



Immunomodulatory Function of Mesenchymal Stem Cells for Rheumatoid Arthritis

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Developments in our comprehension of the autoimmune and inflammation mechanisms in rheumatoid arthritis (RA) have produced targeted therapies that block aberrant immune cells and cytokine networks, and improved treatment of RA patients considerably. Nevertheless, limitations of these treatments include incomplete treatment response, adverse effects requiring drug withdrawal, and refractory cases. Hence, many researchers have redirected efforts towards investigation of other biological aspects of RA, including the mechanisms driving joint tissue repair and balanced immune regulation. This investigation focuses on mesenchymal stem cell (MSC) research, with the ultimate goal of developing interventions for immune modulation and repair of damaged joints. MSCs are multipotent cells capable of differentiating into mesodermal lineage cells. These cells have also attracted interest for their anti-inflammatory and immunomodulatory capacities. They have many distinctive immunological properties, inhibiting the proliferation and production of cytokines by T, B, natural killer, and dendritic cells. Indeed, MSCs have the capacity to regulate immunity-induced peripheral tolerance, suggesting they can be used as therapeutic tools in RA. This review discusses properties of MSCs, in vitro studies, animal studies, and clinical trials involving MSCs. Our review discusses the current knowledge of the mechanisms of MSC-mediated immunosuppression and potential therapeutic uses of MSCs in RA. (*J Rheum Dis* 2016;23:279-287)

Key Words. Inflammation, Mesenchymal stem cells, Rheumatoid arthritis

INTRODUCTION

Rheumatoid arthritis (RA) is a complicated autoimmune disease characterized by joint destruction associated with production of inflammatory mediators [1]. RA results in significantly reduced ability to perform daily activities and is associated with multiple comorbidities, increased mortality, and socioeconomic loss [2]. The etiology of RA is not completely understood. However, numerous investigations of the pathogenic mechanisms of inflammation and autoimmunity and our increased understanding of signal mediators implicated in the pathogenesis of RA have led to the development of agents that block tumor necrosis factor- α (TNF- α), interleukin

(IL)-6 signaling, the immune activity of T cells by costimulation signaling, and, the targeting of pathogenic cells such as B cells and osteoclasts [3]. Based on this research, the current care in RA is to apply a treat-to-target (T2T) strategy immediately after diagnosis. The elements of the T2T concept are adjusting the therapy, guided by an assessment of disease activity, with the aim of clinical remission [4]. Despite significant development in treatments, however, several problems remain unresolved. Up to 30% of RA patients fail to respond to current conventional and biologic-disease-modifying therapies [3]. In addition, some RA patients in sustained clinical remission presented radiographic progression of joint damage [5].

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The cause of RA is unknown, but genetic and environmental factors are contributory. The pathophysiology of RA is chronic inflammation of the joint synovium, which causes cartilage destruction and bone erosion through interactions among infiltrating T cells, B cells, proinflammatory cytokines and the resident fibroblast-like synoviocytes [6]. Various proinflammatory cytokines, such as TNF- α , IL-6, IL-1 β and IL-17, play dominant pathological roles and aberrant T helper cells (Th) 17 and Th1 responses have been connected to pathogenesis of RA [7]. In addition, evidence is increasing that a defect in the number or function of regulatory T cells (Tregs) is crucial in the immune imbalance that has a pivotal role in the pathogenesis of RA [8].

MSCs are non-embryonic stromal cells that exist in the bone marrow, peripheral blood, adipose tissue, and synovium. MSCs can be readily isolated from various tissue sources, handily expanded in culture, and differentiated under appropriate stimulation. These characteristics of MSCs suggest it as an ideal candidate tool for tissue engineering efforts aiming to repair damaged structures. In addition to these advantages, MSCs possess multipotent immunomodulatory and anti-inflammatory effects, through either direct cell-cell interaction or secretion of various factors. MSC are now widely researched for their immunomodulating and protective qualities based on their regenerative capacities [9]. Several clinical trials showed that the administration of MSC in patients with RA in general well tolerated and the treatment induced a significant remission and a reduction in disease activity score (DAS)-28. In addition, the serum levels of inflammatory cytokines decreased after the MSC therapy [9-11]. This review summarizes our knowledge on the mechanisms underlying MSC properties on immune responses and the therapeutic effect of MSCs on RA.

MAIN SUBJECTS

Characteristics of MSC

MSCs are adult stromal cells that they have the ability to differentiate into various mesodermal cell lineages, which are very important in inflammatory arthritis [12]. Despite the many studies on MSCs, there is no uniformly accepted phenotype or surface marker for their isolation. Currently, MSCs are defined retrospectively based on a grouping of characteristics in vitro, including a combination of phenotypic markers and multi-potential differ-

entiation functional properties. The minimal requirements for a population of cells to qualify as MSCs are as follows: (1) they must be plastic adherent under standard culture conditions, (2) they should express CD73, CD105, and CD90 and not express CD45, CD34, CD14, CD11b, CD79 α , CD19, or human leukocyte antigen (HLA)-DR surface molecules, and (3) they should possess mesodermal differentiation capability into osteoblasts, chondrocytes, and adipocytes [13].

MSCs can be expanded ex vivo up to a billion-fold without loss of their multipotent properties and are excellent vehicles since they maintain the expression of transfected genes for up to 40 divisions [14]. Even if MSCs isolated from different tissues show similar phenotypic features, it is not distinct whether these are the same MSCs. In addition, MSCs show different dispositions in proliferation and differentiation capacities in response to stimulation with various growth factors. Culture conditions of surface, medium, seeding density, and, isolation methods, and the presence of various growth factors influence the expansion, differentiation, and immunogenic properties of MSCs [15].

MSCs are hypoimmunogenic or non-immunogenic and so can easily escape host immune elimination. MSCs express low to intermediate major histocompatibility complex (MHC) class I molecules and do not express MHC class II molecules, although an intracellular pool of MHC class II molecules can be stimulated by interferon-gamma (IFN- γ) to be expressed on the cell surface [16]. Because MSCs do not express any costimulatory molecules, including B7-1 (CD80), B7-2 (CD86), or CD40, they do not activate alloreactive T cells [17]. Even under stimulation, MSCs do not express MHC class II molecules after differentiation into adipocytes, osteoblasts, and chondrocytes and remain non-immunogenic [16]. These properties indicate that MSCs should be able to be transplanted into an allogeneic host without immune rejection and not elicit a host immune response. An immunoprivileged capacity can be obtained by suppressing alloreactivity through the modulation of most major immune cell activities. However, the immunoprivileged properties of MSCs seem to be limited. A few mouse studies have reported that allogeneic mismatched MSCs were rejected by the host [18-20].

Immunoregulatory properties of mesenchymal stem cells

Especially for their use in rheumatic diseases, the most

notable and useful features of MSCs, are their potent immunosuppressive and anti-inflammatory effects. The mechanism of the immunoregulatory activities of MSCs is not completely known, although both direct and indirect effects have been suggested through either cell to cell interaction or soluble factors. Immunosuppression of MSCs requires preliminary activation of the MSCs by immune cells through the secretion of the proinflammatory cytokine IFN- γ , alone or with TNF- α , IL-1 α or IL-1 β [21]. A number of studies using bone marrow-derived MSCs have reported that MSC-mediated immunomodulation is dependent on IFN- γ [21], and is mainly mediated by soluble factors such as indoleamine 2,3-dioxygenase (IDO), or prostaglandin E2 (PGE2), which inhibit both T- and B-cell proliferation and function [22]. On stimulation with IFN- γ , MSCs can produce high levels of IDO as a tryptophan-catabolizing enzyme that mediates immune tolerance by limiting the availability of the essential amino acid tryptophan and generating toxic metabolites for T cells [23]. PGE2 acts as a potent immune suppressant, inhibiting T-cell mitogenesis and IL-2 production, and is a cofactor of the induction of Th type 2 lymphocyte activity. Production of PGE2 by MSCs is enhanced by TNF- α or IFN- γ stimulation, and MSC-derived PGE2 was shown to act on macrophages by stimulating the production of IL-10 and on monocytes by blocking their differentiation to dendritic cells (DCs) [24]. Another MSC-derived factor, IL-6, has been re-

ported to be involved in the inhibition of monocyte differentiation to DCs, decreasing their ability to stimulate T cells [25]. Other mediators, such as hepatocyte growth factor, transforming growth factor-beta 1 (TGF- β 1), leukemia inhibitory factor, and heme oxygenase-1, have been found to be produced by activated MSCs [26]. HLA-G5 secreted by MSCs has recently been shown to inhibit T-cell proliferation, natural killer (NK) cell cytotoxicity, and T-cell cytotoxicity and to promote the generation of Tregs [27]. Cell to cell contact between MSCs and activated T cells induces IL-10 production, which is essential to stimulate the release of soluble HLA-G5. Finally, MSC-mediated immunoregulation is the result of the cumulative actions of several molecules.

MSCs suppress proliferation of allogeneic lymphocytes and can interfere with and affect cellular differentiation, maturation, and function of immune cells (Figure 1). Both naïve and memory T cells, as well as CD4+ and CD8+ T-lymphocyte proliferation stimulated by specific antigens, are inhibited by MSCs. Suppression of proliferation depends on the arrest of T cells in the G0/G1 phase of the cell cycle, regardless of apoptosis [28]. MSCs change T-cell functions, such as decrease in production of IFN- γ , IL-2, and TNF- α and increase in IL-4 secretion [26]. MSCs can suppress CD8+ cytotoxic T cell-mediated cytotoxicity [29]. In addition, MSCs have been shown to facilitate the in vivo and in vitro generation of CD4+CD25+ or CD8+ Tregs with functional properties [30]. In addi-

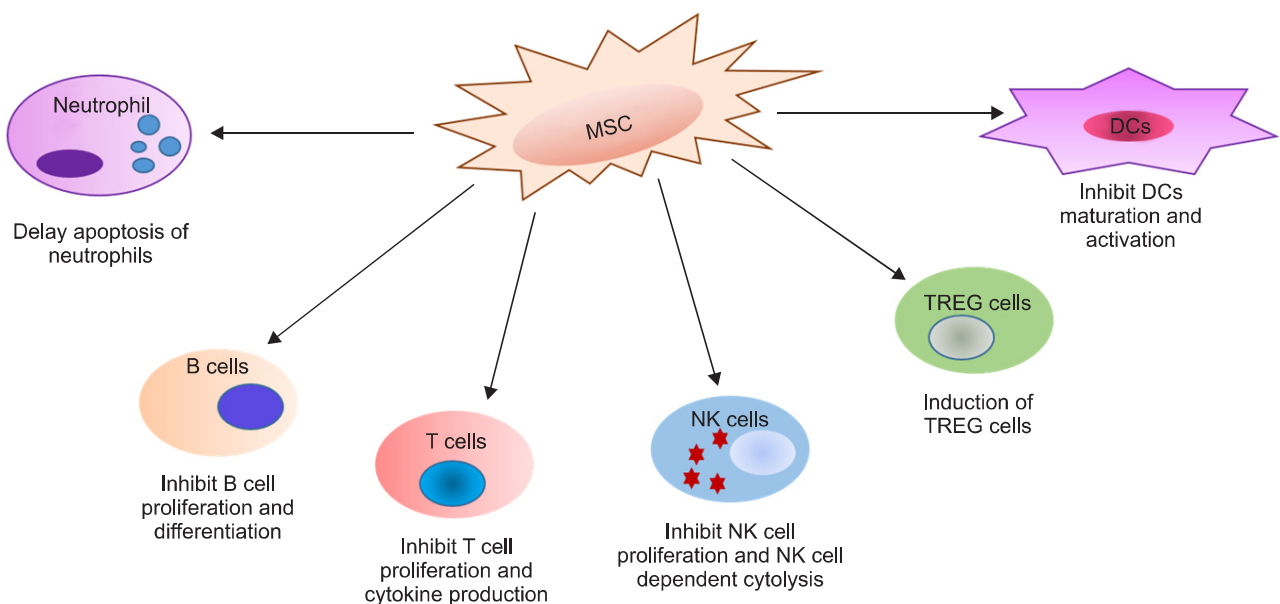


Figure 1. Suppressive effects of MSCs on immune cells. The effects of MSCs on cells of the immune system are anti-inflammatory. DCs: dendritic cells, MSCs: mesenchymal stem cells, NK cell: natural killer cell, TREG cell: regulatory T cell.

tion to T cells, MSCs suppress proliferation of B cells [31], NK cells [32], and DCs [33]. MSCs inhibit the proliferation of B cells, arresting B lymphocytes in the G0/G1 phase of the cell cycle and act their suppressive effect on B-cell terminal differentiation through the secretion of humoral factors [31]. Myeloid DCs as potent antigen-presenting cells are vital in the induction of immunity and tolerance. MSCs inhibit the *in vitro* maturation of monocytes and CD34+ hematopoietic cells into DCs, as shown by a decreased cell surface expression of MHC class II and co-stimulatory molecules, and decreased production of IL-12 and TNF- α [33]. MSCs inhibit IL-2-driven or IL-15-driven NK cell proliferation, and, IFN- γ production and cytotoxicity against HLA class I-expressing targets [34]. These effects depend on cell-to-cell contact and on the release of soluble factors, such as TGF- β 1 and PGE2, implying the existence of various mechanisms of MSC-mediated NK cell inhibition. Overall, the effect of MSCs on immune cells is to skew the immune response toward a tolerant and anti-inflammatory phenotype.

The trafficking and homing properties of MSCs

The trafficking and homing properties of MSCs can be useful tools for clinical applications using non-invasive systemic cell administration to treat RA. MSCs have been reported to express diverse chemokines and chemokine receptors and can move to lesions of inflammation by migrating towards inflammatory chemokines and cytokines [35-37]. However, the first-line accumulation site of intravenously administered MSCs is lungs, followed by liver and spleen. Several studies showed the intraarticular or intraarterial route of administration were effective in avoiding pulmonary entrapment of MSCs and may improve the bioavailability of transplanted MSCs in clinically relevant tissues [35-37]. Acting as evidence that host MSCs can migrate in response to inflammation, systemically injected MSCs are also often observed within the bone marrow or in damaged tissues [35-37]. Although, data are lacking with regard to the bio-distribution of MSCs, their cellular or molecular target structures, and the mechanisms by which MSCs reach these targets, accumulating data indicate that systemic infusion of MSCs can be used for immunosuppressive treatments [36,37].

Therapeutic potential of MSCs in animal studies

Based on their hypoimmunogenicity and immunomodulatory abilities, several studies have reported on the therapeutic effects of allogenic or xenogenic MSC treatment in collagen-induced arthritis (CIA) mice, a representative animal model of RA [38-43]. However, others have failed to demonstrate such effects [44-46] (Table 1). The first study that utilized an immortalized, allogenic mesenchymal cell line, administered intravenously in mice, showed that there was no benefit of MSC therapy for the reduction of the pathogenetic development of CIA, despite the fact that a potent immunosuppressive activity of the cells was observed *in vitro* [44]. However, another study showed that a single intraperitoneal injection of allogeneic MSCs in mice prevented the development of severe arthritis [38]. The study showed reduced levels of proinflammatory cytokines, as compared to the levels in controls, and increased levels of IL-10, an immunosuppressive cytokine produced by Tregs; it also demonstrated that MSC therapy resulted in *de novo* generation of CD4+CD25+FoxP3+ Tregs specific for type II collagen. Further positive results from MSC therapy were reported in a later mouse study, in which it was shown that daily intraperitoneal injection of human or murine allogeneic and syngeneic adipose-derived (AD) MSCs, for 5 days after the onset of disease significantly reduced the severity of arthritis in the CIA model. Intraarticular injection of AD-MSCs was less effective than the intraperitoneal route, adding weight to the argument that the positive effects of MSCs are not simply due to direct tissue repair in the joints. The therapeutic efficacy was associated with decreased antigen-specific Th1/Th17 cell expansion, enhanced secretion of IL-10, and generation of CD4+CD25+FoxP3+ Tregs with the capacity to suppress self-reactive T-effector responses. The effect depended on timing, dose, and route of administration of MSCs [39]. MSCs overexpressing IL-10 have also been shown to attenuate CIA [40]. Results from recent studies [41-43], further support the potential of MSC-based treatment in autoimmune inflammatory arthritis, in that immune modulation and reduction of articular damage following treatment with MSCs have been observed. These findings suggest that MSC therapy is able to reset the immune system by reducing the deleterious Th1/Th17 response and enhancing the protective Tregs response.

However, other studies have failed to demonstrate any improvement in CIA with MSC treatment [45,46]. One study found that Flk-1+ MSCs exacerbated the arthritis in mice, by promoting the secretion of IL-6 and IL-17

Table 1. The literature describing the effects of mesenchymal stem cells (MSCs) in mouse model of rheumatoid arthritis

First author, year [ref]	Source of MSC	Donor-recipient MHC match	Dose of MSCs	Route of administration	Time of treatment	Outcome of treatment
Djouad et al., 2005 [44]	C3 mouse cell line	Allogeneic	1×10^6 , 1×10^6 , 4×10^6	Intravenous	Day 0 or 21, day 0 or 21	UA*
Augello et al., 2007 [38]	Mouse BM	Allogeneic	5×10^6	Intraperitoneal	Day 0 or 21	Positive [†]
Gonzalez et al., 2009 [39]	Adipose tissue: human, mouse	Xenogeneic, allogeneic	1×10^6	Intraperitoneal, intraarticular	Once per day for 5 days ADO	Positive [†]
Choi et al., 2008 [40]	Mouse BM	Syngeneic	1×10^6	Intravenous	Day 21, 28, 35	Positive [†]
Park et al., 2011 [41]	Mouse BM	Syngeneic	1×10^6	Intraperitoneal	Week 7	Positive [†]
Liu et al., 2010 [42]	Human UC	Xenogeneic	5×10^6	Intraperitoneal	Day 31 ADO	Positive [†]
Bouffi et al., 2010 [43]	Mouse BM	Syngeneic, allogeneic	1×10^6	Intravenous	Day 18, 24, 28, 32	Positive [†]
Chen et al., 2010 [45]	Mouse BM	Syngeneic	1×10^6 , 2×10^6	Intravenous	Day 0 or 21	Negative [‡]
Schurgers et al., 2010 [46]	Mouse BM	Syngeneic, allogeneic	1×10^6	Intravenous, intraperitoneal	Day 1, 16, 19, 23, 30	Negative [‡]

Time of treatment is shown as number of days or weeks before or after induction of arthritis, except where specified as number of days after disease onset (ADO). Ref: reference, MHC: major histocompatibility complex, BM: bone marrow, UC: umbilical cord.

*No effect, indicated by an unaffected disease score. [†]Positive effect, indicated by a disease score reduction. [‡]Negative effect, indicated by a disease score increase.

[45]. The other study was unable to demonstrate any benefit from MSC therapy in CIA [46]. Those investigators used both an intravenous route and an intraperitoneal route to administer the MSCs, and also used allogeneic MSCs. None of these changes in MSC therapeutic approaches made any difference to the negative outcome.

Experimental protocols differed between all of these studies (Table 1), which may explain the inconsistencies in results [38-46]. The studies that demonstrated beneficial effects of MSC therapy also had a number of differences in their protocols [38-43]. Potential reasons for the discrepancies among these results include the following: source of MSCs (murine, syngeneic vs. human, allogeneic), tissue of origin (bone marrow, adipose tissue, cord blood), timing of treatment, number of stem cells injected, route of injection (intravenous, intraperitoneal, intraarticular), and treatment regimen (a single injection of MSCs vs. daily injections for 5 consecutive days) [38-47]. They used different administration of routes and used syngeneic, allogeneic, and human sources of MSCs, but the dose of MSCs was fairly consistent between studies. Data showed that human MSCs in completely

MHC-mismatched mice are not rejected by the immune system, even after allogeneic or xenogeneic transplantation. MSCs showed similar cell migration and therapeutic effect given by intravenous or intraperitoneal injection [47,48]. A probable factor was the length of culture of the MSCs. Schurgers et al. [46] used MSCs that had been cultured for several weeks prior to use in these experiments, and the investigators did not observe any therapeutic effect of MSC administration. Allogeneic MSCs, which were used in the study by Augello et al. [38], were from primary culture of mouse MSCs obtained after the first in vitro passage as opposed to the immortalized cell line that was used in the study by Djouad et al. [44]. In addition, non-measurable variables that may have contributed to study differences and could be relevant might include differences in the culture medium used for stem cells, lack of standardization of MSC culture conditions, and differences in animal-housing conditions [47].

Recently, a study investigated the therapeutic efficacy of human bone marrow (BM)-, adipose tissue (AD)-, and umbilical cord (UC)-derived MSCs in CIA to find the best condition for clinical application of human MSCs for RA

treatment [49]. The data showed that BM-, AD-, and UC-derived MSCs significantly suppressed joint inflammation in CIA mice. The cells decreased proinflammatory cytokines and upregulated anti-inflammatory cytokines and induced Tregs. The results show that the immune modulatory effect of MSCs was associated with Tregs induction, consistent with the increase in anti-inflammatory cytokine expression in CIA. The BM-MSC-treated mice showed a greater therapeutic efficacy than AD-MSCs or UC-MSCs in CIA in view of the severity of arthritis and histopathological evaluation. Also, Tregs induced by BM-MSCs had stronger immunosuppressive activity than those induced by AD-MSCs or CB-MSCs. As for clinical application, the study investigated the dose-dependent therapeutic effect of MSCs. Although minimal therapeutic effects were seen with 5×10^5 MSCs, treatment with 5×10^6 cells was sufficient for the suppression of arthritis and the induction of Tregs. The study showed there is no difference in effectiveness between the two administration schedules (daily injection of 1×10^6 MSCs for 5 consecutive days vs. two injections of 2.5×10^6 cells over a 3-day interval) [49]. According to this report, BM-MSCs had a greater therapeutic efficacy than the other types of MSCs in CIA.

The ability of MSCs to generate de novo Tregs may be advantageous therapeutically when compared to neutralizing antibodies against single-cytokine signaling, in terms of both safety and efficacy. These data need to be confirmed in appropriated clinical studies.

The role of MSCs in prevention of bone destruction in RA is still unknown. Recently, one study investigated the effect of AD-MSCs on in vitro formation of bone-resorbing osteoclasts and pathological bone loss in a mouse CIA model of RA [50]. The study showed that AD-MSCs considerably suppressed receptor activator of NF- κ B ligand (RANKL)-induced osteoclastogenesis in both a contact-dependent and -independent manners. Additionally, AD-MSCs inhibited RANKL-induced osteoclastogenesis in the presence of proinflammatory cytokines such as TNF- α , IL-17, and IL-1 β . In addition, treatment with AD-MSCs at the onset of CIA significantly reduced clinical symptoms and joint pathology. Moreover, AD-MSCs inhibited autoimmune T cell responses and increased the proportions of peripheral regulatory T and B cells. Thus, the study provides strong evidence that AD-MSCs improve inflammation-induced systemic bone destruction in CIA mice by reducing osteoclast precursors and improving immune tolerance [50].

Clinical trials with MSCs in patients with rheumatoid arthritis

A few clinical RA studies have been presented [10,11,51]. In a preliminary report on four RA patients with anti-TNF-failing active RA were intravenous injection treated with a single infusion of 1×10^6 cells per kg of allogeneic BM-MSCs or UC-MSCs. The study showed no significant clinical improvement, though no toxicity was observed. Three of four patients experienced reduction in erythrocyte sedimentation rate, DAS-28, and pain visual analogue scale (VAS) score at 1 and 6 months after transplantation. Two of the patients demonstrated a moderate European league against rheumatism (EULAR) response at 6 months but experienced a relapse at 7 and 23 months, respectively. No patient achieved DAS-28-defined remission in the follow-up period. No adverse events were observed during or immediately after infusions of MSC in any of the four patients. No severe infections, malignancies, or death occurred [10].

Another larger, non-randomized comparative trial involving 172 patients with active RA who demonstrated inadequate responses to traditional medication were enrolled to assess the safety and efficacy of human UC-MSCs in the treatment of RA [11]. Patients were divided into two treatment groups: disease-modifying anti-rheumatic drugs (DMARDs) plus medium without UC-MSCs, or DMARDs plus UC-MSCs (4×10^7 cells per time) via intravenous injection. Tests of serological markers were conducted to assess safety and disease activity. Serum levels of inflammatory chemokines/cytokines were measured, and lymphocyte subsets in peripheral blood were analyzed. The serum levels of TNF- α and IL-6 decreased after the first UC-MSC treatment. The percentage of CD4+CD25+ Foxp3+ Tregs in peripheral blood was increased. The treatment induced a significant remission of disease according to the American College of Rheumatology improvement criteria, the DAS-28, and the Health Assessment Questionnaire. The therapeutic effects were maintained for 3~6 months without continuous administration, correlating with the increased percentage of Tregs in peripheral blood. No serious adverse effects were observed during or after infusion, and 4% of the treated patient showed mild adverse effects during the infusion, such as chill and/or mild fever. This study showed that treatment with DMARDs plus UC-MSC can provide safe, significant, and persistent clinical benefits for patients with active RA.

The other study presented three RA patients who re-

ceived autologous AD-MSCs [51]. All patients received multiple injections of MSC, intravenous injection in two cases and intraarticular in the third. The first patient received two intravenous injection of 3×10^8 AD-MSCs. The second patient received 8×10^8 AD-MSCs in total, one intravenous injection of 2×10^8 AD-MSCs and intraarticular injection of 1×10^8 AD-MSCs. This patient then received another intravenous injection of 3.5×10^8 cells and a second intra-articular injection of 1.5×10^8 cells. The third patient received four times intravenous injections of 2×10^8 AD-MSCs at intervals of one month. Clinical benefit was seen in all cases without significant toxicity.

Recently clinical trials are ongoing with MSCs in patients with RA [52-54].

Safety of MSCs in human subjects

A recently published article systematically reviewed clinical trials that examined the use of MSCs in order to evaluate their safety [55]. MEDLINE, EMBASE, and the Cochrane Central Register of Controlled Trials (to June 2011) were searched. A total of 1,012 participants with clinical conditions of ischemic stroke, Crohn's disease, cardiomyopathy, myocardial infarction, graft versus host disease, and healthy volunteers were included. Meta-analysis of all the randomized controlled trials did not detect any association between acute infusional toxicity, organ systemic complications, infection, death or malignancy, although, there was a significant association between MSC and transient fever [55]. We conclude, based on the current clinical trials, that MSC therapy, seems to be safe. However, further larger scale controlled clinical trials with radical reporting of adverse events are required to further define the safety profile of MSCs [55].

CONCLUSION

The current data showed that MSCs represent a promising therapeutic tool in the treatment of RA. Encouraging results have been obtained from clinical trials. However, many questions remain to be identified in order to offer better treatment to control inflammation for the benefit of RA patients. This suggests the need for better comprehension of the underlying mechanisms of immunomodulation and satisfaction of safety concerns. Further studies are necessary to ascertain the concept of MSCs in order to establish the best treatment strategy for use in RA.

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CONFLICT OF INTEREST

No potential conflict of interest relevant to this article was reported.

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