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Concise Review: Stem Cells in Osteoimmunology

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ABSTRACT

Bone remodeling is a lifelong process in which mature bone tissue is removed from the skeleton by bone resorption and is replenished by new during ossification or bone formation. The remodeling cycle requires both the differentiation and activation of two cell types with opposing functions; the osteoclast, which orchestrates bone resorption, and the osteoblast, which orchestrates bone formation. The differentiation of these cells from their respective precursors is a process which has been overshadowed by enigma, particularly because the precise osteoclast precursor has not been identified and because the identification of skeletal stem cells, which give rise to osteoblasts, is very recent. Latest advances in the area of stem cell biology have enabled us to gain a better understanding of how these differentiation processes occur in physiological and pathological conditions. In this review we postulate that modulation of stem cells during inflammatory conditions is a necessary prerequisite of bone remodeling and therefore an essential new component to the field of osteoimmunology. In this context, we highlight the role of transcription factor nuclear factor of activated T cells cytoplasmic 1 (NFATc1), because it directly links inflammation with differentiation of osteoclasts and osteoblasts. STEM CELLS *2017;35:1461–1467*

SIGNIFICANCE STATEMENT

During the last decade an interdisciplinary research field has been developed focused on the crosstalk in between the immune and bone systems. The role of hematopoietic stem cells (HSC) has been largely acknowledged, since HSC give rise to both, all immune cells and osteoclasts. However, only recently some of the mechanisms underlying the effect of inflammation on HSC differentiation have been uncovered. In addition, the activity skeletal stem cells (SSC) is also likely to be affected by the immune system.

INTRODUCTION

During the last decade an interdisciplinary research field has been developed focused on the crosstalk between the immune and bone systems. Although the interplay in between these systems has been recognized since the early 1970s, osteoimmunology started with studies on rheumatoid arthritis, where a strong connection between activation of T lymphocytes and osteoclastogenesis was established [1, 2]. Since then, it has expanded to study the interplay of cells and cross-talk of signaling proteins between bone cells and the immune system [1].

Bone is a dynamic tissue in the body that regenerates itself throughout adult life. This remodeling cycle requires the activity of two cell types with opposing functions; osteoclasts, which orchestrate bone resorption, and osteoblasts, which orchestrate bone formation. Both of these cell types need to be constantly replenished, as terminally differentiated osteoclasts are short-lived and osteoblasts mature into postmitotic osteocytes, which are embedded in bone [3]. The number of osteoclasts and osteoblasts on the bone surface does not get exhausted throughout life as they are constantly replenished by tissue specific stem cells. Albeit significant differences in the dynamics of osteoclast and osteoblast replenishment by stem cells, recent studies in both mice and human cells have allowed a better understanding of the basic principles that govern these processes.

STEM CELLS

In general, stem cells are a class of undifferentiated cells that have the remarkable potential to develop into specialized cell types in the body during development and maintenance of tissue homeostasis. Commonly, stem cells are derived from two main sources: from the inner cell mass of the blastocyst during embryological development (embryonic stem cells) and from adult tissues (adult stem cells). Adult stem cells are found among differentiated cells

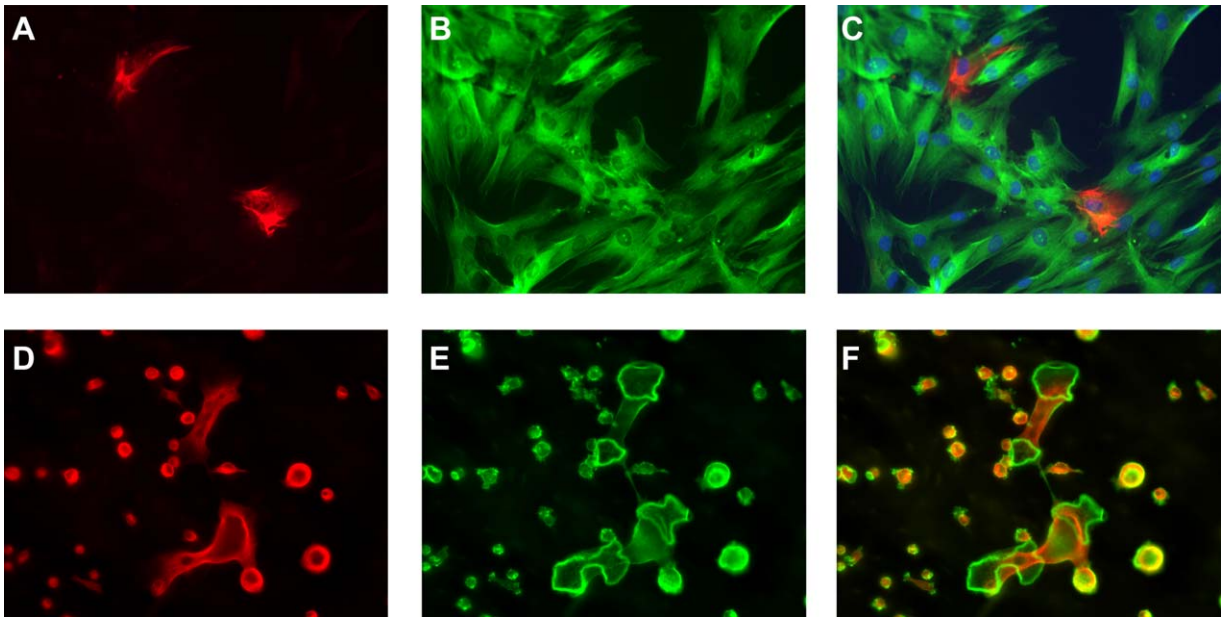

Figure 1. A heterogeneous population of mesenchymal stem cell (MSC) and hematopoietic stem cell (HSC) gives rise to terminally differentiated cells with opposing functions in bone remodeling. Immunofluorescence microscopy of human bone marrow derived MSC **(A–C)**. MSC after 14 days in culture showing homogenous expression of β-III-tubulin (green; A), and differential expression of nestin (red, B), highlighting the heterogeneous population of MSC (merged image, C). Immunofluorescence microscopy of mouse bone marrow derived HSC **(D–F)** cultured in the presence of M-CSF and RANKL showing the homogeneous expression of α-tubulin (red, D), and the presence of multinucleated giant cells forming filamentous-actin ring structures (Phalloidin labeled actin in green, E), highlighting the heterogeneous population of osteoclast precursor cells as not all cells become terminally differentiated osteoclasts merged image (F). Original magnification for (A–C) ×200 and for (D–F) ×60.

in various tissues and organs, and thus are also called somatic stem cells, where somatic refers to cells of the body that differ from germ cells (sperm or eggs). Adult stem cells are of particular interest in many pathological conditions as they have the capacity to self-renew and differentiate to all of the major specialized cell types of the tissue in which they are found and thereby maintain the homeostasis and repair of that tissue.

Stem cells are therefore very important in bone homeostasis, as bone is continuously being remodeled throughout adulthood and undergoes repairs during fracture healing. The bone remodeling process requires both the differentiation and activation of two cell types with opposing functions: the osteoclast, a cell of hematopoietic origin that orchestrates bone resorption, and the osteoblast, a cell claimed to be of mesenchymal origin, which orchestrates bone formation. Two stem cell populations control the differentiation of these cells from their respective precursors in the bone marrow: the hematopoietic stem cells (HSC), which generate all the types of blood cells in the body and the skeletal stem cells (SSC) (which seem to correspond with a subpopulation of mesenchymal stem cells; MSC), which generate bone, stroma, and cartilage and are discussed below. Hence, in the coming years it will be important to establish the role of HSC and SSC on osteoimmunology. In the next sections we describe HSC and SSC and how they differentiate into osteoclasts and osteoblasts, respectively. We then describe our current understanding of the relationship between HSC, SSC and inflammation and address possible implications for bone homeostasis.

Hematopoietic Stem Cells and Osteoclastogenesis

The human body requires the replenishment of more than 50 billion blood cells (leukocytes, erythrocytes, and platelets) daily. This rapid turnover is possible due to HSC harbored in the bone marrow, capable of differentiating into all cells of the hematopoietic lineage, while maintaining a rather constant pool of HSC through self-renewal, thus avoiding cell exhaustion. In mice, different sets of markers have been identified to identify HSC including the original $Lin^{-}Sca^{-}1^{+}c\text{-}kit^{+}$ (LSK) signature [4]. However, the LSK HSC compartment is heterogeneous and is generally divided into at least two multipotent subpopulations, namely long-term (LT)-HSC and short-term (ST)-HSC [5]. LT-HSCs have extensive (life-long) self-renewal potential and on commitment give rise to ST-HSCs with more restricted self-renewal capacity. Recently, the developmental gene homeobox B5 (Hoxb5) was shown to distinguish LT-HSC (cells capable of sustained self-renewal for an organism's lifetime) from ST-HSC (cells with limited self-renewal capacity that do not repopulate secondary recipients in serial transplantation assays in mice) [6]. Other studies have shown that the ST-HSC population also exhibits heterogeneity comprising $CD34^{-}flt3^{-}$, $CD34^{+}flt3^{-}$, and $CD34^{+}flt3^{+}$ cells [7].

HSC are also identified by the signaling lymphocyte activation molecule markers $CD150^{+}CD244^{-}CD48^{-}$ [8]. HSC can commit into multipotent progenitor (MPP) identified as $CD150^{-}CD244^{+}CD48^{-}$, and a late MMP, $CD150^{-}CD244^{+}CD48^{+}$ [8], which are capable of giving rise to both lymphoid and myeloid lineages [9], but show very limited or no self-renewal potential [5]. These cells can then further commit into a common myeloid progenitor (CMP), characterized by the expression of

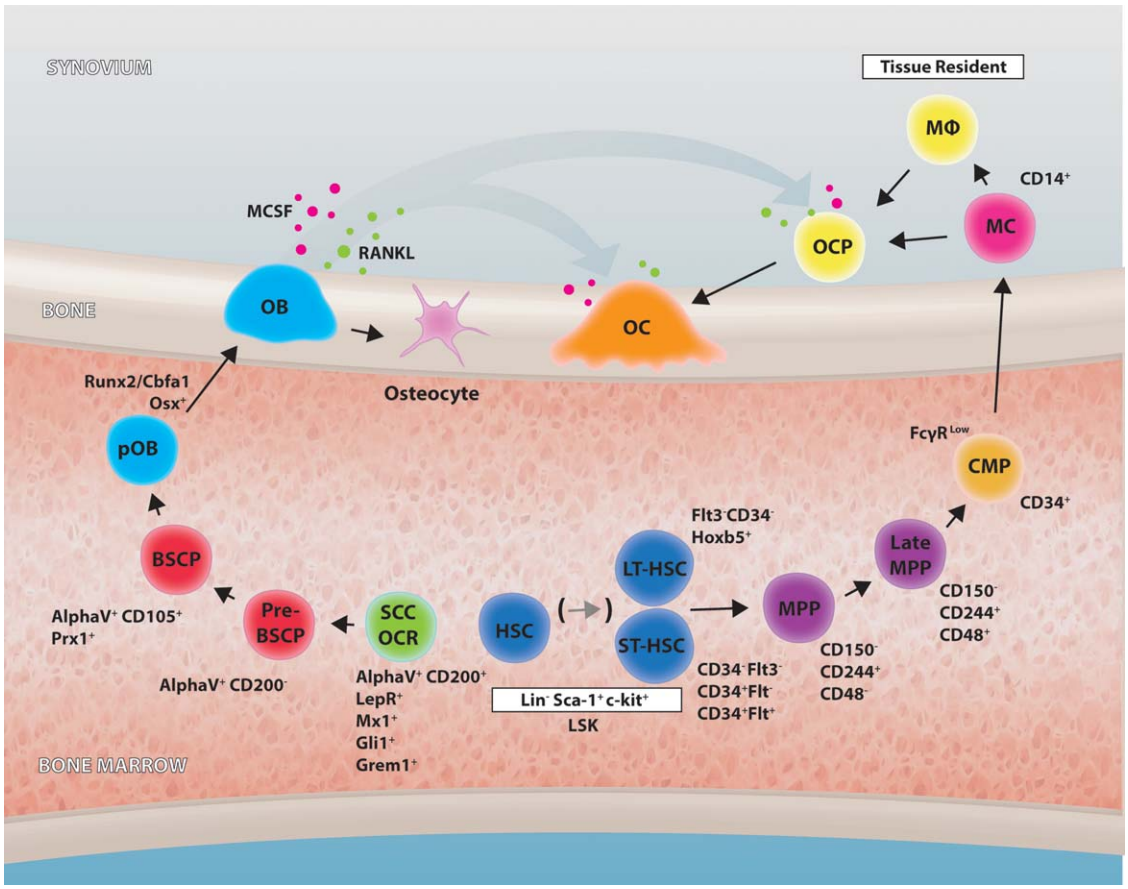

Figure 2. Stem cell progenitors in bone homeostasis. Schematic graphical representation of the bone marrow microenvironment, one of the primary lymphoid organs, harbors immune cells including HSCs, lymphocytes, monocytes/macrophages, and neutrophils, all of which interplay with bone cells such as osteoblasts, osteoclasts, and osteocytes. Schematic depicts the differentiation stages and early markers of HSC and SSC differentiation to osteoclasts and osteoblasts, respectively. LT-HSC, ST-HSC, MPP, late multipotent progenitor (late-MMP), CMP, MC, MΦ, OC. SSC, OCR, Pre-BSCP, BSCP, pOB, OB and osteocyte. Bone remodeling is regulated by MCSF and RANKL at the bone surface. Abbreviations: BSCP, bone, stroma, and cartilage progenitor; CMP, common myeloid progenitor; HSC, hematopoietic stem cell; MC, monocyte; M-CSF, macrophage colony stimulating factor; MPP, multipotent progenitor; MΦ, macrophage; OB, osteoblast; OC, osteoclast; pOB, pre-osteoblast; pBSCP, pre-bone, stroma, and cartilage progenitor; SCC, skeletal stem cell; OCR, osteochondroreticular stem cell; LT-HSC, long-term HSC; ST-HSC, short-term HSC; RANKL, receptor activator for nuclear factor kappa B ligand.

FCγR^{low} and CD34$^+$ [10]. In the presence of macrophage colony stimulating factor, c-fms$^+$ (CD115$^+$), CMP can differentiate in the bone marrow into monoblasts, which then enter circulation and differentiate into immature promonocytes [11] CD13$^+$/CD11b$^+$, and mature monocytes CD11b$^{+/}$CD14$^+$ RANK$^+$ osteoclast precursors which can further be differentiated to osteoclasts in response to receptor activator for nuclear factor kappa B ligand (RANKL) signaling (Fig. 1). Tissue resident CD14$^+$ macrophages also can differentiate to osteoclasts. Terminally differentiated osteoclasts express tartrate-resistant acid phosphatase, cathepsin K, calcitonin receptor, and the $\alpha\nu\beta3$ integrin which facilitate the process of bone resorption. Therefore, during the commitment and differentiation of HSC into osteoclasts [12, 13], some intermediate, immature cell types can be identified based on specific expression of markers, as shown in Figure 2. However, this standard hierarchical model, although widely accepted, has been recently challenged with experiments demonstrating a certain degree of plasticity in the system. For example, different precursors rather than HSC can maintain hematopoiesis [14], and differentiation can occur without going through a specific intermediate cell type [9]. Similarly, various CD11b$^-$ and/or CD14$^-$ and/or CD11c$^+$ populations have also been shown to differentiate to osteoclasts [15, 16]. Hence, it is prudent to consider the hierarchy shown in Figure 1 as a working, model in progress.

Skeletal Stem Cells and Osteoblastogenesis

In addition to HSC, the bone marrow hosts a stem cell type known to differentiate into bone and other skeletal tissues [17, 18]. When bone marrow mononuclear cells are seeded and expanded in tissue culture flasks, a population of cells appears that acquires a fibroblastic morphology and maintains for several passages the potential to differentiate into bone, adipose cells, and cartilage. These cells are commonly called mesenchymal stem cells or marrow stromal cells (MSC) [19] (Fig. 1). However, it has been recently established that ex vivo expanded MSC differ from their in vivo counterparts, which in the context of bone remodeling, are best identified as SSC [20]. The true nature of SSC in vivo has been elusive at least in part due to the lack of markers to identify this cell type in situ [21]. In addition, there is an apparent overlap in between the cells that gives rise to bone (SSC) and cells in the bone

marrow capable of supporting hematopoiesis, serving as a key component of the so-called HSC niche [22]. Furthermore, there is strong evidence that MSC correspond with perivascular cells (pericytes) [23], serving as an explanation for why MSC can be isolated from virtually all vascularized tissues [24]. In consequence, at least three cell types in the bone marrow have been determined functionally, based on the expression of specific marker: SSC, HSC-supporting cells which are identified as $CXCL12^+$ [25] $Nestin^+$ [26], $Prx1^+$ [27], or SCF^+ [28], and pericytes expressing $CD146^+$ [29]. To date, it remains unclear to what extent these three cell types overlap in terms of identity or differ from each other. For example, a population of $CD146^+$ subendothelial cells in human bone marrow contains osteogenic progenitors that are also at the origin of the stromal cells that support hematopoiesis [22].

In mice, SSC have been recently identified as either Integrin $alphaV^+$ $CD200^+$ [30], Leptin-receptor $(LepR)^+$ [31], $Mx1^+$ [32], $Gli\text{-}1^+$ [33], or Gremlin 1^+ [34]. Gremlin 1^+ have been also called osteochondroreticular (OCR) stem cells to highlight the ability of these cells to differentiate into osteoblasts, chondrocytes, and reticular marrow stromal cells, but not adipocytes. Since SSC/OCR have only recently been identified in vivo, it remains unknown if they correspond to the same or different cell types. Chan et al. [30] have recently suggested a hierarchical commitment for SSC into osteoblasts, similar to the progressive stages of HSC into osteoclasts. In this model, integrin $alphaV^+$ $CD200^+$ cells commit into $alphaV^+$ $CD200^-$ pre-bone, stroma and cartilage progenitor (BSCP) cells, which have no self-renewal properties. Pre-BSCP can then further commit into $alphaV^+$ $CD105^+$ BSCP. Experiments with transgenic mice have further demonstrated that $Prx1^+$ cells can give rise to osteoblasts and chondrocytes [35]. Hence, $Prx1^+$ cells approach the definition of $alphaV^+$ $CD105^+$ BSCP [30, 36], although their contribution to stroma is not known. It is then likely that BSCP, with possibly more, undefined intermediate cell types, gives rise to proliferating preosteoblasts, which are characterized by the expression of the transcription factor Runx2/Cbfa1 and are completely committed cells into the osteogenic lineage [37]. Runx2/Cbfa1 is expressed restrictively in fetal development and Cbfa1 mutant mice have completely block of intramembranous and endochondral ossification owing to the maturational arrest of osteoblasts demonstrating the essential role of Cbfa1 in osteogenesis [38, 39]. Cbfa1 activates expression of Osterix [40], and preosteoblasts mature to give rise to calcium precipitating osteoblasts. During osteoblast differentiation, Runx2 and Osterix upregulate the expression of bone matrix protein genes including collagen type I alpha 1, integrin-binding sialoprotein, osteopontin, and fibronectin 1 [41, 42]. Terminally differentiated osteoblasts finally mature into osteocytes (Figure 2).

Inflammation on HSC Differentiation with Implications on Bone Resorption

Inflammation is a protective response of the body to injury or infection. During inflammation, a high demand for myeloid and lymphoid cells causes HSC to divide and differentiate to supply the required cells. This expansion is normally not at the expense of the original HSC pool, because acute inflammation also increases self-renewal of the cells [43]. However, chronic inflammation can lead to HSC exhaustion and anemia [44]. HSC may sense inflammation directly in response to interferons, tumor necrosis factor (TNF), interleukin 1β (IL-1β), and other proinflammatory cytokines, reviewed in [45]. In addition, HSC may respond indirectly by changes triggered by inflammation in the stem cell niche, such as decreased stromal cell derived factor 1 (SDF-1) secretion by osteoblasts induced by granulocyte colony-stimulating factor (G-CSF), or the egress of bone marrow-resident macrophages [46, 47]. Interestingly, during inflammation, circulating HSC are increased and primed to differentiate in situ into myeloid cells [48, 49]. Since HSCs have the capacity to differentiate into osteoclasts, it is not surprising that increased myelopoiesis is directly linked with increased osteoclastogenesis and bone loss in inflammatory conditions [50, 51]. In fact, numerous reports have shown that any disturbance in the number of myeloid precursors will significantly affect the rate of osteoclast formation [15] and inflammatory bone loss.

Although the exact osteoclast precursor(s) remains to be defined, a number of cell types (macrophages, monocytes, immature dendritic cells) and molecules have been described as potential osteoclastogenesis agents both in the presence and/or in the absence of exogenous RANK ligand (RANKL) in vivo and in vitro [52]. RANKL is produced by osteoblasts under physiological conditions, but also activated immune cells, including B and T lymphocytes, have also been described to secrete RANKL [53].

Although the concept that alternative pathways of osteoclastogenesis independent of RANKL exist is still a matter of debate, it is clearly evident that a few proinflammatory cytokines including TNF [54, 55] and IL-23 [56] regulate the activation of calcium signaling and nuclear factor of activated T cells cytoplasmic 1 (NFATc1). $NFATc1^{-/-}$ cells are unable to generate osteoclasts despite normal development into the monocyte/macrophage lineage highlighting the specific needs of osteoclastogenesis [57]. NFATc1 is a transcription factor activated by calcium signaling, as Ca^{2+} activates calcineurin, which in turn dephosphorylates multiple phosphoserines on NFAT, leading to its nuclear translocation and activation. NFATc1 is responsible for the regulation of genes related to osteoclast function as well as numerous genes nonessential to osteoclast function [58, 59]. Therefore, the significance of this pathway may extend beyond our current understanding.

Inflammation on SSC Differentiation with Implications on Bone Formation

SSC, which also give rise to chondrocytes, and reticular marrow stromal cells, differentiate into preosteoblasts and then become osteoblasts on the bone surface. The signals that regulate the decision of progenitor cells to form osteoblasts are complex and partially understood [60]. Osteoblast regulation can be achieved from several signals. Transforming growth factor-β (TGF-β) signaling through activation of receptor type I (R-I) and receptor type II (R-II), transduces signals to Smads, which form a complex with Smad4 and then translocate into the nucleus where they interact with Runx2 also known as Cbfa1 transcription factor to trigger activation of osteoblast specific genes [42, 61]. Commonly Runx2 is considered the major transcription factor to trigger activation of

osteoblast specific genes. However, NFATc1 has also been implicated in osteoblast differentiation, since it forms a complex with Osterix, and together cooperatively regulate osteogenesis [62]. Other groups independently of these studies have also confirmed these observations using the opposite approach where mice expressing a constitutively nuclear NFATc1 variant (NFATc1nuc) in osteoblasts develop high bone mass due to massive osteoblast overgrowth and enhanced osteoblast proliferation [63]. In keeping with these observations it was recently shown that NFATc1 acts downstream of BMP-2 signaling inducing alkaline phosphatase activity and nodule formation in an osteoblastic cell line [64]. Of note, other groups have shown that constitutively active NFATc1 in osteoblast cell lines inhibits osteoblastogenesis in vitro [65]. These data suggest that modulation of NFATc1 may differ in early versus late differentiation and possibly in precursor versus terminally differentiated cells. These findings may serve as explanation for why patients following organ transplantation and treated with inhibitors of the Calcineurin/NFATc1 pathway, such as cyclosporin A and FK506 often develop osteoporosis [66]. Of note, IL-23 which is known to activate NFATc1 also promotes osteogenic differentiation of MSC, by activation of the β-catenin pathway [67]. Similarly, proinflammatory cytokines IL-6 and IL-17 promote osteogenesis of MSC in vitro [68, 69]. Altogether, our current understanding suggests that acute inflammation promotes osteoblastogenesis. In fact, it is well documented that the acute inflammation stage following bone fracture is necessary to promote bone repair [70], and there is impaired fracture healing in the absence of TNF signaling [71] or chronic inflammation [72]. Nonetheless, recent evidence suggests that the anti-inflammatory cytokine IL-27 may promote bone formation, but inhibiting osteoclastogenesis and inhibiting apoptosis of osteoblasts [73]. Future work is required to elucidate if inflammation activates NFATc1 in SSC or the osteoblastic progeny, similarly as observed in osteoclast precursors.

Periosteal and endosteal bone-lining tissues contain resident macrophages (termed osteomacs [74]), which play an important role supporting osteoblastogenesis during bone repair. Macrophages secrete factors including TGF-β, osteopontin, 1,25-dihydroxy-vitamin D3, and BMP-2 [75]. However, the specific role of osteomacs, although likely related to anabolism, on bone repair are poorly understood [76].

Since SSC have only been recently identified, the effect of inflammation on these cells is largely unknown. On the other hand, ex vivo expanded MSC (Figure 2) are widely known to be effective immune suppressors and are promising therapeutic agents for steroid-refractory acute graft versus host disease (GVHD) [77], Crohn's disease [78], and allograft rejection after organ transplantation [79]. Also many other early stage clinical trials are on their way to test the efficacy of MSCs as immune suppressors [80]. MSC affect lymphocytes, monocytes, and dendritic cells by either secretion of factors such as prostaglandin E 2 (PGE2), indoleamine 2,3-dioxygenase (IDO), or cytokines, reviewed by [81]. Of note, a clinical dose of MSC is typically around 20–100 million cells per patient, while the frequency of SSC in the bone marrow is expected to be approximately one in 100,000 mononuclear cells [19]. Therefore, it remains unclear to what extent cells in the bone marrow (SSC, pericytes or others), that seem to correspond with MSC, also exert significant immune suppressive functions.

Conclusion

The role of HSC has been largely acknowledged, since HSC give rise to both immune cells and osteoclasts. However, only recently have some of the mechanisms underlying the effect of inflammation on HSC differentiation been uncovered. In addition, the activity of a second type of stem cell in the bone marrow, identified as SSC, has been demonstrated to be affected by the immune system. This is particularly important since, in chronic inflammatory diseases where excessive bone loss is commonly observed, changes at the bone surface may reflect changes within the bone marrow. Therefore, it is evident that the cellular and molecular interplay is not only present on the bone surface where bone resorption occurs but rather it begins very early and well before bone cells are terminally differentiated.

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Author Contributions

F.A.F. and I.E.A.: conception and design, collection and/or assembly of data, data analysis and interpretation, manuscript writing, and final approval of manuscript; J.A.N.: manuscript writing and final approval of manuscript.

Disclosure of Potential Conflicts of Interest

The authors indicate no potential conflicts of interest.

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