES that could stimulate the homing of T cells to the inflamed area. Together, different cells can directly or via crosstalk work early at the bone injury site to clear the debris and pave the way for healing to start.

Migration of MSCs

MSCs widely exist within the skeleton in the periosteum, BM and bone. Those MSCs that directly participate in the fracture healing can originate from different sources. Whether localised at the fracture site (periosteum and endosteum) or migrating to the bone injury site, MSCs were shown to have a vital activity in fracture healing [33–35].

It has been demonstrated that MSCs are recruited into the injured bone site under the influence of an inflammatory chemokine, Stromal Derived Factor-1 (SDF-1) [36]. The mechanism of SDF-dependent migration of MSCs involves the activation of alpha serine/threonine-protein kinase (AKT) and extracellular-signal-regulated kinases (ERK) signalling pathways [37,38]. Inflammatory TNF-α also mediates the invasion of MSCs into the bone-healing site [39]. Macrophage-derived chemokines, MCP-1 and monocyte inflammatory protein 1 alpha (MIP-1α) have been also linked to MSC migration to the bone healing site [40]. Recently, it has been found that chemokine; CXCL7 that produced by NK cells enhances MSC migration [41]. Overall, immune cells such as macrophages and NK cells as well as inflammatory cytokines and chemokines can act together to help the homing of MSCs into the fracture-healing site.

Preparation for the repair phase; licensing of MSCs

The term licensing is commonly used to describe the activation of MSCs to perform immunosuppressive functions. A group of cytokines that produced within inflammatory milieu are responsible for the licensing of MSCs and can act alone e.g. IFN-γ or in combination [42]. IFN-γ can trigger the proliferation and immunomodulatory function of MSCs via the Kynurenine-dependent mechanism [43]. Likewise, TNF-α induces the proliferation and immunosuppressive function of MSCs using the NF-κβ pathway [44]. Thus, NK cells and T-lymphocytes are linked to the licensing of MSCs as major sources of TNF-α that is highly expressed during both inflammatory and repair phases [45]. Together with IFN-γ and TNF-α, MSCs that are activated by IL-1 can perform immunosuppressive functions associated with the production of prostaglandin 2 (PGE2) and IL-8 [46]. Furthermore, it has been shown that IL-17 is another licensing cytokine that can enhance the immunosuppressive functions of MSCs both in vivo and in vitro [47]. In summary, multiple licensing cytokines help to programme MSCs towards immunosuppressive activity in order to control the inflammatory phase of healing.

Other inflammatory mediators and cells follow the wave of MSC 'licencing' cytokines to directly enhance the osteogenic potential of MSCs. Toll-like receptors (TLRs) stimulate MSC migration and osteogenic differentiation utilising NFκ-β and PI3 kinase signalling pathways [48]. Additionally, macrophages existing in the fractured bone are a source of bone morphogenetic proteins (BMPs) and Oncostatin M that enhance the proliferation and osteogenic function of MSCs [49,50]. Furthermore, activated monocytes induce the expression level of Cbfa1/Runx2 and alkaline phosphatase (ALP) by MSCs and hence drive the bone formation [51]. In contrast, a conditioned media from CD4 T-lymphocytes and not CD8 has been shown to increase the osteogenesis markers of MSCs [52]. Furthermore, innate lymphocytes cells (ILCs) that produce tissue reparative cytokines such as interleukin-22 (IL-22) [53–55] also seem to induce the osteogenic activity of MSCs. Recently, an in vitro study has shown that IL-22 can induce the osteogenic capacity of licensed BM MSCs [56]. Likewise, once MSCs are licensed, IL-17 induces osteogenesis by increase the expression of osteogenic proteins in MSCs, Cbfa1/Runx2 and collagen [14]. In conclusion, the inflammatory microenvironment delivers important signals that help the preparation of MSCs, proliferation and immunomodulation and then the osteogenesis.

Reduction of immune cell response and the end of inflammatory phase

The control of immune cell response is critical to reduce the inflammation and aid the switch into repair phase. Interestingly, the levels of TGF-β2 and TGF-β3 reach the peak at the end of inflammation most likely to control the immune response and finalise the inflammatory phase [57]. As mentioned above, MSCs are licensed to exert their immunosuppressive role. MSCs can induce the generation of anti-inflammatory $CD4^{+}CD25^{+}Foxp3^{+}$ T reg lymphocytes with the production of immunosuppressive cytokine, IL-10 [58]. Beside its effect on T reg lymphocytes, MSCs directly induce the apoptosis and suppress the proliferation and functions of pro-inflammatory Th1 and Th17 subsets [59,60]. Furthermore, MSCs can decrease the function and the migration of B-lymphocytes via the down-regulation of the chemokine receptors; CXCR4, CXCR5 and CCR7 [61]. In addition to adaptive immune cells, MSCs are able to inhibit the proliferation, secretory and cytotoxicity functions of cytokine-activated NK cells [62,63] as well as inhibition of the differentiation functions of monocyte-derived dendritic cells [64].

MSCs employ these immunosuppressive effects via different soluble molecules including TGF-β, indoleamine 2,3-dioxygenase (IDO), inducible Nitric oxide synthases (iNOS), PGE2, IL-1 receptor antagonist and Tumour necrosis factor-inducible gene 6 (TSG6) [65–68]. Recently, other mechanisms of MSC-dependent immunomodulation have been described. It has been shown that MSC-derived extracellular vesicles have a strong immunosuppressive effect on T- and B-lymphocytes as well as NK cell functions [69]. Furthermore, MSCs can programme macrophages to display anti-inflammatory M2 phenotype that suppresses both innate and adaptive immune responses via IL-10 and TGF-β dependent mechanisms [70]. Collectively, MSCs, which licensed by inflammatory signals act in turn to suppress the inflammatory responses of immune cells as a negative feedback mechanism. This mechanism helps the ignition of repair phase of bone healing. Nevertheless, the effects of some immune cells and cytokines continue to have a role during the repair and remodelling phases.

Repair phase

The repair phase involves the differentiation of MSCs into either osteoblasts when the broken bone edges are immaculately aligned (primary healing) [71] or chondroblasts that proliferate forming a cartilaginous structure called soft callus (secondary healing). The soft callus is then mineralised and converted into bone callus with irregularly arranged (woven) bone, which is invaded by new blood vessels in a process called endochondral ossification. In addition to the differentiation function, MSCs support new blood vessel formation via metalloproteinase-dependent mechanisms [72].

Certain immune cells are known to participate in the repair phase. Macrophages participate in the induction of angiogenesis

and a substantial reduction in macrophages is associated with impaired vascularisation and delayed formation of callus as revealed in CCR2$^{-/-}$ mice model [73]. Bone-lining macrophages participate in the intramembranous bone healing as shown in mouse model of tibial fracture [74]. Furthermore, macrophages can regulate MSC differentiation into osteoblasts, as mentioned in previous sections [75]. Macrophages have been detected in invading vessels throughout the ossification of mouse long bones [76] and they efficiently produce matrix metalloproteinases (MMPs) to degrade the cartilage matrix [77,78]. These MMPs have a central role in soft-to-hard callus switch [79–81] and any dysregulation of these enzyme activities has been linked to the fracture non-union [82]. The deposition of collagen type I is another function for macrophages and this is associated with up regulation of macrophage macrosialin protein [74]. The multi-function of macrophages highlights their unique importance during the repair phase of either primary healing or endochondral ossification.

Other immune cells also reappear during the mineralisation of cartilaginous callus. This includes T- and B-lymphocytes that were found to be located in a close contact with osteoblasts and osteoclasts [83]. Both types of adaptive lymphocytes produce TNF-α, which trigger the death of mature chondrocytes aiding the transition from cartilage into bone [84,85]. Importantly, the effect of TNF-α on chondrocytes involves up-regulation of MMPs and angiopoietin coordinating both of angiogenesis and ossification of soft callus [86]. IL-17 is another cytokine that can affect the conversion of soft callus into hard callus. IL-17 can inhibit the chondrogenic differentiation of MSCs via the suppression of a key chondrogenesis transcriptional factor, SRY-box 9 (SOX9) and its activator cAMP-dependent protein kinase (PKA) [87]. Additionally, an in vitro work showed that IL-17 also enhances the MSC differentiation into osteoblasts [88]. This all indicates that adaptive lymphocytes can actively participate in the endochondral ossification.

Several growth factors are needed to support bone healing particularly during the repair phase including platelet-derived growth factor (PDGF), TGF-β, Insulin-like growth Factor (IGF), fibroblast growth factor-1 (FGF-1) and BMPs that promote the proliferation and the chondrogenic differentiation of MSCs as well as deposition of collagen [49,89,90]. Overall, the conversion of soft callus into hard callus is highly controlled by macrophages, T- and B-lymphocytes and various cytokines and growth factors demonstrating the continuation of immune-bone interactions even after the end of the inflammation phase.

Remodelling phase

The remodelling of woven bone into normal lamellar bone is related to the balance between osteoblast and osteoclast functions. The osteoblast/osteoclast function is controlled by MSCs, macrophages and cytokines such as TNF-α and IL-17. As mentioned above, the osteoblast formation from MSCs is influenced by various growth factors such as TGF-β family members, BMPs and IGF [91]. However, MSCs have an inhibitory effect on monocyte differentiation into osteoclast via the production of Osteoprotegerin (OPG) [92]. In contrast, RANKL and M-CSF secreted by osteoblasts can improve the survival and the function of osteoclasts [93,94]. Macrophages seem to maintain the bone forming/resorption balance by augmentation of the osteoblast activity and as being the progenitors of osteoclasts [95]. Osteal macrophages are also responsible for coordinating the crosstalk between osteoclasts and osteoblasts [96]. Together, MSCs and macrophages seem to have contrasting effects on osteoclasts to maintain the balance during the remodelling.

The role of IL-17 in the remodelling of hard callus indicates a possible involvement of T-lymphocytes during this phase. The downstream effect of IL-17 on the osteoblasts includes the up-regulation of the osteogenic mediators, bone sialoprotein, collagen and osteocalcin [14]. At the same time, IL-17 enhances the expression of RANKL on MSCs enhancing the osteoclastogenesis when co-cultured with peripheral blood mononuclear cells (PBMCs) [97]. Another cytokine, TNF-α produced by MSCs and osteoblasts during late phase of bone healing [45] can also influence both osteoblasts and osteoclasts functions demonstrating its vital role in the remodelling phase [98,99]. Overall, specific immune cells and mediators keep the bone healing process under control till the end.

The uncontrolled immune cell response and defective bone healing

Systemic inflammatory diseases and local sepsis at the bone injury site are linked to complicated healing including non-union [100]. MSCs extracted from non-union tissues have an impaired proliferative capacity and function compared to healthy controls [101–104]. Nevertheless, these non-union MSCs retain their osteogenic differentiation when activated in vitro [103,105]. This indicates that the healing microenvironment including the effect of immune response could be the main biological player in fracture non-union.

An exaggerated activation of neutrophils using oxygen free radicals is associated with defective healing of bone fracture [106], while an induced neutropenia in animal models of bone defects shows an enhanced osteogenic repair [107]. Likewise, excessive stimulation of macrophages with lipopolysaccharide can decrease their production of BMP-2 causing a delayed bone healing [108]. Additionally, within a chronic inflammatory milieu, monocytes have a higher potential to differentiate into osteoclasts through TNF-α dependent mechanism [109]. Excessively activated NK cells could mediate cytotoxicity against allogeneic or autologous MSCs [62,110–113]. Both Activated T-lymphocytes and B-lymphocytes are well known to release RANKL, which boosts osteoclast differentiation from their progenitors and subsequently provoke the bone lysis [94,114]. Totally, although critical at the early phase of bone healing, excess activation of immune cells has a strong link to defective bone formation.

Several studies have proven that inflammatory cytokines IFN-γ and TNF-α can block the osteogenic differentiation of MSCs [115,116]. TNF-α also works to enhance the expression of Wnt signalling pathway antagonist, Dickkopf-1 (DKK-1) that has an inhibitory effect on the osteoblast formation [117] and inhibits nephronectin, an extracellular matrix protein, which helps the proliferation of osteoblasts [118]. Furthermore, TNF-α stimulates the production of M-CSF by MSCs that in turn, induce the differentiation of osteoclast progenitors [119]. Similarly, IFN-γ has a positive influence on the osteoclastogenesis [120]. Furthermore, IL-1 and IL-17 has been linked to the bone loss within highly inflammatory milieu [121,122]. Altogether, this shows that excess or prolonged inflammation via immune cells or cytokines can be involved in impaired bone healing. Although cytokines such as IFN-γ and TNF-α are key players for MSC licensing, they can exert a negative effect on osteogenic differentiation of MSCs. Therefore, it is vital to determine the exact timings and levels needed of these cytokines to ensure the correct balance between their actions favouring bone healing.

What is unknown?

Despite all the research advances in the osteoimmunology field, more knowledge about cellular interactions during bone healing

still required. The immune cell-MSC cross talk is essential to complete the inflammatory phase and to initiate the repair phase. However, it is still remaining to reveal if there is a link between MSCs and neutrophils as both cells can be detected early during the inflammation. Furthermore, the in vitro studies indicate complicated interactions between NK cells and MSCs, i.e. NK cells functions can be suppressed by MSCs and NK cells can participate in MSC licensing, but also can kill MSCs [62,63,110–113]. Thus, further research is necessary to understand the biological importance of these interactions NK cells during physiological fracture healing and how this would affect the cell therapy. Also, it will be interesting to locate and identify the functions of innate immune cells, ILCs within healing bone tissues. Similarly, although IL-17 are linked to regulation osteoblast and osteoclast activity [123], Th17 cell location in the final stage of the bone healing remains to be investigated. Also, CD4 and CD8 T-lymphocytes seem to have contrasting effects on bone healing. The impact of the variable effects of these subsets on the MSC osteogenic capacity and the exact molecular mechanisms underlying these effects and the time of their participation are not clear yet. All this knowledge will help significantly to improve the therapeutic strategies of complicated fractures.

Therapeutic implications

The use of cell-therapy for non-union of fractures is a promising alternative to conventional bone autograft. According to the diamond concept [12,124–127], the biological elements of these therapies should involve MSCs and growth mediators including those promoting the new vasculature formation. Whether MSCs are delivered within concentrated or not-concentrated mononuclear bone marrow cells [128,129] or as culture-expanded pure population and loaded on matrices [130] or injected subcutaneously [131], their therapeutic use still needs further optimisation. The inflammation status could affect considerably the effectiveness of the regenerative treatments at least in part via their effect on MSCs [132]. Also, the revascularisation of bone graft or matrices are vital to maintain the survival and function within the healing milieu via supplying the nutrition, oxygen supply and regulatory mediators.

Several studies, in which MSCs were used for bone and cartilage repair, have indicated that the failure of therapeutic effect of allogeneic MSCs was associated with signs of activated immune responses [115,133–135]. However, in other studies where the allogeneic MSCs were loaded into scaffolds, an inflammatory response was similar to that induced by autologous MSCs with better outcomes [136–141]. This could be related to limited accessibility of allogeneic MSCs to host immune cells. For autologous MSCs, in vitro studies have shown that NK cells can lyse autologous MSCs similar to allogeneic MSCs [142]. The NK-cell mediated killing of autologous MSCs is related to the low expression of HLA-I molecules and high expression of NK cell activating ligands on the MSC surface [62,110–113]. Thus, it is remaining to investigate if under certain conditions, this mechanism could threaten the fate of the transplanted MSCs for fracture healing. Overall, this clearly indicates how immune response and inflammatory milieu can greatly affect the activities of both allogeneic and autologous MSCs. Also, the choice of the intervention therapy time in relation to inflammation status is an essential challenge to be addressed.

The in vitro polarisation of macrophages into either pro-inflammatory M1 or anti-inflammatory M2 that are also involved in tissue repair and angiogenesis, becomes possible via differentiation of peripheral blood monocytes utilising specific cytokines [143,144]. Importantly, it has been shown that both subsets together highly support the angiogenesis. This role is mediated via M1 cells that produce the angiogenesis prompting factors, VEGF, IL-8, bFGF and RANTES. Additionally, M2 cells have been proposed to enhance the blood vessel fusion, vascular remodelling and regulation of M1 activity [145]. These findings had a great potential to be applied in the regenerative bone therapies to fabricate scaffolds that help to polarise macrophages and consequently supporting blood vessel formation during bone healing [145] together with promoting the proliferation and polarisation of bone progenitors cells to achieve a completed healing process.

Conclusions

Bone healing constitutes a successive process with three phases starting with critical inflammation; in which both innate and adaptive immune cells as well as cells of macrophage-osteoclast lineage help the removal of bone debris, antisepsis and preparation of MSCs for next repair phase. In turn, licensed MSCs work to control the inflammatory phase and differentiate directly into osteoblasts or most commonly into chondroblasts forming soft callus. During this repair phase, certain immune cells and mediators play an important role to convert soft callus into hard callus and formation of new blood vessels. Finally, bone remodelling is mediated via interplay between osteoclasts and osteoblasts under influence of MSCs, macrophages and probably Th17 lymphocytes. The excess activation of the immune mediators can inhibit the osteogenic differentiation of MSCs. Thus a delicate balance between the functions of immune cells, MSCs and bone cells are critical for healthy bone healing. The therapeutic use of MSCs for bone loss and fractures should consider enhancing the bone forming capacity of MSCs as well as microenvironment particularly the inflammation status. Additionally, a new generation of biomaterials is needed to help the delivery of the appropriate type and concentration of growth factors/cytokines enhancing both osteogenesis and angiogenesis. The developed knowledge about details of cellular interactions during the bone healing will help to improve the outcomes of MSC-based therapy used for complicated bone healing.

References

[1] Giannoudis PV, et al. Tissue loss and bone repair: time to develop an international strategy? Injury 2015;46(Suppl. 8):S1–2.
[2] Alt V, et al. Effects of recombinant human Bone Morphogenetic Protein-2 (rhBMP-2) in grade III open tibia fractures treated with unreamed nails – a clinical and health-economic analysis. Injury 2015;46(11):2267–72.
[3] Ollivier M, et al. Can we achieve bone healing using the diamond concept without bone grafting for recalcitrant tibial nonunions? Injury 2015;46(7):1383–8.
[4] Santolini E, West R, Giannoudis PV. Risk factors for long bone fracture non-union: a stratification approach based on the level of the existing scientific evidence. Injury 2015;46(Suppl. 8):S8–19.
[5] Cheung WH, et al. Fracture healing in osteoporotic bone. Injury 2016;47(Suppl. 2):S21–6.
[6] Watanabe Y, et al. Stem cell therapy: is there a future for reconstruction of large bone defects? Injury 2016;47(Suppl. 1):S47–51.
[7] Giannoudis PV. Treatment of bone defects: bone transport or the induced membrane technique? Injury 2016;47(2):291–2.
[8] Takahara S, et al. Human pseudoarthrosis tissue contains cells with osteogenic potential. Injury 2016;47(6):1184–90.
[9] Zura R, et al. Treatment of chronic (>1 year) fracture nonunion: heal rate in a cohort of 767 patients treated with low-intensity pulsed ultrasound (LIPUS). Injury 2015;46(10):2036–41.
[10] Tsitsilonis S, et al. The effect of traumatic brain injury on bone healing: an experimental study in a novel in vivo animal model. Injury 2015;46(4):661–5.
[11] Roberto-Rodrigues M, et al. Novel rat model of nonunion fracture with vascular deficit. Injury 2015;46(4):649–54.
[12] Moghaddam A, et al. Treatment of atrophic tibia non-unions according to 'diamond concept': results of one- and two-step treatment. Injury 2015;46(Suppl. 4):S39–50.
[13] Askalonov AA. Changes in some indices of cellular immunity in patients with uncomplicated and complicated healing of bone fractures. J Hyg Epidemiol Microbiol Immunol 1981;25(3):307–10.

[14] Nam D, et al. T-lymphocytes enable osteoblast maturation via IL-17F during the early phase of fracture repair. PLoS ONE 2012;7(6):e40044.
[15] Cho SW, et al. Osteal macrophages support physiologic skeletal remodeling and anabolic actions of parathyroid hormone in bone. Proc Natl Acad Sci U S A 2014;111(4):1545–50.
[16] Toben D, et al. Fracture healing is accelerated in the absence of the adaptive immune system. J Bone Miner Res 2011;26(1):113–24.
[17] Xing Z, et al. Rejuvenation of the inflammatory system stimulates fracture repair in aged mice. J Orthop Res 2010;28(8):1000–6.
[18] Richardson J, et al. Fracture healing in HIV-positive populations. J Bone Joint Surg Br 2008;90(8):988–94.
[19] Dominici M, et al. Minimal criteria for defining multipotent mesenchymal stromal cells. The International Society for Cellular Therapy position statement. Cytotherapy 2006;8(4):315–7.
[20] Boyle WJ, Simonet WS, Lacey DL. Osteoclast differentiation and activation. Nature 2003;423(6937):337–42.
[21] Andrew JG, et al. Inflammatory cells in normal human fracture healing. Acta Orthop Scand 1994;65(4):462–6.
[22] Mizuno K, et al. The osteogenetic potential of fracture haematoma. Subperiosteal and intramuscular transplantation of the haematoma. J Bone Joint Surg Br 1990;72(5):822–9.
[23] Claes L, Recknagel S, Ignatius A. Fracture healing under healthy and inflammatory conditions. Nat Rev Rheumatol 2012;8(3):133–43.
[24] Xian CJ, et al. Intramembranous ossification mechanism for bone bridge formation at the growth plate cartilage injury site. J Orthop Res 2004;22(2):417–26.
[25] Segal AW. How neutrophils kill microbes. Annu Rev Immunol 2005;23:197–223.
[26] Timlin M, et al. Fracture hematoma is a potent proinflammatory mediator of neutrophil function. J Trauma 2005;58(6):1223–9.
[27] Kong YY, et al. Activated T cells regulate bone loss and joint destruction in adjuvant arthritis through osteoprotegerin ligand. Nature 1999;402(6759):304–9.
[28] Connor JR, et al. Human osteoclast and giant cell differentiation: the apparent switch from nonspecific esterase to tartrate resistant acid phosphatase activity coincides with the in situ expression of osteopontin mRNA. J Histochem Cytochem 1995;43(12):1193–201.
[29] Soderstrom K, et al. Natural killer cells trigger osteoclastogenesis and bone destruction in arthritis. Proc Natl Acad Sci U S A 2010;107(29):13028–33.
[30] Manabe N, et al. Connection between B lymphocyte and osteoclast differentiation pathways. J Immunol 2001;167(5):2625–31.
[31] Weitzmann MN, et al. B lymphocytes inhibit human osteoclastogenesis by secretion of TGFbeta. J Cell Biochem 2000;78(2):318–24.
[32] Tso GH, et al. Phagocytosis of apoptotic cells modulates mesenchymal stem cells osteogenic differentiation to enhance IL-17 and RANKL expression on CD4+ T cells. Stem Cells 2010;28(5):939–54.
[33] Kumagai K, et al. Circulating cells with osteogenic potential are physiologically mobilized into the fracture healing site in the parabiotic mice model. J Orthop Res 2008;26(2):165–75.
[34] Malizos KN, Papatheodorou LK. The healing potential of the periosteum molecular aspects. Injury 2005;36(Suppl. 3):S13–9.
[35] Colnot C, Huang S, Helms J. Analyzing the cellular contribution of bone marrow to fracture healing using bone marrow transplantation in mice. Biochem Biophys Res Commun 2006;350(3):557–61.
[36] Kitaori T, et al. Stromal cell-derived factor 1/CXCR4 signaling is critical for the recruitment of mesenchymal stem cells to the fracture site during skeletal repair in a mouse model. Arthritis Rheum 2009;60(3):813–23.
[37] Liu X, et al. SDF-1/CXCR4 axis modulates bone marrow mesenchymal stem cell apoptosis, migration and cytokine secretion. Protein Cell 2011;2(10):845–54.
[38] Guiducci S, et al. Bone marrow-derived mesenchymal stem cells from early diffuse systemic sclerosis exhibit a paracrine machinery and stimulate angiogenesis in vitro. Ann Rheum Dis 2011;70(11):2011–21.
[39] Bocker W, et al. IKK-2 is required for TNF-alpha-induced invasion and proliferation of human mesenchymal stem cells. J Mol Med (Berl) 2008;86(10):1183–92.
[40] Ito H. Chemokines in mesenchymal stem cell therapy for bone repair: a novel concept of recruiting mesenchymal stem cells and the possible cell sources. Mod Rheumatol 2011;21(2):113–21.
[41] Almeida CR, et al. NAP-2 secreted by human NK cells can stimulate Mesenchymal Stem/Stromal Cell recruitment. Stem Cell Rep 2016;6(4):466–73.
[42] Krampera M. Mesenchymal stromal cell 'licensing': a multistep process. Leukemia 2011;25(9):1408–14.
[43] Croitoru-Lamoury J, et al. Interferon-gamma regulates the proliferation and differentiation of mesenchymal stem cells via activation of indoleamine 2,3 dioxygenase (IDO). PLoS ONE 2011;6(2):e14698.
[44] Dorronsoro A, et al. Human mesenchymal stromal cells modulate T-cell responses through TNF-alpha-mediated activation of NF-kappaB. Eur J Immunol 2014;44(2):480–8.
[45] Kon T, et al. Expression of osteoprotegerin, receptor activator of NF-kappaB ligand (osteoprotegerin ligand) and related proinflammatory cytokines during fracture healing. J Bone Miner Res 2001;16(6):1004–14.
[46] Fan H, et al. Pre-treatment with IL-1beta enhances the efficacy of MSC transplantation in DSS-induced colitis. Cell Mol Immunol 2012;9(6):473–81.
[47] Han X, et al. Interleukin-17 enhances immunosuppression by mesenchymal stem cells. Cell Death Differ 2014;21(11):1758–68.
[48] Delarosa O, Dalemans W, Lombardo E. Toll-like receptors as modulators of mesenchymal stem cells. Front Immunol 2012;3:182.
[49] Nakase T, Yoshikawa H. Potential roles of bone morphogenetic proteins (BMPs) in skeletal repair and regeneration. J Bone Miner Metab 2006;24(6):425–33.
[50] Guihard P, et al. Induction of osteogenesis in mesenchymal stem cells by activated monocytes/macrophages depends on oncostatin M signaling. Stem Cells 2012;30(4):762–72.
[51] Omar OM, et al. The stimulation of an osteogenic response by classical monocyte activation. Biomaterials 2011;32(32):8190–204.
[52] Grassi F, et al. T cell subsets differently regulate osteogenic differentiation of human mesenchymal stromal cells in vitro. J Tissue Eng Regen Med 2016;10(4):305–14.
[53] Sawa S, et al. RORgammat+ innate lymphoid cells regulate intestinal homeostasis by integrating negative signals from the symbiotic microbiota. Nat Immunol 2011;12(4):320–6.
[54] Dudakov JA, et al. Interleukin-22 drives endogenous thymic regeneration in mice. Science 2012;336(6077):91–5.
[55] Scandella E, et al. Restoration of lymphoid organ integrity through the interaction of lymphoid tissue-inducer cells with stroma of the T cell zone. Nat Immunol 2008;9(6):667–75.
[56] El-Zayadi AA, Jones EA, Churchman SM, Baboolal TG, Cuthbert RJ, El-Jawhari JJ, Badawy AM, Alase AA, El-Sherbiny YM, McGonagle D. IL-22 drives the proliferation, migration and osteogenic differentiation of human bone marrow mesenchymal stem cells (MSCs): a novel cytokine that may contribute to aberrant new bone formation in human SpA. Rheumatology (Oxford) 2016 [in press].
[57] Cho TJ, Gerstenfeld LC, Einhorn TA. Differential temporal expression of members of the transforming growth factor beta superfamily during murine fracture healing. J Bone Miner Res 2002;17(3):513–20.
[58] Luz-Crawford P, et al. Mesenchymal stem cells generate a CD4+CD25+Foxp3+ regulatory T cell population during the differentiation process of Th1 and Th17 cells. Stem Cell Res Ther 2013;4(3):65.
[59] Akiyama K, et al. Mesenchymal-stem-cell-induced immunoregulation involves FAS-ligand-/FAS-mediated T cell apoptosis. Cell Stem Cell 2012;10(5):544–55.
[60] Klyushnenkova E, Mosca JD, McIntosh KR. Human mesenchymal stem cells suppress allogeneic T cell responses in vitro: implications for allogeneic transplantation. Blood 1998;92(10). 642a–642a.
[61] Corcione A, et al. Human mesenchymal stem cells modulate B-cell functions. Blood 2006;107(1):367–72.
[62] Spaggiari GM, et al. Mesenchymal stem cell-natural killer cell interactions: evidence that activated NK cells are capable of killing MSCs, whereas MSCs can inhibit IL-2-induced NK-cell proliferation. Blood 2006;107(4):1484–90.
[63] Spaggiari GM, et al. Mesenchymal stem cells inhibit natural killer-cell proliferation, cytotoxicity, and cytokine production: role of indoleamine 2,3-dioxygenase and prostaglandin E2. Blood 2008;111(3):1327–33.
[64] Jiang XX, et al. Human mesenchymal stem cells inhibit differentiation and function of monocyte-derived dendritic cells. Blood 2005;105(10):4120–6.
[65] Nemeth K, et al. Bone marrow stromal cells attenuate sepsis via prostaglandin E(2)-dependent reprogramming of host macrophages to increase their interleukin-10 production. Nat Med 2009;15(1):42–9.
[66] Nemeth K, et al. Bone marrow stromal cells use TGF-beta to suppress allergic responses in a mouse model of ragweed-induced asthma. Proc Natl Acad Sci U S A 2010;107(12):5652–7.
[67] DelaRosa O, et al. Requirement of IFN-gamma-mediated indoleamine 2,3-dioxygenase expression in the modulation of lymphocyte proliferation by human adipose-derived stem cells. Tissue Eng A 2009;15(10):2795–806.
[68] Rafei M, et al. Mesenchymal stromal cells ameliorate experimental autoimmune encephalomyelitis by inhibiting CD4 Th17 T cells in a CC chemokine ligand 2-dependent manner. J Immunol 2009;182(10):5994–6002.
[69] Di Trapani M, et al. Differential and transferable modulatory effects of mesenchymal stromal cell-derived extracellular vesicles on T, B and NK cell functions. Sci Rep 2016;6:24120.
[70] Chiossone L, et al. Mesenchymal stromal cells induce peculiar alternatively activated macrophages capable of dampening both innate and adaptive immune responses. Stem Cells 2016;34(7):1909–21.
[71] Marsell R, Einhorn TA. The biology of fracture healing. Injury 2011;42(6):551–5.
[72] Ghajar CM, et al. Mesenchymal cells stimulate capillary morphogenesis via distinct proteolytic mechanisms. Exp Cell Res 2010;316(5):813–25.
[73] Xing Z, et al. Multiple roles for CCR2 during fracture healing. Dis Model Mech 2010;3(7–8):451–8.
[74] Alexander KA, et al. Osteal macrophages promote in vivo intramembranous bone healing in a mouse tibial injury model. J Bone Miner Res 2011;26(7):1517–32.
[75] Gong L, et al. The macrophage polarization regulates MSC osteoblast differentiation in vitro. Ann Clin Lab Sci 2016;46(1):65–71.
[76] Blumer MJ, Longato S, Fritsch H. Localization of tartrate-resistant acid phosphatase (TRAP), membrane type-1 matrix metalloproteinases (MT1-MMP) and macrophages during early endochondral bone formation. J Anat 2008;213(4):431–41.
[77] Huang WC, et al. Classical macrophage activation up-regulates several matrix metalloproteinases through mitogen activated protein kinases and nuclear factor-kappaB. PLoS ONE 2012;7(8):e42507.

[78] Dreier R, et al. Paracrine interactions of chondrocytes and macrophages in cartilage degradation: articular chondrocytes provide factors that activate macrophage-derived pro-gelatinase B (pro-MMP-9). J Cell Sci 2001;114(Pt 21):3813–22.
[79] Colnot C, et al. Altered fracture repair in the absence of MMP9. Development 2003;130(17):4123–33.
[80] Kosaki N, et al. Impaired bone fracture healing in matrix metalloproteinase-13 deficient mice. Biochem Biophys Res Commun 2007;354(4):846–51.
[81] McDonald MM, et al. Matrix metalloproteinase-driven endochondral fracture union proceeds independently of osteoclast activity. J Bone Miner Res 2013;28(7):1550–60.
[82] Fajardo M, et al. Matrix metalloproteinases that associate with and cleave bone morphogenetic protein-2 in vitro are elevated in hypertrophic fracture nonunion tissue. J Orthop Trauma 2010;24(9):557–63.
[83] Konnecke I, et al. T and B cells participate in bone repair by infiltrating the fracture callus in a two-wave fashion. Bone 2014;64:155–65.
[84] Gerstenfeld LC, et al. Impaired fracture healing in the absence of TNF-alpha signaling: the role of TNF-alpha in endochondral cartilage resorption. J Bone Miner Res 2003;18(9):1584–92.
[85] Gerstenfeld LC, et al. Impaired intramembranous bone formation during bone repair in the absence of tumor necrosis factor-alpha signaling. Cells Tissues Organs 2001;169(3):285–94.
[86] Lehmann W, et al. Tumor necrosis factor alpha (TNF-alpha) coordinately regulates the expression of specific matrix metalloproteinases (MMPS) and angiogenic factors during fracture healing. Bone 2005;36(2):300–10.
[87] Kondo M, et al. IL-17 inhibits chondrogenic differentiation of human mesenchymal stem cells. PLOS ONE 2013;8(11):e79463.
[88] Croes M, et al. Proinflammatory T cells and IL-17 stimulate osteoblast differentiation. Bone 2016;84:262–70.
[89] Mountziaris PM, Mikos AG. Modulation of the inflammatory response for enhanced bone tissue regeneration. Tissue Eng B Rev 2008;14(2):179–86.
[90] Chen G, Deng C, Li YP. TGF-beta and BMP signaling in osteoblast differentiation and bone formation. Int J Biol Sci 2012;8(2):272–88.
[91] James AW. Review of signaling pathways governing MSC osteogenic and adipogenic differentiation. Scientifica (Cairo) 2013;2013:684736.
[92] Oshita K, et al. Human mesenchymal stem cells inhibit osteoclastogenesis through osteoprotegerin production. Arthritis Rheum 2011;63(6):1658–67.
[93] Fan X, et al. Macrophage colony stimulating factor down-regulates MCSF-receptor expression and entry of progenitors into the osteoclast lineage. J Bone Miner Res 1997;12(9):1387–95.
[94] Kong YY, et al. OPGL is a key regulator of osteoclastogenesis, lymphocyte development and lymph-node organogenesis. Nature 1999;397(6717):315–23.
[95] Wu AC, et al. Unraveling macrophage contributions to bone repair. Bonekey Rep 2013;2:373.
[96] Cho SW. Role of osteal macrophages in bone metabolism. J Pathol Transl Med 2015;49(2):102–4.
[97] Huang H, et al. IL-17 stimulates the proliferation and differentiation of human mesenchymal stem cells: implications for bone remodeling. Cell Death Differ 2009;16(10):1332–43.
[98] Lam J, et al. TNF-alpha induces osteoclastogenesis by direct stimulation of macrophages exposed to permissive levels of RANK ligand. J Clin Invest 2000;106(12):1481–8.
[99] Gilbert L, et al. Inhibition of osteoblast differentiation by tumor necrosis factor-alpha. Endocrinology 2000;141(11):3956–64.
[100] Bhandari M, et al. Predictors of reoperation following operative management of fractures of the tibial shaft. J Orthop Trauma 2003;17(5):353–61.
[101] Bajada S, et al. Decreased osteogenesis, increased cell senescence and elevated Dickkopf-1 secretion in human fracture non union stromal cells. Bone 2009;45(4):726–35.
[102] Seebach C, et al. Number and proliferative capacity of human mesenchymal stem cells are modulated positively in multiple trauma patients and negatively in atrophic nonunions. Calcif Tissue Int 2007;80(4):294–300.
[103] Mathieu M, et al. Decreased pool of mesenchymal stem cells is associated with altered chemokines serum levels in atrophic nonunion fractures. Bone 2013;53(2):391–8.
[104] Bajada S, et al. Successful treatment of refractory tibial nonunion using calcium sulphate and bone marrow stromal cell implantation. J Bone Joint Surg Br 2007;89(10):1382–6.
[105] Tawonsawatruk T, Kelly M, Simpson H. Evaluation of native mesenchymal stem cells from bone marrow and local tissue in an atrophic nonunion model. Tissue Eng C Methods 2014;20(6):524–32.
[106] Gokturk E, et al. Oxygen-free radicals impair fracture healing in rats. Acta Orthop Scand 1995;66(5):473–5.
[107] Grogaard B, Gerdin B, Reikeras O. The polymorphonuclear leukocyte: has it a role in fracture healing? Arch Orthop Trauma Surg 1990;109(5):268–71.
[108] Champagne CM, et al. Macrophage cell lines produce osteoinductive signals that include bone morphogenetic protein-2. Bone 2002;30(1):26–31.
[109] Herman S, et al. Induction of osteoclast-associated receptor, a key osteoclast costimulation molecule, in rheumatoid arthritis. Arthritis Rheum 2008;58(10):3041–50.
[110] Crop MJ, et al. Human mesenchymal stem cells are susceptible to lysis by CD8(+) T cells and NK cells. Cell Transplant 2011;20(10):1547–59.
[111] Gotherstrom C, et al. Fetal and adult multipotent mesenchymal stromal cells are killed by different pathways. Cytotherapy 2011;13(3):269–78.
[112] Sotiropoulou PA, et al. Interactions between human mesenchymal stem cells and natural killer cells. Stem Cells 2006;24(1):74–85.
[113] Poggi A, et al. Interaction between human NK cells and bone marrow stromal cells induces NK cell triggering: role of NKp30 and NKG2D receptors. J Immunol 2005;175(10):6352–60.
[114] Harre U, et al. Induction of osteoclastogenesis and bone loss by human autoantibodies against citrullinated vimentin. J Clin Invest 2012;122(5):1791–802.
[115] Dighe AS, et al. Interferon gamma and T cells inhibit osteogenesis induced by allogeneic mesenchymal stromal cells. J Orthop Res 2013;31(2):227–34.
[116] Huang H, et al. Dose-specific effects of tumor necrosis factor alpha on osteogenic differentiation of mesenchymal stem cells. Cell Prolif 2011;44(5):420–7.
[117] Diarra D, et al. Dickkopf-1 is a master regulator of joint remodeling. Nat Med 2007;13(2):156–63.
[118] Tsukasaki M, et al. Expression of POEM, a positive regulator of osteoblast differentiation, is suppressed by TNF-alpha. Biochem Biophys Res Commun 2011;410(4):766–70.
[119] Kitaura H, et al. M-CSF mediates TNF-induced inflammatory osteolysis. J Clin Invest 2005;115(12):3418–27.
[120] Gao Y, et al. IFN-gamma stimulates osteoclast formation and bone loss in vivo via antigen-driven T cell activation. J Clin Invest 2007;117(1):122–32.
[121] Zwerina J, et al. TNF-induced structural joint damage is mediated by IL-1. Proc Natl Acad Sci U S A 2007;104(28):11742–47.
[122] Sato K, et al. Th17 functions as an osteoclastogenic helper T cell subset that links T cell activation and bone destruction. J Exp Med 2006;203(12):2673–82.
[123] Lee Y. The role of interleukin-17 in bone metabolism and inflammatory skeletal diseases. BMB Rep 2013;46(10):479–83.
[124] Ray S, et al. Effects of macroporous, strontium loaded xerogel-scaffolds on new bone formation in critical-size metaphyseal fracture defects in ovariectomized rats. Injury 2016;47(Suppl. 1):S52–61.
[125] Giannoudis PV, Einhorn TA, Marsh D. Fracture healing: the diamond concept. Injury 2007;Suppl. 4(38):S3–6.
[126] Giannoudis PV, et al. The diamond concept – open questions. Injury 2008;Suppl. 2(39):S5–8.
[127] Giannoudis PV, et al. Long bone non-unions treated with the diamond concept: a case series of 64 patients. Injury 2015;Suppl. 8(46):S48–54.
[128] Gomez-Barrena E, et al. Bone fracture healing: cell therapy in delayed unions and nonunions. Bone 2015;70:93–101.
[129] Guimaraes JA, et al. The effect of autologous concentrated bone-marrow grafting on the healing of femoral shaft non-unions after locked intramedullary nailing. Injury 2014;Suppl. 5(45):S7–13.
[130] Marcacci M, et al. Stem cells associated with macroporous bioceramics for long bone repair: 6- to 7-year outcome of a pilot clinical study. Tissue Eng 2007;13(5):947–55.
[131] Kim SJ, et al. A multi-center, randomized, clinical study to compare the effect and safety of autologous cultured osteoblast (Ossron) injection to treat fractures. BMC Musculoskelet Disord 2009;10:20.
[132] Wang Y, et al. Plasticity of mesenchymal stem cells in immunomodulation: pathological and therapeutic implications. Nat Immunol 2014;15(11):1009–16.
[133] Prigozhina TB, et al. Mesenchymal stromal cells lose their immunosuppressive potential after allotransplantation. Exp Hematol 2008;36(10):1370–6.
[134] Okuno M, et al. Meniscus regeneration by syngeneic, minor mismatched, and major mismatched transplantation of synovial mesenchymal stem cells in a rat model. J Orthop Res 2014;32(7):928–36.
[135] Isakova IA, et al. Allo-reactivity of mesenchymal stem cells in rhesus macaques is dose and haplotype dependent and limits durable cell engraftment in vivo. PLOS ONE 2014;9(1):e87238.
[136] Arinzeh TL, et al. Allogeneic mesenchymal stem cells regenerate bone in a critical-sized canine segmental defect. J Bone Joint Surg Am 2003;85-A(10):1927–35.
[137] Guo SQ, et al. Immunological study of allogeneic mesenchymal stem cells during bone formation. J Int Med Res 2009;37(6):1750–9.
[138] Udehiya RK, et al. Comparison of autogenic and allogenic bone marrow derived mesenchymal stem cells for repair of segmental bone defects in rabbits. Res Vet Sci 2013;94(3):743–52.
[139] Streckbein P, et al. Reconstruction of critical-size mandibular defects in immunoincompetent rats with human adipose-derived stromal cells. J Craniomaxillofac Surg 2013;41(6):496–503.
[140] Gu H, et al. Bone regeneration in a rabbit ulna defect model: use of allogeneic adipose-derivedstem cells with low immunogenicity. Cell Tissue Res 2014;358(2):453–64.
[141] Xie F, et al. Ectopic osteogenesis of allogeneic bone mesenchymal stem cells loading on beta-tricalcium phosphate in canines. Plast Reconstr Surg 2014;133(2):142e–53e.
[142] Reinders ME, Hoogduijn MJ. NK cells and MSCs: possible implications for MSC therapy in renal transplantation. J Stem Cell Res Ther 2014;4(2):1000166.
[143] Spiller KL, et al. The role of macrophage phenotype in vascularization of tissue engineering scaffolds. Biomaterials 2014;35(15):4477–88.
[144] Vogel DY, et al. Human macrophage polarization in vitro: maturation and activation methods compared. Immunobiology 2014;219(9):695–703.
[145] Spiller KL, et al. Sequential delivery of immunomodulatory cytokines to facilitate the M1-to-M2 transition of macrophages and enhance vascularization of bone scaffolds. Biomaterials 2015;37:194–207.