Mesenchymal Stromal Cells in Rheumatoid Arthritis: Biological Properties and Clinical Applications

Maria-Christina Kastrinaki and Helen A. Papadaki*

Department of Hematology, University of Crete School of Medicine, Heraklion, Crete, Greece

Abstract: Mesenchymal stromal cells (MSC) isolated from a variety of adult tissues including the bone marrow (BM), have the capacity to differentiate into different cell types such as bone and cartilage and have therefore attracted scientific interest as potential therapeutic tools for tissue repair. MSC display also immunosuppressive and anti-inflammatory properties and their putative therapeutic role in a variety of inflammatory autoimmune diseases is currently under investigation. Joint destruction, caused by persistent inflammation, renders rheumatoid arthritis (RA) a possible clinical target for cartilage and bone repair using BM MSCs for their tissue repair and immunoregulatory effects. A number of studies, based mainly on experimental animal models, have recently provided interesting data on the potential of BM-MSCs to suppress local inflammation and tissue damage in RA whereas tissue engineering and cell-scaffold technology represents an emerging field of research. This review deals with the biological repair/regeneration of joint tissues in RA *via* MSC-based therapies. In view of the current interest in the autologous usage of BM MSC in RA, all available data on the biological properties of patient MSCs including the immunoregulatory characteristics, differentiation capacity towards osteocytes/chondrocytes, clonogenic/proliferative potential and molecular/protein profile and the possible influence of the RA milieu will be also summarized.

Keywords: Mesenchymal stromal cells (MSCs), bone marrow, rheumatoid arthritis, autoimmune diseases, tissue regeneration.

INTRODUCTION

As continuous cellular rejuvenation is needed for all animal tissues to remain vital, progenitor, tissue-specific stem/progenitor cells are needed to provide various mature phenotypes. Mesenchymal progenitors, more frequently referred as mesenchymal stromal cells (MSC) originally isolated from the bone marrow (BM) [1], can also be found in a variety of tissues and organs such as the synovial membrane, [2] synovial fluid [3], muscle [4], cartilage [5-7], bone [2, 8], adipose tissue [9,10], placenta [11], amniotic fluid [12] and umbilical cord [13-15]. MSCs display the capacity to generate stem progeny through symmetric or asymmetric divisions, as well as cells differentiated into the lineages of the tissue that they reside. However, compared to the traditional tissue-specific stem cells, MSCs display a high degree of plasticity as they can differentiate upon proper inductive signals to several lineages of different tissues or organs or even to switch between lineages of different embryonic origin [16,17].

BM is an easily accessible source of MSCs and, therefore, BM MSC properties have extensively been studied [18-21]. In contrast to the hematopoietic stem cells (HSCs), BM MSCs do not display a unique cellular identification marker. Instead, they express several cell surface antigens upon *in vitro* expansion such as the Stro-1 [22,23], CD105 (endoglin; SH2) [24], CD73 (SH3/SH4) [22,25], CD44 (hyaluronate receptor) [26,27], CD90 (Thy-1) [26], CD106 (vascular cell adhesion molecule-1, VCAM-1) [26], CD166 (activated leukocyte cell adhesion molecule, ALCAM) [28], CD29/CD49 (integrin family) [29], and CD200 [30] whereas they are negative for CD45, CD14 and CD34, markers specific for leukocytes, monocytes and HSCs, respectively [31]. The true *in vivo* phenotype of BM MSCs is still elusive. The low-affinity nerve growth factor receptor (LNGFR/CD271) and the carbohydrate embryonic stem cell antigen SSEA-4 have been recently emerged as highly specific markers for native BM MSCs [32-34]. Regarding their differentiation potential, it has been clearly shown that BM MSCs can differentiate towards osteoblasts [35], chondrocytes and adipocytes [35], cardiomyocytes [29], tendon cells and fibroblasts [36], among others.

BM MSC frequency, calculated by means of colony-forming unit fibroblast (CFU-F) or limiting dilution assays, has been estimated as 1 per 10^5 BM nucleated cells [37,38]. Although rare, MSCs influence the local environment by secreting several growth factors such as stem cell factor (SCF), interleukin (IL)-6, leukemia inhibitory factor (LIF), granulocyte-macrophage and macrophage colony stimulating factors (GM-CSF and M-CSF, respectively), chemokines such as IL-11, IL-15, and stromal derived factor-1 (SDF-1), adhesion molecules such as the intracellular adhesion molecule-1 (ICAM-1), VCAM-1, CD44 and extracellular matrix components such as fibronectin, collagen and glycosaminoglycans. By producing all these regulatory factors, MSC actually control the fate of the neighboring hematopoietic cells [39-41], as well as their own. They also respond to local homeostatic and/or traumatic demands and enter a more proliferative state resulting in tissue maintenance and rebuilding.

On the basis of their *in vitro* potential to differentiate into osteocytes and chondrocytes, BM MSCs have emerged as particularly promising therapeutic tools for bone and carti-

*Address correspondence to this author at the Professor of Hematology, University Hospital of Heraklion, PO Box 1352, Heraklion, Crete, Greece; Tel: +30 2810 394-629; Fax: +30 2810 394.632;
E-mail: epapadak@med.uoc.gr

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lage disorders whereas their regenerative potential and immunoregulatory effects render them potentially more powerful for degenerative and inflammatory diseases [42,43]. Rheumatoid arthritis (RA) is a candidate disease for articular repair requiring both cartilage/bone regeneration and local/systemic immunoregulation, potentially through MSCs. This review summarizes the available data on the biological properties of BM MSCs in RA as well as the hitherto clinical experience and future perspectives of this treatment approach. It should be stated that in this review, data from both human and murine research were included; hence some of the published research relating to murine models may not be relevant in humans.

MSCs IN RA

The Synovial Defect

RA is a common chronic autoimmune inflammatory disease (AID) mainly affecting the synovial membrane and the underlying cartilage and bone. The trigger of the autoimmunity process leading to the inflammation and destruction of the affected joints remains elusive [44]. RA patients, estimated as 1% in the general population, have a poor long-term prognosis and reduced overall life expectancy [45].

The synovial membrane in the affected RA joints, consisting mainly of fibroblast-like synoviocytes (FLS) and inflammatory cells, becomes hyperplastic (pannus) and the normally cell-free synovial fluid is gradually populated by numerous infiltrating inflammatory cells such as activated T- and B-lymphocytes, monocytes, macrophages and FLS. The T-lymphocytes are considered as the key cell-components of the autoimmune process [44]. Specifically, activated $CD4^+$ T-cells stimulate monocytes, macrophages and synovial FLS to produce pro-inflammatory cytokines such as IL-1β, IL-6, tumor necrosis factor (TNF)α and matrix metalloproteinases (MMPs). Inflammation is installed in the joint and attacks the underlying cartilage and bone. The cartilage is invaded by aggressive, highly proliferating FLS that cause cartilage destruction by producing MMPs whereas the native osteoclasts are triggered to damage further the cartilage and bone [46]. It seems that the combination of the accelerated tissue damage due to the underlying autoreactive/inflammatory process and the ineffectiveness of the tissue regeneration machinery are responsible for the joint destruction. The pathogenetic mechanisms underlying tissue damage in RA joint are depicted in Fig. (**1**).

TNFα is one of the cardinal mediators of the local tissue damage and systemic manifestations of RA and, therefore, the cytokine has been considered as a key-target of anti-arthritic biological therapies [47]. Most RA patients respond effectively to anti-TNFα treatments with a marked recess of the inflammation and improvement of the local and systemic symptoms [48]. However, by the time of diagnosis, a significant proportion of RA patients may have already acquired cartilage and bone deformities. Autologous or even allogeneic BM MSCs locally injected or implanted in biomaterials upon *in vitro* expansion and differentiation, might be an efficient therapeutic approach for the repair of the articular damage in RA patients [42,49-52]. The MSC-based technology of tissue engineering using biocompatible scaffolds appropriate to induce both bone and cartilage formation as well as cell-free approaches using bioactive materials with the capacity to recruit and/or to induce resident MSCs, represent an emerging area of research for RA patients requiring skeletal reconstruction [53-56].

MSCs in RA Synovial Tissues

Enumerated MSCs expressing bone morphogenic protein (BMP) receptors have been isolated in RA synovial membrane [57]. Although the recruitment/influx of these cells in the affected joints may represent a physiological response to the local tissue injury, it might also imply a contributory effect of MSCs in the disease process [58]. Specifically, it has been suggested that BM MSCs are recruited in the inflamed joint through bone-joint interconnecting canals and gradually repopulate the synovial membrane. These cells have been shown to express embryonic growth factors, normally regulating limb bud mesenchyme and BM stem cell development, such as wingless (wnt) and frizzled (fz) molecules. The wnt/fz signaling pathway has been implicated in the transcriptional control of stem cell renewal/differentiation process but also in inflammation induction through protein kinase C activation [59-62]. It has even been hypothesized that the abnormal RA FLS may be transformed BM MSCs "frozen" at early stages of differentiation by the inflammatory mediators. This hypothesis was exploited by a recent study in animal models of RA which showed that arthritic FLS contain an increased fraction of BM-derived MSCs and that the differentiation potential of these FLS towards the adipogenic and osteogenic lineages is abolished by the inflammatory cytokine IL-1β, present in the inflamed joint, presumably through activation of the nuclear factor-κB (NF-κB) [63].

In collagen–induced arthritis (CIA) animal models, MSCs are found in early stages of disease in the periosteum, cortical bone, epiphysial region, synovial membrane and in enlarged bone-joint interconnecting canals. Anti-TNFα treatment has been shown to reduce MSC numbers in BM and synovium indicating a direct effect of this cytokine in the recruitment of MSCs to the inflamed joint [58]. The gradual invasion and proliferation of these immature MSCs by a plethora of local inflammatory signals may further augment the cellular hyperplasia and destruction process through autocrine and/or paracrine production of cytokines, chemokines, cell cycle regulators, adhesion proteins and MMPs [62,64]. Collectively, BM derived immature MSCs in an attempt to restore the tissue damage and regenerate the articular structures through a cellular differentiation process, may also induce a cascade of events and subcellular pathways, associated with their immature phenotype, that sustain the chronic inflammatory process (Fig. **1**).

The Properties of BM MSCs in RA

A critical question is whether BM MSCs are depleted or functionally altered and therefore incapable to repair the joint damage in RA. This might be due to a primary or, most likely, to a secondary defect associated to the chronic inflammatory process or even to the long-standing immunosuppressive medication. This has been considered in the case of BM HSCs in RA. Specifically, it has been shown that the

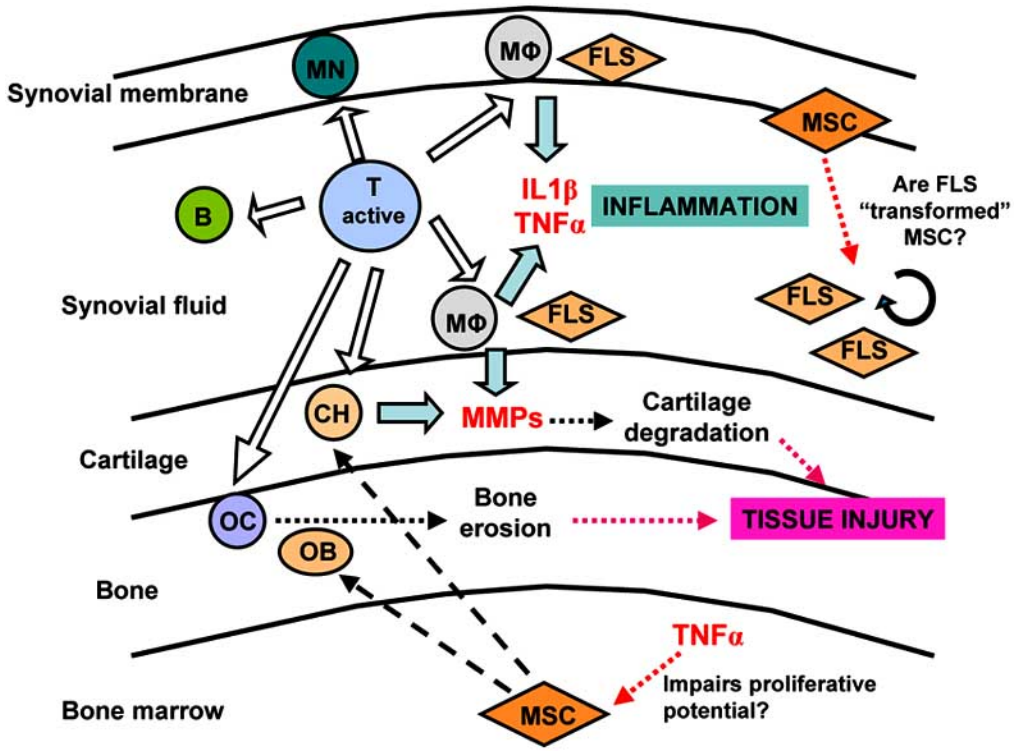

Fig. (1). A model for joint destruction in RA. In this simplistic scheme, an unknown antigen has triggered the autoimmune process mainly through T self-reactive cells, which further stimulate the immune responses by activating B-cells and recruiting monocytes (MN) and macrophages (MΦ). Activated T-cells sustain inflammation and indirectly cause tissue damage by inducing the production of the pro-inflammatory cytokines IL-1β, TNFα and matrix metalloproteinases (MMPs) by MN, MΦ, and fibroblast-like synoviocytes (FLS). Chondrocytes (CH) are also triggered to produce MMPs, contributing therefore to cartilage degradation. Triggered osteoclasts (OC) cause bone erosions. Synovial fluid MSCs become aggressive and may transform into FLS. Bone marrow MSCs may contribute to cartilage and bone repair but cell proliferative potential is affected by the inflammatory marrow microenvironment.

reserves, the clonogenic potential and overall survival of HSCs and their progeny are defective in RA patients and these abnormalities reverse following anti-TNFα therapy suggesting a cytokine-mediated effect on patients' hematopoiesis [65,66]. Interestingly, it has been recently shown that BM MSCs display reduced proliferative capacity and defective chondrogenic and adipogenic activity in another degenerative arthritis, namely the osteoarthritis [67]. These abnormalities might explain, at least in part, the increased bone density and loss of cartilage characterizing the articular abnormalities in osteoarthritis.

We have recently studied the reserves, function and differentiation potential of BM MSCs in RA patients and we have also characterized their proteomic and molecular profile in comparison to healthy subjects [38]. The number, immunophenotypic and survival characteristics as well as the osteogenic, chondrogenic and adipogenic potential of patient MSCs were within normal limits. *In vitro* differentiation of patient MSCs are shown in Fig. **2**. MSCs' proteomic profile and production of inflammatory cytokines, chemokines and growth factors such as IL-1β, IL-6, IL-8, IL-15, TNFα, SDF-1 and transforming growth factor β1 (TGF-β1) was also normal suggesting that BM MSCs do not display primarily the abnormal phenotype of the RA FLS. Interestingly, however, patient MSCs had defective clonogenic and proliferative potential and decreased expansion growth rate through passages compared to controls probably due to a premature, age-inappropriate telomere loss. We have postulated that under the influence of the inflammatory BM milieu, MSCs of RA patients undergo accelerated proliferation that finally result in premature replicative exhaustion and cell senescence. Alternatively, the previously reported stochastic, genetically-determined variation of telomere shortening in RA patients may also have a role in the inappropriate MSC senescence [68]. In agreement with the observed attenuation of cell growth, MSC gene expression profile revealed a possible repressive influence of the inflammatory microenvironment on G1/S transition. The commonly used anti-rheumatic therapeutic agents such as methotrexate, corticosteroid, anti-cytokine and disease modifying anti-inflammatory agents did not seem to affect the survival and functional characteristics of BM MSCs. Overall, despite some restrictions related to the reduced clonogenic/proliferative potential, the data of this study encourage the use of autologous MSCs for cartilage and bone damage associated with RA.

THE IMPACT OF INFLAMMATION ON THE PROPERTIES OF MSCs

An important question is the effect of the inflammatory articular environment on the biologic properties of the locally infused or implanted MSCs. Normally, human BM MSCs display immunosuppressive and immunoregulatory functions while escape immune recognition as they express MHC Class I but not Class II or co-stimulatory molecules

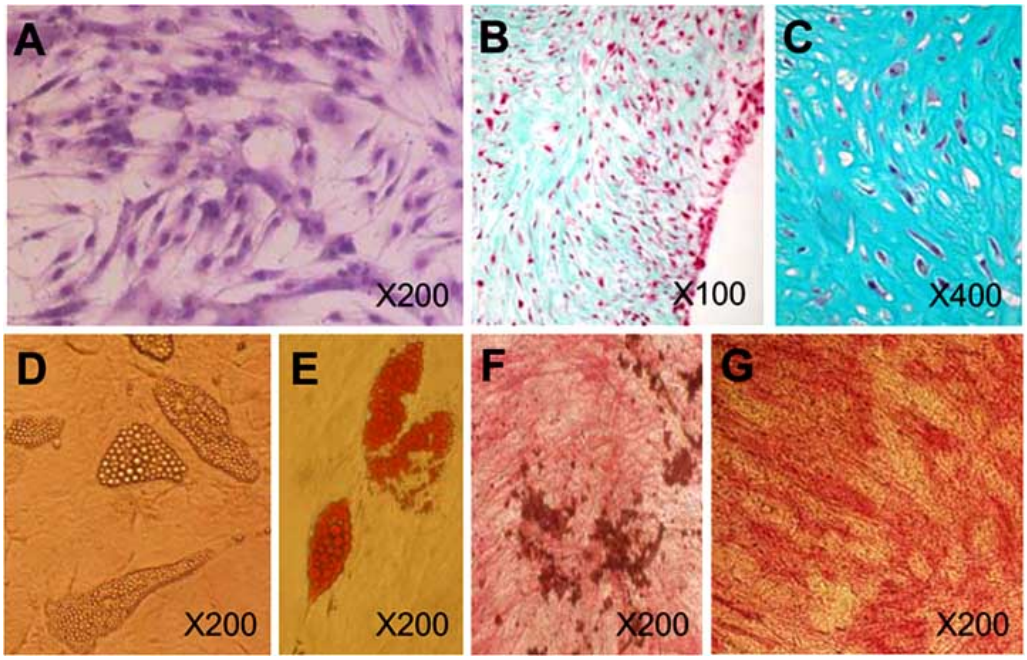

Fig. (2). Undifferentiated and differentiated MSCs from a RA patient. Culture expanded BM MSCs exhibiting the characteristic spindle-shaped morphology (A), and differentiated cells towards the chondrogenic (B,C), adipogenic (D,E) and osteogenic (F,G) lineages. Chondrogenic differentiation was identified with Masson (B) and Alcian blue (C), adipogenic differentiation with Oil red O (E) and osteogenic differentiation with Alkaline Phosphatase/Von Kossa (F,G) staining. Undifferentiated MSCs have been stained with Giemsa.

[69-72]. They inhibit T-cell and B-cell proliferation, promote the T-regulatory cell function, affect the production of TNFα from Dendritic Cells type 1 (DC-1), increase IL-10 production from DC-2, decrease IFNγ release from T-helper-1 (TH1) and Natural Killer (NK) cells, and increase IL-4 release from TH2 cells [73,74]. They also inhibit the effective maturation of antigen presenting cells by downregulating the CD40 and CD86 co-stimulatory molecule expression [73,75-77]. MSC immunoregulation is mainly mediated through soluble factors rather than cell-to-cell contact interactions. The hepatocyte growth factor (HGF), IL-10, TGF-β1, indoleamine 2,3-dioxygenase (IDO), prostaglandins, and nitric oxide have been recognized as immunoregulatory MSC-derived molecules [75,78-82].

Interestingly, a number of studies have shown that inflammatory mediators may alter the immunoregulatory properties of MSCs. Specifically, IFNγ has been shown to upregulate MHC class II expression on MSCs, however the cytokine also induces the production of HGF, IL-10, TGF-β1, and IDO by MSCs promoting therefore their immunosuppressive capacity [83-85]. No conclusive evidence, however is available on the TNFα-mediated effect on the MSC-induced immunosuppression as contradictory results have been reported so far in animal models of RA [86,87]. Data from RA patients, however, indicate that MSCs derived from the inflammatory BM environment [65] display immunosuppressive properties similar to their normal counterparts in terms of the capacity to inhibit T-cell proliferation [51,82,88]. Nevertheless, the local immunoregulatory effects upon local infusion or implantation of autologous or allogeneic MSCs in the inflamed RA joint remains to be elucidated.

On the basis of experimental data it is anticipated that the damaged joint environment will provide chondrogenic and osteogenic differentiation signals on MSCs [89]. A concern, however, is whether the inflammatory microenvironment might affect the differentiation potential of MSCs. It has been reported that both TNFα and IL-1β inhibit the multilineage differentiation of MSC lines [90,91]. Although a major suppressive effect by these cytokines has been shown for the adipogenic induction [92], indirect evidence suggests that these cytokines may also suppress the chondrogenic and osteogenic formation, since TNF receptor-1 (TNFR1) deficient mice form more cartilage and bone than normal [93]. Recent evidence also suggests that TNFα may inhibit chondrogenic differentiation of synovial fibroblasts through p38 mitogen activating protein kinase pathway [94]. Furthermore, in CIA animal models it was shown that TNFα induces the expression of Dickkopf-1 (DKK-1), an inhibitor of the wnt signaling, in the synovial membrane and may therefore inhibit the osteogenic differentiation normally mediated through this pathway [95,96]. Interestingly, the same pathway has been implicated in chondrogenic differentiation during embryonic development [97]. Finally, another possibility is that molecules from the joint microenvironment and extracellular matrix components may direct the MSC differentiation towards specific pathways [98].

The effect of the inflammatory microenvironment on the survival characteristics of MSCs is also an issue. It has been shown that MSC-differentiated osteoblasts express TNFR family members including Fas and TNFR1, however they are resistant to apoptosis under conditions favoring cell growth [99].We have also shown that BM MSCs constitutively and stably express high levels of Fas and TNFR1 through passages. Interestingly, ligation of Fas may result in

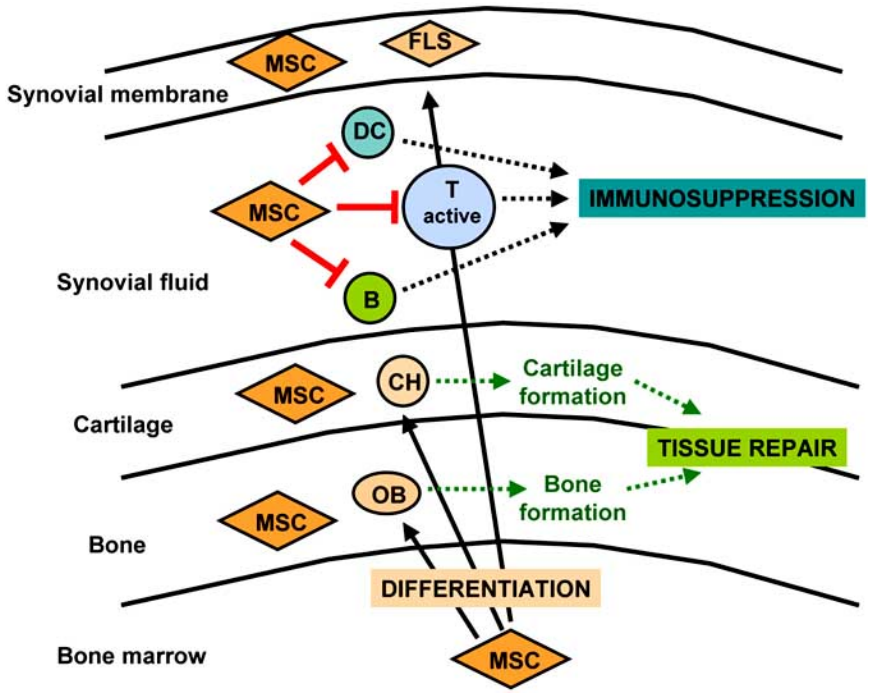

Fig. (3). MSCs in RA treatment. MSCs might contribute to RA treatment by suppressing the local (and even systemic) immune responses and by promoting the cartilage and bone formation through their tissue repair and differentiation effects.

MSC apoptosis in serum deprivation or low serum conditions whereas the presence of TNFα does not seem to affect the survival characteristics of MSCs even at high concentrations [100].

PERSPECTIVES ON MSC-BASED THERAPIES IN RA

MSCs can be used under the prism of a variety of treatment strategies in RA. Local delivery of autologous or allogeneic MSCs or induction of resident MSCs by appropriate biomaterials may promote cartilage and bone regeneration and may also alleviate arthritis through production of immunosuppressive factors (Fig. **3**). An alternative approach might be the local administration of MSCs genetically engineered to produce appropriate tissue remodeling factors [101]. For example, MSCs expressing factors influencing the skeletal repair, such as BMP-2 [102,103], BMP-4 [104], or TGF-β1 [105], have enhanced cartilage and bone formation properties. Local cell administration of MSCs, rather than systemic, seems to be the appropriate way for orthopaedicians to handle specific bone/cartilage defects. Alternatively, systemic administration of MSCs could be used, on the basis of their immunoregulatory properties, to modify and reorganize the disturbed immune response in RA. Following the encouraging results from the therapeutic application of MSCs in acute graft versus host disease (GvHD) [106,107], a number of experimental studies have investigated the potential of MSCs to treat AID including RA. Interestingly, in an animal lupus model, complete regression of the disease was demonstrated upon co-transplantation of BM cells and MSCs from the same donor [108] whereas MSCs were also shown to ameliorate experimental autoimmune encephalomyelitis [109]. Regarding RA, two related studies have been reported so far. In the first study the administration of an immortalized MSC cell line did not result in any beneficial effect in a mouse model of CIA [87] whereas in the second study allogeneic BM MSCs prevented or even treated CIA, depending on the day of administration [110]. In this study, BM MSCs displayed the potential to suppress the autoreactive T-cells *in vitro* and also the capacity to reduce the levels of INFγ, IL-4, IL-10 and TNFα in animal sera and to increase the proportion of T-regulatory cells in animal spleens. Different study design seems to be the cause for the apparent controversial results of the aforementioned trials: the immortalized cell line used in the first study appears not to possess immunosuppressive characteristics. Preliminary clinical data also suggest that despite some functional abnormalities, the immunosuppressive properties of BM MSCs are intact in a number of AID encouraging therefore the concept of systemic administration of MSCs in these diseases [88,111].

In conclusion, the use of MSCs for cartilage and bone repair in RA is a very promising and exciting area of research. Recently published studies on MSCs in RA are summarized in Table **1**. Despite, however the continuous delineation of the biological properties and mechanisms of action of MSCs [112-121] there are still several unanswered questions, concerns, and open fields for research. These include, for example, the definition of the appropriate tissue-source of MSCs as cells of different origin appear to have diverse differentiation capacity. This is not unexpected, as MSCs residing in different tissues, have already been exposed to specific differentiation-cues propagated by local homeostasis. It has been reported that cartilage-isolated cells have a more restricted cell fate to chondrocytes [122] whereas synovial fluid MSCs display reduced osteogenic and enhanced chondrogenic potential compared to BM MSCs [123]. MSC therapeutic efficacy also depends on the enrichment procedure as *in vitro* expansion of MSCs may

Table 1. List of References Relating MSCs and RA

Reference No.	Brief Description
[57, 58, 63, 86, 87, 110]	MSCs in murine models of RA
[38, 67, 88]	BM MSCs in RA and other AID
[69-82, 106, 107]	MSC immunosuppressive/immunoregulatory functions
[65, 81, 83-85, 90-94, 96]	MSCs and inflammation
[42, 49, 52-56]	MSC-based tissue engineering including trials in RA patients

affect the multipotentiality and may also drive cells to senescence or even to spontaneous transformation [124-127]. Therefore, investigation of MSC enrichment procedures are currently a challenge [30,32,33,128-131]. Additional studies are also required for the detailed definition of bone and cartilage differentiation pathways and tissue regeneration events as well as for the development of appropriate scaffolds and biomaterials. Finally, the long-term efficacy and safety of the MSC-based therapies remains to be evaluated through appropriated clinical studies.

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