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The potential role of adult stem cells in the management of the rheumatic diseases

Tiziana Franceschetti and Cosimo De Bari

***Abstract*:** Adult stem cells are considered as appealing therapeutic candidates for inflammatory and degenerative musculoskeletal diseases. A large body of preclinical research has contributed to describing their immune-modulating properties and regenerative potential. Additionally, increasing evidence suggests that stem cell differentiation and function are disrupted in the pathogenesis of rheumatic diseases. Clinical studies have been limited, for the most part, to the application of adult stem cell-based treatments on small numbers of patients or as a 'salvage' therapy in life-threatening disease cases. Nevertheless, these preliminary studies indicate that adult stem cells are promising tools for the long-term treatment of rheumatic diseases. This review highlights recent knowledge acquired in the fields of hematopoietic and mesenchymal stem cell therapy for the management of systemic sclerosis (SSc), systemic lupus erythematosus (SLE), rheumatoid arthritis (RA) and osteoarthritis (OA) and the potential mechanisms mediating their function.

Keywords: stem cells, arthritis, rheumatic diseases, immune modulation, cartilage repair, cell therapy, regenerative medicine

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Introduction

Rheumatic diseases are a range of conditions affecting one or more areas of the musculoskeletal system, such as joints, bones, cartilage, muscles, ligaments and tendons, as well as internal organs. Rheumatic diseases are among the most prevalent conditions in industrialized countries, which affect both genders and can occur at all ages. They can have a profound impact on the quality of life, as they can cause pain and disability and reduce life expectancy, and they constitute a significant financial burden on the individual and the healthcare system. Great effort has been made to identify predicting factors and the molecular mechanisms responsible for the development of rheumatic diseases. This research has led to improved treatment options that help manage pain and other symptoms, and in some cases modify disease progression. However, a substantial number of patients fail to respond to therapeutics. Stem cells represent a promising tool for the long-term treatment of rheumatic diseases due to their immunomodulatory and regenerative features. Different types of stem cells have been utilized over the last two decades in preclinical and clinical trials for a variety of rheumatic diseases, also contributing to our understanding of the pathogenesis of these disorders. This article provides a brief overview of stem cells and their taxonomy, and critically reviews the recent literature on adult stem cell therapy as a treatment for systemic sclerosis (SSc), systemic lupus erythematosus (SLE), rheumatoid arthritis (RA) and osteoarthritis (OA).

Stem cells

Stem cells are broadly described as undifferentiated cells that have the potential to give rise to several different cell types in the organism during early life and postnatal growth. Furthermore, they are responsible for tissue and organ remodeling and repair throughout life. One of the main characteristics of stem cells is 'self-renewal'. This is defined as their ability to divide for long periods of time and generate daughter cells with identical proliferative and developmental potential.[1] Another critical stem cell feature is their capacity to differentiate into specialized cells under certain physiologic or experimental conditions. Because

Correspondence to:
Cosimo De Bari
Arthritis & Regenerative Medicine Laboratory, Aberdeen Centre for Arthritis and Musculoskeletal Health, Institute of Medical Sciences, University of Aberdeen, Aberdeen, UK
c.debari@abdn.ac.uk

Tiziana Franceschetti
Arthritis & Regenerative Medicine Laboratory, Aberdeen Centre for Arthritis and Musculoskeletal Health, Institute of Medical Sciences, University of Aberdeen, Aberdeen, UK

Table 1. Types of stem cells.

Stem cell type	Potency	Tissue source	Reference
Embryonic stem cells	Pluripotent	Blastocyst	Martello and Smith,[4] Evans and Kaufman,[116] Martin,[117] Thomson *et al.*[118]
Induced pluripotent stem cells	Pluripotent (reprogrammed)	Skin fibroblasts, keratinocytes, T cells, hepatocytes, other somatic cells	Takahashi and Yamanaka,[10] Takahashi *et al.*,[11] Brouwer, Zhou and Nadif Kasri[119]
Fetal stem cells	Multipotent	Fetal blood, bone marrow, liver, lung, kidney, pancreas	Leary and Ogawa,[120] O'Donoghue and Fisk[121]
Adult stem cells	Multipotent	Hematopoietic stem cells, mesenchymal stem cells: umbilical cord, adult tissues (peripheral blood, bone marrow, synovial membrane, periosteum, adipose tissue, dental pulp)	Till and McCulloch,[1] Bryder, Rossi and Weissman,[19] Pittenger *et al.*,[36] De Bari *et al.*,[37] De Bari , Dell'Accio and Luyten,[38] Zuk *et al.*,[39] Gronthos *et al.*,[40] Wang *et al.*,[41] Lv *et al.*[42]

of these features, stem cells represent powerful tools for exploring several aspects of cell biology, and hold considerable promise as therapeutic tools for drug discovery and tissue regeneration. Stem cells can be classified according to their potency, which indicates how committed they are to becoming any specific cell type and typically correlates to the developmental stage from which they are obtained (Table 1).[2] For example, the zygote and blastomere are totipotent, denoting potential to give rise to all embryonic and extra-embryonic tissues.[3] As an organism develops, the potential of a stem cell to produce any cell type in the body is gradually restricted. Embryonic stem cells (ESCs) derived from the inner cell mass of mid-blastocyst-stage embryos are pluripotent, and can develop into all of the three embryonic germ layers: ectoderm, endoderm, and mesoderm.[4] Because of their extensive differentiation capacity, ESCs have clinical potential in tissue repair and restoration of normal tissue function for a wide range of common pathological conditions (reviewed by Buzhor *et al.*).[5] Fetal stem cells can be isolated from fetal and extra-embryonic tissues. They appear to have greater differentiation plasticity and replicative potential, better homing and engraftment, and lower immunogenicity than adult stem cells.[6–9] Intriguingly, the notion of cell plasticity led to the use of transcription factors to reprogram somatic cells into induced pluripotent stem cells (iPSCs)[10,11] which, like ESCs, have the potential to specialize into any somatic cells and are able to maintain self-renewal (reviewed by Takahashi and Yamanaka).[12] Different types of stem cells are summarized in Table 1. Additional information on the state-of-the-art application of ESCs and iPSCs in the clinic are reviewed in references 13 and 14.

Adult stem cells

Numerous adult tissues have been determined to harbor stem cells. Animal studies have been particularly useful in observing that *in vivo* adult stem cells usually reside in specific areas called 'niches'.[15] These are specialized microenvironments in which adult stem cells remain quiescent (non-dividing) for long periods of time, until they are re-activated upon tissue injury or disease. Local and system cues are integrated in the niche to maintain stem cell self-renewal. Besides stem cells, the niche is typically formed by cells that provide physical support and regulatory signals *via* cell–cell interactions and secreted soluble factors, as well as extracellular matrix proteins for scaffolding. Stem cell niches are usually found in the vicinity of blood vessels, which convey nutrients and systemic signals from other organs and allow the recruitment of circulating stem cells to and from the niche.[16] Additionally, recent studies have highlighted the role of neural inputs in transmitting cues for stem cell homing and mobilization from the niche.[17] Altered niche function has been observed during aging and in certain pathological conditions, and can result in abnormal stem cell renewal, differentiation, and migration with systemic effects.[18]

Hematopoietic stem cells. Hematopoietic stem cells (HSCs) were the first type of tissue-specific stem cells to be isolated, and probably are the most characterized.[1] Postnatally, HSCs can be mainly identified in the bone marrow (BM) and,

in small numbers, in peripheral blood. HSCs can give rise to all blood cell types of the myeloid and lymphoid lineages through the process of hematopoiesis. Numerous studies contributed to the identification of specific cell markers that allowed HSC tracing, isolation, and functional characterization.[19,20] The knowledge gained of HSC biology and these methods have been routinely applied in the clinic – for example, in the treatment of a variety of hematopoietic malignancies.

The potential application of HSC transplantation in the treatment of rheumatic autoimmune diseases was first suggested by preclinical observations on both genetically determined (diabetes and lupus) and inducible (acute arthritis) animal models of autoimmune disease.[21–23] These studies first suggested that ablation of the aberrant autoreactive immune cells through a conditioning regimen can 'reset' the BM, thus preventing or reversing autoimmune conditions. Subsequently, BM transplant could allow for normal, healthy HSCs to repopulate the BM and peripheral blood. Initial clinical observations in patients affected by severe forms of autoimmune conditions, such as SSc, SLE, RA, multiple sclerosis, vasculitis, and juvenile idiopathic arthritis also showed promising results for both autologous and allogeneic HSC transplantation, such as the elimination of aberrant self-reacting immune cells such as plasma cells producing autoantibodies, and the induction of regulatory T cells.[24–26] Most of the early phase I/II studies, aimed to assess safety and efficacy of the transplantation, presented some limitations, such as being performed on a restricted number of therapy-refractory patients and variability in the type and severity of the autoimmune disease, source of HSCs, age of the donor and recipient, conditioning treatment, and follow-up period. However, these pilot studies were useful for refining the criteria of patient selection, immunoablative treatment, and preferred use of autologous HSCs in order to reduce complications. An international program was started from the European Group for Blood and Marrow Transplantation (EBMT) and the European League against Rheumatism (EULAR) to explore the role of immunosuppression followed by HSC transplantation in the treatment of severe autoimmune diseases, including SSc and SLE. This collaboration outlines the basic guidelines for disease categories, selection of patients, stem cell mobilization, *in vitro* manipulation, conditioning and treatment.

Several clinical trials focused on investigating the potential use of HSC transplantation in SSc patients. SSc is a rare autoimmune rheumatic disease presenting vasculopathy and extensive fibrosis, which result in thickening and tightening of the skin and inflammation and scarring of many internal organs. Its pathogenesis is complex and incompletely understood. Most SSc patients are refractory to conventional therapeutics and have poor prognosis. To date, three major controlled, prospective, randomized studies testing autologous stem cell transplantation as a treatment for SSc have been completed or are underway. These trials have similar patient-selection criteria, but different conditioning treatments, stem cell mobilization and selection techniques, and length of follow-up. Results from the single-center American Scleroderma Stem Cell versus Immune Suppression Trial (ASSIST) were published in 2011.[27] This phase II study enrolled 19 patients to assess safety and efficacy of autologous non-myeloablative hematopoietic stem cell transplantation (HSCT) compared with high-dose standard immunosuppression with cyclophosphamide for 6 months, with follow-up to 2 years. No transplant-related deaths were reported at follow-up, and complications were limited to transient infections and heart dysfunctions controlled with medications. The results were promising and revealed that patients that received peripheral blood HSC transplantation showed improved skin condition and pulmonary function, whereas patients who received standard treatment showed disease progression. However, the small number of patients and short follow-up duration are limitations of this study.

A new phase III ASSIST II trial, aimed to compare the conditioning regimen used in ASSIST I with a less cardiotoxic regimen, has been ongoing in North America since 2011 (ClinicalTrials.gov identifier: NCT01445821). The first phase III study was the multicenter Autologous Stem Cell Transplantation International Scleroderma (ASTIS) trial.[28] This study also compared autologous non-myeloablative HSCT with cyclophosphamide administration in early-stage diffuse SSc patients over 12 months. This trial included a total of 156 SSc patients in 29 European centers, randomly selected for transplantation with peripheral blood CD34+ HSCs, with follow-up to approximately 6 years. The ASTIS trial had an early treatment-related mortality rate of 10% and serious adverse effects, including renal failure, cardiac involvement and respiratory distress. Nevertheless, the study showed significantly improved long-term event-free survival and overall survival in patients who received HSCT.

Finally, the Scleroderma: Cyclophosphamide or Transplantation (SCOT) trial is a North American phase III study designed to compare high-dose immunosuppressive therapy with cyclophosphamide and HSCT to cyclophosphamide treatment for 12 months, with follow-up to 54 months. The SCOT trial, which enrolled 75 patients, has recently concluded and the results have not been published at the time of writing (ClinicalTrials.gov identifier: NCT00114530). Further analyses are needed to determine whether HSCT should be offered as first treatment to a selected group of patients rather than an alternative option for severe cases not responding to conventional therapy. Therefore, additional studies are essential to optimize patient selection to reduce transplant-related mortality and adverse effects, and identify patients with poor prognosis already at the early stages of the disease who would benefit the most from the transplantation. In addition, long-term follow-up of transplanted patients is required to identify known severe complications, such as the development of secondary autoimmune diseases and malignancies.

HSCT has also been investigated in the treatment of severe forms of SLE, a potentially life-threatening disease with variable inflammatory manifestations, ranging from relatively minor skin and joint symptoms to major visceral organ involvement, including the neural, cardiovascular and renal systems. Despite recent advances, SLE treatments, typically including glucocorticoids and immunosuppressants, are insufficient to control the disease in a subset of patients with poor prognosis. Therefore, there is a need for newer therapeutic approaches to improve long-term outcomes and reduce relapse. Safety and efficacy of autologous HSCT in treatment-refractory SLE patients have been determined in two retrospective studies based on the EBMT/EULAR registry[29,30] and in a single-center study by Northwestern University.[31] These trials have enrolled over 100 patients in total and reported improved disease activity, increased responsiveness to previously failed conventional therapy, and 50% probability of 5-year remission. Further, these and other results suggest that non-myeloablative conditioning regimens have lower risk of infection and treatment-related morbidity and mortality.[31,32] However, while smaller phase II studies are still being pursued, controlled randomized trials powered to assess the efficacy of HSCT in SLE patients are needed.

Autologous HSCT has also been used in several studies for severe RA patients not responding to conventional treatments. Retrospective analyses on the EBMT registry showed that sustained remission at 6 months post-transplant was achieved in 67% of patients.[33] However, relapse rates were considerable due to the incomplete ablation of the T cell repertoire, and disease-modifying anti-rheumatic drugs (DMARDs) had to be reintroduced in most of the patients within 6–12 months post-transplant.[33,34] Nevertheless, as a consequence of the immune modulation by HSCs, following transplantation there was a better response to biologic and non-biologic therapeutics. Furthermore, overall survival at 5 years post-transplantation was 94%, which gives an indication of the safety of this approach in severe RA.[35] However, progression-free survival at the end of 5 years was only 18%.[35] Thus, also due to the availability of new effective biological agents, the use of autologous HSCT for RA has become less attractive and the number of transplants performed has significantly declined.

Mesenchymal stem cells. Mesenchymal stem cells (MSCs) were originally derived from the BM stroma as plastic-adherent colonies of spindle-shaped fibroblast-like cells with single-cell inherent mesenchymal multipotency.[36] Since then, BM-MSCs have been the most investigated, but there have been many reports indicating that MSCs are present in a wide variety of other adult tissues, including the synovial membrane,[37] periosteum,[38] adipose tissue,[39] dental pulp,[40] and umbilical cord (UC).[41,42] Currently, there is no consensus on a protocol or specific cell surface markers for the prospective identification and isolation of MSCs. In 2006 the International Society for Cellular Therapy established essential criteria for MSCs: they should adhere to plastic under standard culture conditions, have tri-lineage potential (differentiate into osteoblasts, chondrocytes, and adipocytes), and express CD105, CD73, and CD90 while lacking expression of endothelial and hematopoietic markers and HLA-DR molecules.[43] Because of their multipotency, ease of isolation and expansion potential, MSCs have been investigated as promising candidates for tissue regeneration.

Furthermore, the potential of MSCs for translational medicine takes into account their immunomodulatory properties. MSCs secrete a multitude of cytokines and growth factors with immunosuppressive properties, which inhibit B

and T cell proliferation and monocyte maturation and promote the generation of regulatory T cells and M2 macrophages.[44–46] In addition, MSCs have been considered to have low immunogenicity because of their limited expression of major histocompatibility complex (MHC) I, lack of expression of MHC II and costimulatory molecules, and inability to stimulate T cell activation.[47–49] This has prompted the exploration of MSC transplantation in clinical trials for a range of diseases, including graft-*versus*-host disease, autoimmune disorders and cardiovascular disease. However, more recent *in vivo* observations indicate that MSCs may not be immunologically 'privileged'. Mismatched allogeneic MSCs do not persist following infusion in patients, and were shown to elicit a humoral and cellular immune response in host mice and to stimulate the formation of memory T cells.[50,51] Furthermore, stimulation with inflammatory cytokines induces MSCs to express elevated levels of MHC I, MHC II and VCAM-1 and to significantly increase cytotoxic lysis.[48,51] In certain circumstances, MSCs do not exert their immunosuppressive properties, but rather can act as antigen-presenting cells and promote inflammation.[52] The route of delivery may also influence MSC immunogenicity. Allogeneic MSC transplantation *via* the intra-cranial, intra-cerebral and intra-articular routes, and implantation into skin wounds appear to be non-immunogenic or very weakly immunogenic, in contrast to intravenous, intra-peritoneal, subcutaneous and intramyocardial administration.[53] Conversely, no adverse events suggestive of an immunogenic response were recorded upon transplantation of allogenic MSCs in patients affected by cardiovascular disease.[54–57] These conflicting results indicate that the complexity and variability of MSC immunogenicity *in vivo* are yet to be fully defined.

Allogeneic BM-MSCs and UC-MSCs have been transplanted in patients with severe SLE, with promising outcomes regarding the safety and efficacy of this approach. Approximately 50% of the 87 patients, who were unresponsive to conventional medications, were in clinical remission at the 4-year follow-up. Additionally, the overall survival rate was 94%, and the overall rate of relapse was 23%.[58] Despite this encouraging clinical evidence, the biological mechanisms by which MSCs exert their therapeutic effect in SLE are undefined. One of the postulated mechanisms is the secretion of soluble immunomodulatory factors that, for example, regulate the balance of T helper (Th)1 and Th2 cytokines[59,60] and promote the expansion of regulatory T cells and the eradication of autoreactive lymphocytes.[61,62] Some authors have also suggested that MSCs could directly differentiate into endothelial cells in nephrons to elicit tissue repair and improve renal function,[63] although no long-term renal engraftment of MSCs was identified in a lupus mouse model.[64]

Limited clinical data are available for MSC therapy in patients with severe refractory SSc, described in two case reports and one case series.[65–67] No major adverse events related to MSC administration were reported and all three reports described skin improvement after intravenous administration of allogeneic BM-MSCs. Therefore, MSC administration in SSc patients appears to be safe, but the small number of patients does not allow drawing any conclusions regarding efficacy.

MSCs in RA. RA is the most common autoimmune joint disease, characterized by chronic inflammatory synovitis and progressive joint destruction that causes severe morbidity.[68] The introduction of new classes of therapeutics, DMARDs and biologics targeting inflammatory cytokines has significantly improved patient outcomes. These treatments are most successful in newly diagnosed patients and clinical remission can be achieved. However, they are not able to address damage that has already occurred at the joints or other tissues. Furthermore, over one-third of patients fail to respond to these treatments.[69]

Because of their regenerative and immunomodulatory properties, MSC therapy has been the focus of several investigations as potential therapeutic tools to correct the aberrant immune tolerance both in animal models of inflammatory arthritis and in RA patients. At the same time, our understanding of the role of endogenous MSCs in the pathogenesis of RA is still limited. Resident MSCs in the joint tissues, and in particular in the synovium, have been identified.[70] In homeostatic conditions, these MSCs would contribute to the maintenance and repair of the joint tissues, and current research is aimed to address their identity and function. In RA, the interaction of infiltrating immune cells and resident fibroblast-like synoviocytes (FLSs) results in the thickening and inflammation of the synovium (synovitis) and has deleterious effects on the cartilage and bone tissues, which are consequently degraded.[71] The relationship between resident MSCs and FLSs is still under debate. Additional studies are required to define whether

MSCs and FLSs represent the same population of cells at distinct stages of differentiation or that have acquired different functions. Furthermore, the role of synovial MSCs (SMSCs) in this microenvironment, as well as MSCs recruited from the BM, is not fully defined. The inflammatory setting may inhibit the regenerative ability of MSCs by repressing their differentiation. In addition, MSCs could be directly involved in the secretion of pro-inflammatory cytokines or acquire an aberrant invasive phenotype, therefore contributing to the pathogenesis of RA.[72]

The efficacy of MSC administration in preclinical models of inflammatory arthritis has been demonstrated (reviewed by Ansboro *et al.*) (Table 2).[73] The beneficial effects of MSC transplantation in autoimmune disorders have been accredited to their anti-inflammatory and immunomodulatory properties. Thus, recent reports have attempted to characterize the underlying cellular and molecular basis of this function. For example, MSC transplantation resulted in the reduction of pathogenic T cell subsets, such as GM-CSF+CD4+, T follicular helper (Tfh) and Th1/Th17 cells, and consequently in decreased secretion of inflammatory cytokines.[74–76] This anti-inflammatory function was demonstrated to act, at least in part, through the inhibition of the Nuclear factor κB (NFκB) signaling pathway.[77] In addition to rescuing T cell homeostasis, MSCs were shown to induce the apoptosis of activated T cells *via* the Fas ligand (FasL)/Fas pathway.[76] An increased proportion of regulatory T cells was detected after MSC administration, which is indicative of enhanced immune tolerance.[61,74,76] Remarkably, MSC transplantation also conferred protection from bone loss *via* direct inhibition of receptor activator of NF-kB ligand (RANKL)-induced osteoclastogenesis.[78]

Two large clinical trials addressing the safety of MSCs in severe RA patients unresponsive to standard therapies have been reported to date (Table 3). A total of 136 RA patients with active disease were enrolled in a single-center phase I/II study and treated with intravenous injection of UC-MSCs and DMARDs. The procedure was shown to be safe, as no serious adverse effects were observed, and significant remission was achieved in comparison to the non-randomized control group of 36 patients receiving only DMARDs and cell medium. The clinical improvement correlated with decreased expression of inflammatory cytokines and increased presence of regulatory T cells in peripheral blood, and was maintained up to 3–6 months without continuous administration.[79] More recently, a multicenter, dose-escalation, randomized, single-blind (double-blind for efficacy), placebo-controlled, phase Ib/IIa trial was reported. The study has demonstrated the safety and tolerability of adipose tissue (Ad) MSCs injected intravenously in 48 patients with active RA up to 6 months after administration. In addition, a tendency for clinical efficacy was observed, although it did not persist after three months, suggesting that repeated cell administration may be required. Conversely, anti-HLA-I antibodies against Ad-MSCs were detected in a few patients without apparent clinical consequences, which may be indicative of sensitization upon multiple cell injections.[80] However, the small size of the placebo control group does not allow any conclusions concerning efficacy.

MSCs in OA. OA is a common degenerative joint disease and the leading cause of disability in the elderly. It is characterized by progressive cartilage degradation, subchondral bone sclerosis and aberrant formation of bone outgrowths (osteophytes) that result in joint pain. Treatment options for OA are limited because of the restricted regenerative capacity of the articular cartilage and the lack of specific diagnostic and prognostic biomarkers and therapeutic targets. Some evidence suggests that OA is associated with a depleted local population of MSCs with reduced proliferative and differentiation capacity.[81–83] Conversely, other studies have reported the presence of an increased number of MSCs in the synovial fluid[84] or sub-chondral bone of OA patients and in a mouse anterior cruciate ligament transection model of OA.[85,86] Additionally, OA-derived MSCs showed altered gene expression profiles, suggesting altered MSC function and possibly impaired regenerative potential.[85–87] These discrepancies could reflect variations in the MSC status in different disease stages or experimental conditions, and overall indicate that a deeper understanding of the role of MSCs in OA pathogenesis is needed. Routine treatments for OA are aimed to reduce pain and preserve joint function with no effect on progression of structural damage. This has fostered the investigation of therapeutic strategies, such as the ones involving the use of chondrogenic growth factors (BMP7, FGF18) or compounds (Kartogenin),

Table 2. Recent preclinical MSC-based studies for RA and OA treatment.

Rheumatic disease	Experimental model	Source of MSCs	Dose of MSCs	Route and frequency of administration	Control	End-point after MSC injection	Reference
Inflammatory arthritis	CIA (8 w.o. DBA/1 mice)	hAd-MSCs	1×10^6	i.v.	Ringer's Lactate solution	7 days	Lopez-Santalla *et al.*[74]
	CIA (6–8 w.o. DBA/1 mice)	hUC-MSCs	1×10^6	i.v.	Not injected	62 days	Liu *et al.*[75]
	CIA (10 w.o. DBA/1 mice)	hESC-derived MSCs	1×10^6	i.p. (1×, 3×)	PBS	10 days after arthritis onset	Gonzalo-Gil *et al.*[122]
	CIA (8–10 w.o. DBA/1 mice)	Syngeneic mAd-MSCs	2×10^6	i.p.	PBS	14 days	Garimella *et al.*[78]
	CIA (8–12 w.o. DBA/1 mice)	Syngeneic mBM-MSCs and mouse Tr1 cells	5×10^5	i.p. (MSCs), i.v. (Tr1 cells); (2×)	PBS; 5×10^5 mouse Tr1 cells (i.v.)	6.5 weeks	Lim *et al.*[123]
	CIA (8 w.o. DBA/1 mice)	Syngeneic mBM-MSCs with AAV-miR-548e	1×10^6	i.p.	PBS; AAV-antisense-miR-548e	4 weeks	Yan *et al.*[77]
	CIA (8 w.o. DBA/1 mice)	CD146+ or CD146$^-$ hUC-MSCs	1×10^6	i.a.	PBS	14 days	Wu *et al.*[124]
	CIA (6–8 w.o. DBA/1 mice)	C57BL/6 mouse gingival MSCs (wt, FasL$^{-/-}$, FasL overexpression)	1×10^6	i.v.	PBS	35 days	Gu and Shi[76]
	CII-immunized IL-1Ra-KO BALB/c mice	hAd-MSCs (wt, sRAGE-overexpressing)	1×10^6	i.v. (3×)	PBS	6 weeks	Park *et al.*[125]
OA – osteochondral defects	MMR (New Zealand rabbits)	Equine UC-MSCs	3.5×10^6	i.a.	PBS	Up to 53 days	Saulnier *et al.*[100]
	ACTL (10–12 w.o. Lewis rats)	hSMSCs	1×10^6	i.a. (single or weekly)	PBS	Up to 12 weeks	Ozeki *et al.*[102]
	MMx (Sprague-Dawley rats)	hAd-MSCs	2.5×10^6	i.a.	PBS	Up to 10 weeks	Li *et al.*[126]
	Bilateral medial anterior hemimeniscectomy (12 m.o. New Zealand rabbits)	hAd-MSCs	2×10^6; 6×10^6	i.a.	Ringer's lactate solution	4 weeks	Riester *et al.*[127]
	Full-thickness cartilage defect (4–6 w.o. C57BL/6 mice)	Sca-1+ mSMSCs from C57BL6 or MRL/MpJ mice	1×10^5	i.a.	PBS	4 weeks	Mak *et al.*[112]

AAV, adeno-associated virus; ACTL, anterior cruciate ligament transection; CIA, collagen-induced arthritis; CII, type II collagen; hAd-MSCs, human adipose tissue mesenchymal stem cells; hESC-MSCs, human embryonic stem cell-derived mesenchymal stem cells; i.a., intra-articular; i.p., intra-peritoneal; i.v., intravenous; mAd-MSCs, mouse adipose tissue mesenchymal stem cells; m.o., months old; MMR, medial meniscal release; MMx, medial meniscectomy; PBS, phosphate buffered saline; sRAGE, soluble receptor for advanced glycation end products; Tr1 cells, IL-10-producing type 1 regulatory T cells; UC-MSCs, umbilical cord mesenchymal stem cells; w.o., weeks old.

Table 3. Recent clinical MSC-based studies for RA and OA treatment.

Rheumatic disease	Type of study	Intervention	Comparator	Reference
RA	Clinical phase I/II; follow-up at 3, 6 and 8 months	136 patients, i.v. injection of 4×10^7 UC-MSCs and DMARDs	36 patients, intravenous injection of DMARDs and cell medium	Wang *et al*.[58]
	Clinical phase Ib/IIa; follow-up at 6 months	3 i.v. injection of allogeneic Ad-MSCs: 16 patients, 1×10^6 cells/kg; 19 patients, 2×10^6 cells/kg; four patients, 4×10^6 cells/kg	Four patients, placebo (Ringer's lactate solution)	Álvaro-Gracia *et al*.[80]
OA	Clinical phase I/II; follow-up at 2 years	12 patients, i.a. injection of 40×10^6 autologous BM-MSCs	None	Orozco *et al*.[105]
	Clinical phase I/II; follow-up at 12 months	50 patients, i.a. injection of 40×10^6 autologous BM-MSCs	None	Rich *et al*.[106]
	Clinical phase I/II; follow-up at 6 months	i.a. injection of autologous Ad-MSCs: 3 patients, 1×10^7 cells; 3 patients, 5×10^7 cells; 12 patients, 1×10^8 cells	None	Jo *et al*.[107]
	Clinical phase I/II; follow-up at 12 months	15 patients, i.a. injection of 40×10^6 cells	15 patients, intra-articular injection of hyaluronic acid	Vega *et al*.[108]

Ad-MSCs, adipose tissue mesenchymal stem cells; BM-MSCs, bone marrow mesenchymal stem cells; DMARDs, disease-modifying anti-rheumatic drugs; i.a., intra-articular; i.v., intravenous; UC-MSCs, umbilical cord mesenchymal stem cells.

inhibitors of matrix degradation (MMP13 inhibitor, Syndecan-4 antibody), or inhibitors of inflammation (IL-1β receptor antagonist, IL-1β receptor antibody).[88–94]

Several investigations have focused on cell-based therapy for joint surface defects and OA using mature cells (chondrocytes) and stem cells, because it could offer a long-term solution to repair and regenerate cartilage, improve symptoms and delay disease progression. Implantation of cells, in suspension or in combination with three-dimensional scaffolds (such as type I collagen gels or fibrin glue), in the osteochondral defect has been achieved through surgical procedures.[95,96] Autologous chondrocyte implantation or transplantation (ACI or ACT), based on the isolation and *in vitro* expansion of autologous healthy chondrocytes that are subsequently re-implanted in the patient, is largely used in the clinic in full-thickness joint surface defect repair.[97] However, chondrocytes undergo de-differentiation during culture, affecting their ability to generate hyaline cartilage. Furthermore, unlike in focal cartilage defects, OA often results in a generalized alteration of joint homeostasis that could minimize the efficacy of chondrocyte-mediated regeneration. Thus, several investigators have focused on exploring different approaches for cartilage repair in OA.

Numerous preclinical studies have extensively investigated the potential application of MSCs for cartilage repair (Table 2).[98,99] MSC multipotency does not appear to be as relevant as paracrine signaling in promoting tissue regeneration, although it is yet to be clearly defined which trophic molecules would mediate this effect. MSC-secreted factors are thought to target both the synovium and articular chondrocytes to regulate their gene expression and promote, for example, the down-regulation of matrix-degrading metalloproteinases.[100,101] Furthermore, intra-articular injected MSCs were shown to localize to the synovium and express molecules with anti-inflammatory and chondrogenic properties.[102] Therefore, MSCs could contribute to establishing a regenerative microenvironment at the delivery site, which would enhance the recruitment, activation and differentiation of endogenous stem cells with the potential to repair the articular cartilage.[103,104]

There has been an increasing trend to localized stem cell delivery *via* minimally invasive intra-articular injections. Several case reports have

described the outcomes of direct intra-articular injection of MSCs into the knee of OA patients unresponsive to conventional treatment (Table 3). Safety and feasibility of intra-articular injection of autologous BM-MSCs were confirmed in a pilot study in 12 OA patients.[105] No serious adverse effects were observed up to 2 years post-intervention, and indications of clinical efficacy were identified, including pain relief and improved cartilage quality.[105] The same procedure was applied to 50 patients, and results at 12 months were reported last year. Again, no serious adverse events occurred, and the authors described indications for improved pain relief, functionality and cartilage quality.[106] Jo and colleagues recently reported the intra-articular injection of autologous Ad-MSCs in 18 osteoarthritic patients. No treatment-related adverse effects were reported at 6 months follow-up, validating the safety of the procedure. In contrast, decreased knee pain and functional improvement were described. In particular, the reduction of cartilage defect size and the formation of hyaline-like cartilage were observed by MRI imaging and histology.[107]

Recently, the first randomized, controlled multicenter study aimed to investigate the safety of intra-articular injection of allogeneic BM-MSCs was published.[108] Injection of hyaluronic acid, one of the current treatment options for OA, was used as a control. In the 30 patients enrolled in the trial, no serious adverse events were detected upon intervention, reinforcing the safety data of allogeneic MSCs previously reported in numerous clinical studies.[58,109] Furthermore, the study provided an indication of efficacy, as pain reduction and increased cartilage repair were observed up to 12 months after MSC injection.

The effect of MSCs in autoimmune diseases is thought to take advantage of their immunosuppressive and anti-inflammatory properties. In contrast, the specific role of MSCs in the treatment of OA and joint repair is not fully defined. One of the potential mechanisms that have been suggested is MSC engraftment on the damaged articular cartilage and direct differentiation of MSCs into chondrocytes.[110–112] However, evidence suggests the possibility that the administration of exogenous MSCs could stimulate the proliferation and activity of the articular chondrocytes or promote the recruitment of stem/progenitor cells to the injury site, as well as their chondrogenic differentiation.[103,113–115] This function could be the result of direct cell–cell interactions or depend on the secretion of paracrine factors, such as cytokines and growth factors, to induce/mediate tissue regeneration.[83]

Conclusion

In this review we presented the most recent advancements of cell-based therapy for the treatment of rheumatic diseases. Long-term data have demonstrated that HSC transplantation in SSc and SLE in the treatment of refractory disease is safe and associated with improved disease-free survival. Nevertheless, none of the trials has achieved complete remission, and additional investigations are required to better understand HSC biology and the pathogenesis of these conditions. Furthermore, adequately powered randomized controlled trials are necessary to confirm the efficacy of HSC transplantation in comparison to the currently accepted standard therapy.

Clinical data available on the use of MSCs in therapeutic settings are limited. Despite encouraging preliminary results for safety and efficacy, the potential application of MSCs in the clinic for the treatment of rheumatic diseases needs to be evaluated through larger randomized, double-blind, controlled trials and longer follow-up periods. In addition to the small number of patients, the case reports and clinical studies described in the literature are variable in the source of MSCs and in the procedure used. Autologous MSCs have been prevalently used so far because of their safety, although allogeneic MSCs may represent a more readily available source of stem cells also for older and diseased patients. Current data are insufficient to conclude which source would be preferable. Similarly, most studies utilized MSCs isolated from the Ad, UC or, in particular, the BM, but it is yet to be established which MSCs are more potent for each clinical indication. This is in part because the exact role of the injected MSCs in immune modulation and tissue repair is incompletely understood. Moreover, investigators have used different cell doses and a variety of vehicles and scaffolds, as well as different frequency and procedures for injection. The approach used in the studies summarized here mainly involves *ex vivo* expanded cells, which allows for better standardization of MSCs, but is more labor intensive and potentially introduces contamination with animal products during culturing.

Alternatively, other studies have used 'one-step' procedures during which the patient receives complete treatment in a single sitting. However, MSCs are delivered mixed with other cell types from the tissue of origin, thereby posing challenges related to the inconsistency of the cellular therapeutic.

The knowledge gained from current preclinical and clinical studies will allow better insight into the molecular mechanisms by which exogenous stem cells can exert their therapeutic functions. This expertise will provide the opportunity to develop alternative approaches targeting the endogenous pathways that support the homing and activation of native stem cells and intrinsic tissue repair. Establishing cell-free regenerative strategies would also simplify the regulatory pathway toward clinical application.

In conclusion, stem cell-based therapies offer an exciting prospect for the treatment of rheumatic diseases. Initial studies demonstrate a satisfactory safety profile and potential for clinical efficacy. However, larger multicenter clinical studies are needed for sound evidence and to position (stem) cell-based therapies in the treatment algorithm of the rheumatic diseases.

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Conflict of interest statement

The authors declare that there is no conflict of interest.

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