pISSN: 2093-940X, eISSN: 2233-4718
Journal of Rheumatic Diseases Vol. 25, No. 3, July, 2018
https://doi.org/10.4078/jrd.2018.25.3.158

Review Article

Adult Stem Cell Treatment for Rheumatoid Arthritis

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Since methotrexate began to be used in the treatment of rheumatoid arthritis (RA) 30 years ago, RA treatments have advanced rapidly from only reducing joint pain and inflammation to suppressing disease progression and joint destruction. In particular, the development of biologics and targeted anti-rheumatic drugs has almost made it possible to induce remission in patients with RA. On the other hand, the current RA treatments are still limited by adverse effects and treatment failure. Stem cell therapy has been suggested as an alternative treatment of RA, and preclinical studies and clinical trials using representative adult stem cells (ASCs), hematopoietic stem cells (HSCs) and mesenchymal stem cells (MSCs), are currently underway. HSC therapy in RA has mostly progressed based on the concept of 'immune reset', in which the existing immune cells are replaced with healthy ones. HSC transplantation was completed relatively safely, and the patients showed a positive treatment response. Nevertheless, the treatment response of HSCs in RA depends on the conditioning regimen, and the efficacy did not persist for a long time. The MSCs possessed a hypo-immunogenicity, immune modulation effect and tissue regeneration capability, making them another promising candidate for the RA treatment. MSC transplantation in RA was found to be safe with few adverse effects, such as immune rejection or embolism, but it showed a partial and transient response. This review addresses the characteristics of ASCs, focusing specifically on HSCs and MSCs, and summarizes the results of preclinical studies and clinical trials of ASC therapy in RA. **(J Rheum Dis 2018;25:158-168)**

Key Words. Rheumatoid arthritis, Adult stem cell, Hematopoietic stem cell, Mesenchymal stem cell, Clinical trial

INTRODUCTION

Rheumatoid arthritis (RA) is a representative autoimmune disease characterized by chronic synovitis of the entire joints. The activity and severity of arthritis vary among individuals over time, and if joint inflammation cannot be properly controlled, it can lead to physical disability and severely reduced quality of life due to joint destruction and deformity [1]. Recently, emphasis on early diagnosis and treatment has led to autoimmune response modulation being performed using disease-modifying antirheumatic drugs (DMARDs), a type of immunosuppressant, from the time of diagnosis [2]. In particular, effective suppression of disease progression by single or concomitant administration of conventional DMARDs, starting with methotrexate (MTX), revolutionized RA treatment. Moreover, the development of biologic agents that directly block pro-inflammatory cytokines, such as tumor necrosis factor (TNF)-α and interleukin (IL)-6, as well as targeted synthetic DMARDs that block intracellular signaling pathways, such as Janus kinases, provides alternatives for near-remission treatment in patients who experienced treatment failure with conventional DMARDs [3].

However, despite the use of these drugs, they do not enable regeneration of already damaged joints, and some patients have to keep changing their drugs because they do not show a satisfactory response to treatment [4]. Moreover, long-term drug use can cause complications from common adverse effects, such as gastrointestinal

Received : May 21, 2018, **Revised** : May 29, 2018, **Accepted** : May 30, 2018

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complications, to severe adverse effects, such as hepatic- and nephrotoxicity, infection or malignancy due to immune suppression. Even if biologics or targeted DMARDs induce clinical remission, attempts to reduce the dose or change the treatment interval can worsen the disease [5]. In this regard, there is still a need for RA treatments that are safe and have no adverse effects and approach the cure of the disease without these medications.

Stem cells are cells with multipotency, which means that several different types of cell can be produced from a single cell. Stem cells can be broadly categorized into embryonic stem cells (ESCs), adult stem cells (ASCs), and induced pluripotent stem cells (iPSCs). ESCs are stem cells obtained during embryonic development at the blastocyst stage. They have the pluripotency to differentiate into almost any cell in the body. However, because ESCs are obtained from others, genetic modifications are required for use in treatments. The risk of tumor development is also high. And, ethical issues regarding the use of embryos still need to be resolved [6]. iPSCs are artificially manufactured stem cells made by obtaining somatic cells in adult skin or blood that have already finished differentiating and injecting the four reprogramming factors Oct4, Sox2, Klf4, and c-Myc intracellularly to provide the cells with the same type of pluripotency as ESCs [7]. Since the patient's own somatic cells are used, immune rejection can be avoided. However, the risk of tumor development cannot be excluded due to ex vivo genetic engineering [6]. Therefore, the use of these stem cells seems to be very limited in RA treatment.

Conversely, ASCs are cells from an adult body without any ex vivo manipulation. This means that they are safer than the two types of stem cell discussed above. Moreover, several studies have demonstrated immune regulation and tissue regeneration effects for ASCs, and they have been used in treatments for not only rheumatic diseases such as systemic sclerosis, lupus, and RA, but also various autoimmune diseases such as multiple sclerosis, graft-versus-host disease (GvHD), and type I diabetes [8,9]. Representative ASCs include hematopoietic stem cells (HSCs), which have the ability to produce all blood cells such as white blood cells, red blood cells, and platelets as well as mesenchymal stem cells (MSCs), which are the origin of stromal cells in the tissues other than the skin, blood vessels, and internal organs [10]. Types and characteristics of stem cells are summarized in Table 1. In this review, we briefly describe the characteristics of ASCs, dividing the review broadly into two parts focusing on HSCs and MSCs and analyze the results of preclinical studies and clinical trials in the treatment of RA to evaluate their availability and considerations for use in RA treatment.

Table 1. Types and characteristics of stem cells

Stem cell type	Cell source	Potency (target cells)	Strong point	Weak point	Ref.
Embryonic stem cell	Blastocyst of embryo	Pluripotent (all kinds of cells)	High replicable capability, Large quantity production	Immune rejection, Ethical issue, Tumor formation	[6,16]
Induced pluripotent stem cell	Skin fibroblast, keratinocyte, T cell, hepatocyte, other somatic cells	Pluripotent (all kinds of cells)	Patient-specific, Large quantity production, No ethical issue	Tumor formation, Contamination, High cost	[7,17]
Adult stem cell					
Hematopoietic stem cell	BM, UCB, peripheral blood	Multipotent (myeloid and lymphoid blood cells)	Proven safety, No ethical issue, Restore blood cell	Limited differentiation, Limited quantity production	[9,18,23]
Mesenchymal stem cell	BM, UCB, UC, placenta, adipose tissue, dental pulp, periosteum	Multipotent (osteoblast, chondrocyte, adipocyte)	Proven safety, No ethical issue, Hypo-immunogenic, Immune modulation	Limited differentiation, Limited quantity production, Tissue sequestration	[11,16,48]

Target cells are those cells in which the stem cells can be differentiate. BM: bone marrow, UCB: umbilical cord blood, UC: umbilical cord, Ref.: reference.

MAIN SUBJECTS

Adult stem cells

ASCs, which are also called 'somatic stem cells', are undifferentiated cells existing in parts of the body after the end of embryonic development and they are detected in the bone marrow (BM), umbilical cord (UC), skin, adipose tissue, nerve, liver, and pancreas [11]. The majority of stem cells exist quietly without differentiation for a long time in a specialized microenvironment known as a 'niche' within the tissue. And, they become activated and participate in the healing process in cases of tissue damage or disease [10,12].

One of the important characteristics of stem cells is 'self-renewal', which refers to the ability to produce daughter cells with the same proliferation and differentiation ability after multiple divisions [13]. When stem cells are actually cultured in the laboratory, they maintain their characteristics while proliferating through a large number of passages and this makes it possible to mass culture and obtain enough cells to use in treatments.

In HSC transplantation (HSCT) for RA, self-renewal capability is important not only to determine the number of stem cells showing successful engraftment after conditioning, but also to maintain long-term tissue regeneration and engraftment [14]. Moreover, to maximize the immune modulation and tissue regeneration effects in RA treatment using MSCs, it is important to maintain the highest possible number of cells in the body that do not differentiate into undesired cell types [15,16]. Therefore, self-renewal capability can have an important effect on treatment success in RA.

The other important characteristic of stem cells is that it is possible to differentiate into several desired cell types under specific conditions [16,17]. This is referred to as 'multipotency' or 'stem cell plasticity'. In particular, HSCs can differentiate into blood cells in myeloid lineages, including macrophages, neutrophils, erythrocytes, and platelets, and in lymphoid lineages, including T cells, B cells, and natural killer (NK) cells [18]. Ultimately, it is important to remove auto-reactive immune cells and change to normal cells in RA treatment. And, this is achieved by a conditioning protocol using cyclophosphamide (CYC) or total body irradiation (TBI) and inducing differentiation to healthy immune cells by using multipotent HSCs. Additionally, the normal erythrocytes and platelets removed during conditioning can also be restored by HSCT.

Another important objective in RA treatment, alongside suppression of autoimmunity, is regeneration of damaged joint tissues. Conventional DMARDs, such as MTX, and biologic agents are unable to regenerate the cartilages and bone tissues that have already been damaged. In this regard, MSCs, which not only have an immune modulation effect but can also differentiate into chondrocytes or osteoblasts, have the advantage of regenerating damaged joints [15,19]. Therefore, they are being actively studied as a therapeutic tool in RA.

Hematopoietic stem cells

1) Characteristics of HSCs

HSCs were first discovered and identified in mouse BM in 1961 [20]. During development, HSCs originate in the embryonic mesoderm and eventually migrate to the red BM located in the trabecular region of the long bones [21,22]. These cells are also present in the umbilical cord blood (UCB) and peripheral blood. The cells can be identified by the cell surface markers they express. In humans, HSCs characteristically express CD34, in addition to CD59, CD90, and CD117, but do not express CD38 or blood lineage markers (Lin-) [23,24].

The HSCs engraft mostly in the BM to contribute to maintaining hematopoiesis, while the other cells migrate to the peripheral blood and lymphatic system [25]. Most of the engrafted HSCs are kept in an undifferentiated state by various niche-related factors within a specific microenvironment, and only a fraction of HSCs differentiate [26]. Of a particular importance in this regard are stromal cell-derived factor-1 (SDF-1, also termed as CXCL12), which is a chemokine secreted by stromal cells in the BM, and its receptor, CXC chemokine receptor 4 (CXCR4), which is expressed by HSCs [25-27]. In fact, SDF-1/CXCR4 signaling has a major effect on stem cell quiescence, proliferation, retention within niches, and migration to the outside.

In the study by Petit et al. [28], granulocyte colony-stimulating factor, which is used in HSCT for the mobilization of stem cells from inside the BM to the periphery, was reported to promote peripheral movements of HSCs by activating neutrophil elastase to induce SDF-1 degradation.

HSCs that have migrated to the peripheral blood go towards damaged tissues, with SDF-1 secreted by cells in the damaged tissues playing an important role in the local infiltration of stem cells. Kim et al. [29] reported that SDF-1 is overexpressed by synovial fibroblasts in RA owing to IL-17 secreted by T lymphocytes. This implies that

when HSCs are grafted into a patient with RA, they will be able to go towards inflamed joints overexpressing SDF-1.

The main objective of HSCT in autoimmune diseases was originally the so-called 'immune reset', in which pathologic immune cells are replaced with healthy blood cells. However, some studies have also suggested that HSCs themselves display an immunosuppressive effect [30-32]. One mechanism is that since all HSCs express MHC class I and class II, but not the B7 family of co-stimulatory molecules, they are able to induce anergy by direct contact with cytotoxic T cells [31]. Another mechanism is that grafted HSCs activate Notch signaling by direct intracellular contact and secrete soluble factors, such as granulocyte-macrophage colony-stimulating factor, thereby inducing proliferation of peripheral Foxp3+ regulatory T (Treg) cells [32]. Since inhibition of the autoimmune response is important in RA treatment, both of these mechanisms are thought to provide a sufficient theoretical basis for the use of HSCT in RA.

2) Preclinical studies of HSCs in RA animal models

The use of HSCT in RA has advanced on the basis of positive results in experiments using an animal model. Dirk et al. used TBI (8.5 Gy) to remove abnormally activated immune cells from an adjuvant-induced arthritis rat model, extracted BM-derived HSCs (5×10^7) from syngeneic or allogeneic donors, and administered the HSCs intravenously [33]. The results showed that both syngeneic and allogeneic transplantations significantly improved arthritis, while reducing concerns on graft rejection based on the lack of adverse events and demonstrating a greater effect when HSCs were grafted earlier in the progression of arthritis. Moreover, another animal study that performed autologous stem cell transplantation using a similar method showed a strong remission induction [34].

There has also been studied on conditioning regimens. Combination regimens using multiple fractionated TBI (6×2.5 Gy), CYC (2×60 mg/kg) with low dose TBI (4 Gy), or CYC (2×60 mg/kg) with busulfan (BU) (10 mg/kg) have been shown to be as effective as the conventional lethal single dose TBI (9 Gy), providing evidence for the use of conditioning regimens in the treatment of patients with RA [35].

Allogeneic BM-derived HSCT significantly suppressed arthritis in a collagen-induced arthritis (CIA) model using DBA/1J mice and effectively prevented arthritis in a spontaneous arthritis model using New Zealand black/KN mice, suggesting that there are no differences among animal models [36].

3) Clinical trials of HSCs in patients with RA

Although the use of HSCT in patients has mostly progressed on the basis of animal experiments, there is additional evidence provided by serendipitous cases such as cases of patients with RA and aplastic anemia or blood cancer, including lymphoma, who received HSCT to treat the blood disorder and showed simultaneous improvement in arthritis [37]. Patients with RA and aplastic anemia have received allogeneic BM-derived HSCT from a sibling donor before being medicated with immunosuppressants, such as MTX or cyclosporine, to prevent GvHD and showed RA remission of 2～20 years [38-40]. By contrast, patients with RA who received autologous HSCs after chemo-conditioning to treat lymphoma showed a significant improvement in arthritis. However, the disease relapsed within 5 weeks to 2 years, suggesting that HSCT alone cannot be expected to provide a long-term suppressive effect on arthritis [41,42].

Nevertheless, allogeneic stem cell transplantation, which has potential adverse effects, including BM failure and GvHD, cannot be readily considered in patients with only RA, which is less directly life threatening [37]. Thus, most clinical trials of RA have been conducted using autologous transplantation.

With regard to HSCT for autoimmune diseases, the European Society for Blood and Marrow Transplantation (EBMT) registry and the Autologous Blood and Marrow Transplant Registry Database (ABMTR) in Europe and the Center for International Blood and Marrow Transplant Research (CIBMTR) registry in North and South America have been launched to collect and analyze patient cases [43,44].

In the case of RA, the EBMT/ABMTR registries have registered 78 patients between 1996 and 2011, while the CIBMTR registry has registered 10 patients. This indicates that HSCT has been performed in approximately 88 patients with RA worldwide. Of these, only two patients received allogeneic transplantation, and all the other patients were treated using autologous methods.

Snowden et al. [45] first published phase I/II clinical trial results demonstrating the safety and efficacy of autologous HSCT in patients with severe, active RA. A total of eight patients were divided into two groups according to the dose of CYC used in the conditioning regimen, with four patients receiving 100 mg/kg CYC and the other four patients receiving 200 mg/kg CYC. Thereafter, all pa-

tients underwent mobilization with filgrastim and received a graft of HSCs (2×10^6 CD34+ cell/kg) collected from the peripheral blood. While the former group showed a temporary American College of Rheumatology (ACR)20 or ACR50 response that lasted only 3～4 months, the latter group had one patient who showed complete remission for over a year and three patients who maintained an ACR50 or ACR70 response that lasted for 17～19 months. In all patients, HSCT was tolerable. However, relapse eventually occurred, indicating that it will be difficult to cure RA completely with a single HSCT.

Since then, various conditioning regimens and graft manipulation methods have been studied [46,47]. In 2004, a retrospective analysis was conducted on 73 patients with RA who received autologous HSCT and were registered in the EBMT/ABMTR registries [43]. The majority of patients received a high dose of CYC (200 mg/kg) (62 patients) for the conditioning regimen, while the other patients received CYC+anti-thymocyte globulin (ATG) (seven patients), CYC+BU (two patients), CYC+ATG+TBI (one patient), or fludarabine+ATG (one patient). In most cases, the HSCs used were collected from the peripheral blood by mobilization, while stem cells derived from an autologous BM were used in one patient. Forty-nine patients (67%) showed an ACR50 response and disability improvement for at least 18 months, and treatment was more effective in seronegative RA than in seropositive RA. However, the majority of patients showed persistence or recurrence of disease activity, and the disease could be controlled by re-administration of DMARDs within 6 months in only approximately half of the patients. In terms of adverse effects, the treatment was tolerable in most patients. However, one patient who was treated with CYC+BU died of infection and non-small cell lung cancer 5 months after transplantation.

Later, the EBMT working party had planned a large-scale phase III trial to investigate the effects of autologous HSCT in severe, active, and anti-TNF resistant RA. However, the patient recruitment was limited owing to persistent development and widespread use of biologics and targeted synthetic DMARDs with excellent therapeutic effects in RA. Therefore, this clinical trial was ultimately cancelled. Major clinical trials of HSCs transplantation in the patients with RA are summarized in Table 2.

Mesenchymal stem cells

1) Characteristics of MSCs

The term 'mesenchymal stem cell' was first coined by Caplan in 1991 [48] to describe a rare population of BM-derived, plastic adherent cells discovered by

Table 2. Major clinical trials of hematopoietic stem cell therapy in rheumatoid arthritis

No. of patients	Transplantation type	Cell source (n)	Graft manipulation (n)	Conditioning regimen (n)	Response (n)	Ref.
8	Autologous	Peripheral blood	None	CYC 100 mg/kg (4) CYC 200 mg/kg (4)	Arthritis improving (8) - only lasting 2～3 mo in CYC 100 mg/kg (4) - beyond 17～19 mo in CYC 200 mg/kg (4)	[45]
4	Autologous	Peripheral blood	CD34+ selection	CYC 200 mg/kg +ATG (3)+TBI (1)	Arthritis improving (3) - ACR70 (3) within 3 mo - ACR70 (1), ACR50 (1) after 6 mo	[46]
6	Autologous	Peripheral blood	CD34+ selection	CYC 200 mg/kg	Arthritis improving (6) - ACR20 (3), ACR50 (2), ACR70 (1) : all relapsed at 1.5～9 mo	[47]
73	Autologous	BM (1) Peripheral blood (72)	Unmanipulated (28) CD34+ selection (45)	Various: CYC 200 mg/kg (62)	ACR50 (49) HAQ score ↓ Most restarted DMARDs within 6 mo	[43]

BM: bone marrow, CD: cluster of differentiation, CYC: cyclophosphamide, ATG: anti-thymocyte globulin, TBI: total body irradiation, ACR: American College of Rheumatology, HAQ: Health Assessment Questionnaire, ↓: decrease, DMARD: disease-modifying antirheumatic drug, Ref.: reference.

Friedenstein et al. [49]. MSCs are adult multipotent stem cells that have the ability to differentiate into cells of a mesodermal origin such as adipocytes, chondrocytes, or osteoblasts. They have a characteristic spindle and undifferentiated shape that appears similar to fibroblasts [50]. Since MSCs were first discovered in the BM, they have been isolated and cultured from various tissue types, including the adipose tissue, UC and UCB, placenta, skin, tendon, muscle, and dental pulp [9,51-53]. The Mesenchymal and Tissue Stem Cell Committee of the International Society for Cellular Therapy proposed the following three minimal criteria to define MSCs: (1) MSCs must be plastic adherent when maintained in standard culture conditions. (2) They must express CD105, CD73, and CD90 and lack expression of CD45, CD34, CD14 or CD11b, CD79a or CD19, and HLA class II surface molecules. (3) They must be able to differentiate to mesodermal cells, such as osteoblasts, chondrocytes, and adipocytes [54].

One major advantage of MSCs is that the cells themselves are hypo-immunogenic or non-immunogenic. Therefore, host immune attacks can be avoided, enabling allografts. Specifically, MSCs express MHC class I (HLA-A, B, and C) molecules on the cell surface, allowing them to avoid attacks by host NK cells [55]. Referentially, NK cells recognize, attach to, and remove infected cells and tumor cells that have downregulated MHC class I expression. MSCs also express the nonclassical MHC class I molecule, HLA-G5, inducing production of regulatory T cells and inhibiting NK cell activity by binding to their major inhibitory receptors, killer-Ig-like receptor (KIR)1 and KIR2 [56]. MSCs can avoid recognition by alloreactive CD4+ T cells because they do not express MHC class II (HLA-DR) molecules. Moreover, they do not even express the co-stimulatory molecules required to induce effector T cells, such as CD40, CD40L, CD80, and CD86. This means that they actually neutralize T cells and can be expected to show immune tolerance [57].

Taking these characteristics into account, it seems that immunosuppressants in MSC therapy may not be absolutely necessary to prevent immune rejection even for allogeneic transplantation. However, some animal experiments have reported immune rejection by the host following allogeneic transplantation of MSCs, demonstrating that they may not be perfectly immune privileged cells [58,59]. In that sense, if conventional DMARDs, such as MTX or leflunomide, were combined with allogeneic stem cell transplantation to treat RA, we expect it would be possible to improve the therapeutic effect against arthritis while also slightly reducing the rate of transplantation rejection.

The main reason that MSCs have been used to treat autoimmune diseases is because these cells demonstrate immune modulation effects. The mechanisms of these effects have not been fully elucidated. However, they are known to be mediated by direct intercellular contact and the secretion of soluble factors such as indoleamine 2,3-dioxygenase (IDO), prostaglandin E2 (PGE2), and nitric oxide. In particular, IDO, which is secreted in response to TNF-α or IFN-γ stimulation, is a tryptophan-catabolizing enzyme that suppresses lymphocyte growth by depleting the essential amino acid tryptophan [60]. PGE2 promotes IDO secretion while also directly inhibiting T lymphocyte mitogenesis and IL-2 secretion and acting on macrophages to inhibit TNF-α and IL-1β secretion while increasing secretion of the anti-inflammatory cytokine IL-10 [61]. In addition, IL-6 secreted by MSCs inhibits monocyte differentiation into dendritic cells and also suppresses maturation and activation of dendritic cells [62]. In summary, via the various mechanisms described above, MSCs have the ability to suppress both innate and adaptive immunity and can thus be considered as ideal candidate for treating RA.

Another advantage of MSCs is that they are able to differentiate into osteoblasts and chondrocytes, thereby regenerating the joint tissues that have been damaged by RA [63]. Unfortunately, when MSCs are injected systemically via an intravenous route, most cells are sequestered by the lungs and liver without ever reaching the damaged joint tissue [64]. This phenomenon is thought to be closely related to the size and surface adhesion of MSCs. The stem cells would need to be injected via intra-articular or intra-arterial routes to overcome this limitation. However, the former would only allow localized treatment, reducing the effectiveness, and the latter would be difficult to implement in the clinical field owing to the risk of arterial puncture. Therefore, it is appropriate to understand the main RA treatment mechanisms of intravenous MSCs in terms of paracrine effects owing to various soluble factors secreted by sequestrated stem cells.

2) Preclinical studies of MSCs in RA animal models

Based on the immune-modulating ability of MSCs, there have been several preclinical studies in which allogeneic or xenogeneic MSCs were transplanted into CIA mice. Apart from a few studies, the majority of research has

demonstrated significant improvements in arthritis.

Augello et al. provided CIA mice a single intraperitoneal injection of 5×10^6 BM-derived allogeneic MSCs and reported clinical and histologic improvements in arthritis [65]. In particular, the MSC treatment group showed a large decrease in the serum TNF-α level, and analysis of the spleen tissues revealed inhibition of antigen-activated T lymphocyte proliferation and increased levels of CD4+CD25+FoxP3+Treg cells.

González et al. [66] collected adipose tissue-derived MSCs from humans and administered 1×10^6 cells to CIA mice every day, either via intraperitoneal or intra-articular injection, for 5 days after arthritis induction. All groups that received stem cells showed improvements in arthritis, and the therapeutic effect was greater when MSCs were administered sooner after arthritis induction and when administered via intraperitoneal injection compared with intra-articular injection. The groups that received stem cells also showed significantly reduced circulating TNF-α and IL-1β levels, elevated IL-10 level, and increased CD4+CD25+FoxP3+Treg population in the joint, spleen, and lymph node cell analyses.

Liu et al. [67] administered 1×10^6 human UC-derived MSCs to CIA mice intraperitoneally every day for 5 days after arthritis induction, and these mice showed improved clinical arthritis scores and histologic findings compared with a control group. In the group that received stem cells, the serum levels of TNF-α, IL-6, and monocyte chemoattractant protein-1 significantly decreased, while the levels of IL-10 significantly increased. When the mouse spleens were harvested and the immune cells were analyzed, the stem cell implantation group showed a shift from a Th1-type response to a Th2-type response, alongside a reduced Th17-type response and an increase in the Treg cell level. These results indicate that the hypo-immunogenicity and immunosuppressive effects of MSCs enable allogeneic transplantation, and since the UC is naturally obtained as a by-product during birth, there is no need for invasive procedures as in harvesting BMs or adipose tissues, which makes this an attractive source for stem cells.

3) Clinical trials of MSCs in patients with RA

Despite the immune-modulating capability of MSCs and the positive results in preclinical trials, there have been a few studies on MSCs in actual patients with RA. This is because the risk of immune rejection cannot be completely excluded although allogeneic transplantation of MSCs is relatively safe, and there are also concerns regarding the risk of embolism due to cell aggregation. Moreover, as mentioned above, widespread use of biologic and targeted synthetic DMARDs could be an obstacle to the use of MSCs in RA treatment.

The first outcomes of MSC therapy in humans were published by a Korean research team [68]. In a study of various autoimmune diseases, MSCs were extracted from autologous adipose tissues, cultured, expanded, and administered to patients via intravenous or intra-articular injection. There were three patients with RA included in this study. The first patient received two intravenous injections of 3×10^8 adipose tissue-derived MSCs and showed an improvement in the pain visual analog scale (VAS) score from 10 to 2~3 and in the Korean Western Ontario and McMaster Universities arthritis index from 73 to 28. The second patient received an intravenous injection of 2×10^8 MSCs and an intra-articular injection of 1×10^8 MSCs, followed by an additional intravenous injection of 3.5×10^8 MSCs and an intra-articular injection of 1.5×10^8 MSCs. The patient previously had a difficulty in walking, but was able to walk after stem cell treatment and stopped taking steroids. Finally, the third patient received four intravenous injections of 2×10^8 MSCs. As in the previous patient, this patient was able to walk normally after treatment and stopped taking steroids. This study verified the efficacy and safety of autologous adipose tissue-derived MSCs in treating RA. However, the study was limited by the small number of patients, absence of objective indices to evaluate the response, such as ACR response or DAS28 score, and short follow-up duration (3~13 months).

Liang et al. [69] reported their experience of allogeneic MSC therapy in four patients with severe RA who had not responded to anti-TNF therapy. These patients were administered with a single intravenous dose of 1×10^6 cells/kg, with one patient receiving BM-derived MSCs from her husband, and the other three patients receiving UC-derived MSCs. Three out of the four patients showed decreased erythrocyte sedimentation rates, DAS28 score, and pain VAS score 1~6 months after MSC transplantation. Two patients showed a moderate response according to the European League Against Rheumatism (EULAR) response criteria 6 months after the treatment, but experienced relapse at 7 and 23 months after the intervention, respectively. The other two patients did not show a EULAR response after treatment. In addition, none of the patients satisfied the

DAS28 remission criteria during monitoring after MSC therapy. Severe adverse reactions were not observed in any patients, demonstrating that treatment with allogeneic MSCs is safe. However, the treatment showed a lack of therapeutic effect against RA.

A Chinese research team published large-scale research results evaluating the safety and efficacy of UC-derived MSCs (4×10^7 cells per time, intravenous route) with DMARDs and DMARDs only in 172 patients with active RA who had not responded to conventional treatment [70]. The group that received MSCs showed a significant decrease in the DAS28 score, with approximately 50% of the patients achieving remission and 30% continuing to show a low disease activity. When the treatment effect was evaluated using the ACR response criteria, the percentage of patients in the MSC group achieving ACR20, ACR50, and ACR70 responses was 38%, 18%, and 8%, respectively. By contrast, only 14% of the patients in the DMARDs only group achieved an ACR20 response. For a single dose, the treatment effect lasted 3∼6 months, and similar treatment effects could be induced again by repeated doses. Only 4% of the patients complained of mild fever and/or chills after MSC treatment, and there were no other severe adverse reactions. The results of this study show that treatment with allogeneic MSCs in combination with DMARDs can improve the therapeutic effect and that repeated doses can maintain remission longer than single doses.

Results from a multicenter, randomized, placebo-controlled, phase I/II clinical trial using allogeneic adipose tissue-derived MSCs in patients with refractory RA were recently reported [71]. Fifty-three patients with RA were divided into a placebo group and a stem cell therapy group; the latter was further divided into three subgroups, receiving three intravenous doses of 1×10^6 cells/kg (group A), 2×10^6 cells/kg (group B), or 4×10^6 cells/kg (group C) at 1-week intervals. The percentage of patients in groups A, B, and C and the placebo group showing an ACR20 response after 1 month was 45%, 20%, 33%, and 29%, respectively; that at 3 months was 25%, 15%, 17%, and 0%, respectively. However, disappointingly, the percentage of patients showing an ACR50 or ACR70 response in the stem cell therapy group was very low. Of the 46 patients who underwent stem cell therapy, as many as 38 patients (82%) experienced at least one adverse event, including three serious adverse events (i.e., lacunar infarction, peroneal nerve palsy, and fever). Major clinical trials of MSCs therapy in the patients with RA are summarized in Table 3.

Taken together, MSCs can be considered effective and relatively tolerable for RA treatment. However, therapeutic effects and adverse effects differ by the MSC type, dose, route of administration, dose frequency, and combination with DMARDs. Therefore, well-designed large-scale

Table 3. Major clinical trials of mesenchymal stem cell therapy in rheumatoid arthritis

No. of patients	Transplantation type	Cell source (n)	Total cell dose (n)	Follow-up duration (mo)	Response (n)	Ref.
3	Autologous	Adipose tissue	6×10^8 (1) 8×10^8 (2)	3∼13	Pain VAS ↓, KWOMAC ↓ (1) Walking improving (2) Off steroid (2)	[68]
4	Allogeneic	Bone marrow (1) Umbilical cord (3)	1×10^6/kg	24	ESR ↓, DAS28 ↓, Pain VAS ↓ (3) EULAR response but relapse (2) No EULAR response (2) No DAS28 remission (4)	[69]
136	Allogeneic	Umbilical cord	4×10^7 (112) 8×10^7 (24)	3∼8	DAS28 remission (68) DAS28 low-activity (40) ACR20 (53), ACR50 (31), ACR70 (12)	[70]
46	Allogeneic	Adipose tissue	3×10^6/kg (20) 6×10^6/kg (20) 12×10^6/kg (6)	6	ACR20 (9), ACR50 (5), ACR70 (2) EULAR response (6) DAS28 low-activity (6)	[71]

VAS: visual analog scale, ↓: decrease, KWOMAC: Korean Western Ontario and McMaster Universities arthritis index, ESR: erythrocyte sedimentation rate, DAS28: 28 joints disease activity score, EULAR: The European League Against Rheumatism, ACR: American College of Rheumatology, Ref.: reference.

studies are required to determine these parameters more clearly.

CONCLUSION

We examined ASC therapy for RA by analyzing HSCs and MSCs in two separate parts. Both cell types are effective against RA by resetting or suppressing autoimmunity. However, given the observations of relapse either a short or long time after treatment, the genetic predisposition to RA cannot be overlooked, and it seems that it will be difficult to eradicate autoimmune tendencies completely with stem cell therapy. Nevertheless, stem cells demonstrate several advantages over conventional treatment, so it is too early to exclude stem cells in treatment of RA. The limitations of stem cell therapy can be overcome by optimizing stem cell types and methods for RA treatment through various preclinical studies and clinical trials. In the near future, we expect that it will be possible to control or cure RA completely even without administering oral DMARDs or injecting biological agents, but using only stem cell transplantation.

ACKNOWLEDGMENTS

This study was supported by a grant from the Basic Science Research Program through the National Research Foundation of Korea (NRF) funded by the ministry of Education (NRF-2014R1A1A1004857).

CONFLICT OF INTEREST

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

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