Recent Advances in Cell Therapeutics for Systemic Autoimmune Diseases

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ABSTRACT

Systemic autoimmune diseases arise from loss of self-tolerance and immune homeostasis between effector and regulator functions. There are many therapeutic modalities for autoimmune diseases ranging from conventional disease-modifying anti-rheumatic drugs and immunosuppressants exerting nonspecific immune suppression to targeted agents including biologic agents and small molecule inhibitors aiming at specific cytokines and intracellular signal pathways. However, such current therapeutic strategies can rarely induce recovery of immune tolerance in autoimmune disease patients. To overcome limitations of conventional treatment modalities, novel approaches using specific cell populations with immune-regulatory properties have been attempted to attenuate autoimmunity. Recently progressed biotechnologies enable sufficient in vitro expansion and proper manipulation of such ‘tolerogenic’ cell populations to be considered for clinical application. We introduce 3 representative cell types with immunosuppressive features, including mesenchymal stromal cells, Tregs, and myeloid-derived suppressor cells. Their cellular definitions, characteristics, mechanisms of immune regulation, and recent data about preclinical and clinical studies in systemic autoimmune diseases are reviewed here. Challenges and limitations of each cell therapy are also addressed.

Keywords: Autoimmune disease; Cell therapy; Mesenchymal stromal cells; Regulatory T cells; Myeloid-derived suppressor cells

INTRODUCTION

Immune systems of human bodies are very complex and sophisticated. Various immune cells and their soluble factors present in immune tissues and organs respond to everyday foreign Ags breaking into hosts. While most of such immune cells can exert ‘effector’ functions to fight against external pathogens, others can act with regulatory properties to suppress excessive inflammation that can potentially result in unintended host damages. Autoimmune diseases can arise from breakage of such immune homeostasis between immune ‘effector’ and ‘regulator’ (1). Although the exact pathogenetic mechanisms of most autoimmune diseases have not been fully understood yet, it is believed that when hosts with genetic susceptibility are exposed to specific environmental conditions, they can acquire unintentional autoimmunity toward themselves (1). In some autoimmune diseases, specific
autoantibodies such as anti-citrullinated protein Abs in rheumatoid arthritis (RA) and anti-DNA Abs in systemic lupus erythematosus (SLE) have been found. However, questions of why autoimmunity toward certain self-proteins or tissues is generated in 'some' people and how these factors contribute to the development of a diseased state in some people but not in others are largely unsolved.

Despite such pathogenetic uncertainty, treatments for autoimmune diseases have shown great advances recently. At first, objectives of managing autoimmune diseases were mainly focused on reducing symptoms such as pain and fever. The discovery of glucocorticoid and disease-modifying anti-rheumatic drugs as well as other immunosuppressants then accelerated therapeutic approaches to attenuate inflammation due to autoimmunity beyond symptomatic care (2,3). Since the early 2000s, multiple biologic agents and small molecule inhibitors aiming at specific cytokines or intracellular signal pathways in certain conditions have introduced; for instance, TNF, IL-6, IL-17, IL-12/23, costimulatory signals or Janus kinase signal pathways are targeted in RA, ankylosing spondylitis, psoriasis, psoriatic arthritis, or inflammatory bowel diseases (4). Such targeted therapies can provide much improved clinical responses with less side effects than global immunosuppressive treatments. Despite such optimistic advances, there are still unmet needs in autoimmune disease treatment. For instance, some patients with autoimmune diseases remain unresponsive to all kinds of targeted therapies available as well as conventional immunosuppressants (5). Furthermore, even if they reach ‘clinical remission’ under such treatments, a genuine ‘immunological remission’ and a treatment-free state could not be guaranteed because their underlying immune intolerance toward self-Ags could not be controlled by these treatments. In fact, after clinical remission is achieved by anti-cytokine treatment, tapering of these agents can lead to recurrence of the disease in many cases (6). More importantly, other than certain diseases such as RA, specific therapies with proven effectiveness for several systemic autoimmune diseases including SLE and systemic sclerosis (SSc) are currently unavailable (7).

Considering limitations of current therapeutic modalities, alternative approaches have been attempted to treat autoimmune diseases by regaining immune tolerance. Around late 1990s, several cell populations were reported to be able to confer immunosuppressive activities (8). In 2010s, the progress of techniques enabling in vitro generation and expansion of specific cells provided investigators with opportunities to apply immune-regulatory cell therapies in autoimmune disease treatments (8). Theoretically, ‘tolerogenic’ cell therapies can provide immunological re-establishment from autoimmunity toward immune tolerance in affected patients. Such therapeutic attempts are more ideal than currently available treatments that independently target separate cytokines or pathways related to the disease pathogenesis.

In this review, we summarized definitions and mechanisms of cell therapies using 3 representative cell types (mesenchymal stromal cells [MSCs], Tregs, and myeloid-derived suppressor cells [MDSCs]) with immune-regulatory activities. Although some cell therapies reviewed here have been largely investigated in other medical conditions such as organ transplantation and specific organ-targeted autoimmune diseases including multiple sclerosis (MS) and type I diabetes mellitus (DM), we mainly focused on results of recent preclinical and clinical studies regarding systemic autoimmune diseases, especially those in the rheumatologic field, such as RA, SLE, SSc, and Sjogren’s syndrome (SjS).
MSCs

Definition of MSCs
MSCs are multipotent progenitor cells firstly known to be capable of differentiating into diverse stromal cells such as osteocytes, chondrocytes, and adipocytes present in most mesenchymal tissues (9). Later, these cells are proven to play various immunomodulatory roles by interacting with not only innate immune cells, but also adaptive immune cells (10). After their first discovery by Friedenstein in 1970s (11), MSCs were defined by their ability to adhere to plastic surfaces under specific culture conditions. Their typical surface phenotypes are positive for CD73, CD90, and CD105 but negative for CD11b, CD14, CD19, CD34, and CD45 according to the International Society for Cell & Gene Therapy in 2006 (9). Although MSCs were noticed for use in tissue repair based on their regenerative potency at first, their anti-inflammatory properties have attracted more attention in the field of systemic autoimmune diseases.

Immunomodulatory mechanisms of MSCs
According to previous studies, MSCs can acquire enhanced immune suppressive properties under specific conditions such as exposure to pro-inflammatory signals including IL-1β, TNF-α, and IFN-γ (12). This process is called as ‘MSCs licensing’ (Fig. 1). Because ‘licensed’ MSCs can exert more potent immunomodulatory activities, current methods for adoptive transfer of MSCs in each inflammatory disease need ‘licensing’ for in vitro expansion (12). MSCs exert their immunomodulatory functions through 2 different pathways (Fig. 1): i) secretion of various soluble factors; and ii) direct cell-to-cell interaction. Various mediators such as TGF-β, inducible nitric oxide (NO) synthase, prostaglandin E2, and indoleamine-2,3-dioxygenase (IDO) have been suggested as potential secretory factors for immune-regulatory properties of MSCs (13). These factors can modulate functions of effector immune cells such as macrophages, neutrophils, and T cells. Previous studies have also reported that cell-to-cell contact via various surface proteins such as vascular cell adhesion molecule 1 and intercellular adhesion molecule 1 is another mechanism of immune-regulatory functions of MSCs (14,15). Interactions of these adhesion proteins can suppress neutrophils and effector T cells. Recently, some studies have suggested that MSC-derived extracellular vesicles including exosomes and microvesicles are the third mechanism involved in the immunomodulation of MSCs (16). These vesicles contain immunosuppressive proteins. And they can transfer their contents by membrane fusion and intracellular endocytic system of targeted cells. Through such pathways, MSCs can induce differentiation of naïve T cells into regulatory phenotypes and inhibit proliferation and differentiation of effector T cell and B cell, consequently exerting their immune-regulatory functions (17,18).

Types of MSCs
Because MSCs express no MHC class II or costimulatory molecules on their cellular surfaces, they exert low immunogenicity. Therefore, they can be relatively freely chosen as therapeutic modalities between autologous and allogenic transplantation (19,20). Because MSCs were firstly isolated from bone marrow (BM), BM-MSCs are the most well-established types of MSCs. However, many other tissues and organs can also be sources for MSCs (21). Umbilical cord (UC) blood or tissues and adipose tissues (AD) are also frequently adopted and well-characterized sites for harvesting MSCs (22,23). With increased accessibility and distinct characteristics, alternative sites such as nasal turbinate are also potential sources for MSCs (24). While MSCs share some common functional features among different cellular sources, pluripotent capacities and immunomodulatory properties could vary depending on culture conditions (25).

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Preclinical and clinical studies using MSCs: collagen-induced arthritis (CIA) and RA

Numerous attempts have been made to use MSCs to treat systemic autoimmune diseases in various preclinical and clinical studies. The first preclinical study using MSCs in a CIA murine model showed negative results with increased inflammatory features under TNF-α stimulation (26). However, 2 later studies using the same animal model demonstrated improvement in arthritis severities by adoptive transfer of allogenic murine BM-MSCs and human AD-MSCs, respectively (27,28). In these 2 studies, reduction of Ag-specific effector T cell population and induction of Tregs by MSC treatments were observed. Based on positive results from preclinical studies using allogenic or xenogenic MSCs, several clinical trials involving human RA patients have been performed. Intravenous injection of allogenic UC-MSCs presented some clinical efficacies by improving Disease Activity Score values combined with serological reduction of inflammatory cytokines such as TNF-α and IL-6 in 2 separate trials (29,30). Both trials showed no significant safety issues after single or multiple injections with therapeutic effects persisting for at least 3 to 6 months. Another multi-centered trial using allogenic AD-MSCs also showed clinical improvement (31). However, one case of high-grade adverse event was observed (31). The most recently published clinical trial for RA applying autologous BM-MSCs as a therapeutic modality demonstrated that adoptive transfer of patient-originated BM-MSCs could also induce immune-regulatory phenotypes with increased soluble factors such as serum levels of IL-10 and TGF-β (32). Multiple early-phase clinical trials using other sources of MSCs and more advanced stages of clinical trials using BM or UC-MSCs are currently ongoing worldwide.

Preclinical and clinical studies using MSCs: SLE

Studies performed in animal models for SLE also showed efficacy of MSC treatments. Transplantation of human BM-MSCs acquired from healthy donors reduced serum levels of anti-double stranded DNA Ab and proteinuria in MRL/lpr mice, one of lupus-prone murine models (33). Similar results were replicated in other studies using different animal models for SLE such as NZB/NZW F1 mice or different sources of MSCs including AD- and UC-MSCs (34,35). Considering these optimistic results from preclinical studies of SLE, several pilot
studies using both allogenic UC and BM-derived MSCs have been performed in refractory SLE patients (36-38). Most studies since initial clinical trials demonstrated good safety profiles and improvement of clinical parameters including serological markers and SLE disease activity index scores (39-41). However, because several events of clinical relapse or unresponsiveness have been observed in follow ups (42,43), data from additional clinical studies which are newly ongoing or extended should be carefully analyzed in the future.

**Preclinical and clinical studies using MSCs: SSc and SjS**

In SSc and SjS, attempts of MSCs application were more preliminary than in RA and SLE. Although some preclinical studies using animal models for SSc such as chemicals-induced mice have been performed, data acquired from these trials are rather inconclusive because animal models fully recapitulating all important clinical features of SSc including autoimmunity, fibrosis, and microangiopathies have not been introduced yet (44).

Nevertheless, several case reports using MSCs in SSc patients have been published. Although 2 cases of autologous BM-MSC treatment in refractory SSc patients have been reported, outcomes were unsatisfactory (45,46). Allogenic transplantation using BM-MSC in severe SSc patients showed some positive results with improvement of skin fibrosis and perfusion without major adverse events (47,48). Based on these results, very early-phase clinical trials for SSc using allogenic BM or UC-MSCs are on-going. For SjS, experiments using animal models such as non-obese diabetes (NOD) mice mimicking clinical phenotypes of SjS have been performed to investigate effects of MSC on sicca syndrome. The results of such experiments applying MSCs in SjS animal models have been systematically reviewed elsewhere (49). According to this systematic review, preclinical studies of MSC treatments for SjS demonstrated increase of salivary flow, decrease of serum autoantibody levels, and decrease of inflammation in salivary glands of affected mice (49). In one preliminary clinical study, intravenous injection of allogenic UC-MSC resulted in improvements of SjS Disease Activity Index scores, salivary flow rates and autoantibody profiles of SjS patients (50).

**Challenges of MSC application in autoimmune diseases**

Although there have been many attempts of preclinical and clinical studies for autoimmune diseases as introduced above, several limitations make clinical application of MSCs treatment challenging. One of the most important issues is the heterogeneous characteristic of MSC populations themselves. Therapeutic potentials of MSCs are significantly dependent on medical state of donors and MSC isolation and culture protocols (51). Therefore, well-organized and optimized methods to acquire and prepare homogeneous MSC products should be established to ensure consistent and solid therapeutic effects of MSCs.

Furthermore, considering some reports suggesting decreased therapeutic abilities of MSCs acquired from compromised donors even under ‘MSC-licensing’ conditions, allogenic transplantation is regarded to be more suitable for treating autoimmune diseases (52). However, recent studies raised concerns about ‘graft rejection’ after repeated injections of allogenic MSCs in the same target (53). Such ‘rejection’ issues might limit persistent therapeutic effects of multiple transplantations of allogenic MSCs.

**TREGs**

**Definitions of Tregs**

Autoimmune diseases arise from loss of self-tolerance. To prevent occurrence of self-reactive immune cells, there are multiple cellular populations with immunosuppressive
activities. Among them, Tregs are one of the most important cell populations that can maintain immunological homeostasis and tolerance by inhibiting effector immune cells and suppressing excessive inflammation (54). Many studies have demonstrated that decreased numbers and dysregulated functions of Tregs are associated with various systemic autoimmune diseases including RA and SLE (55). Considering the critical roles of Treg in the development of such conditions, therapeutic approaches that can restore immunosuppressive functions of Tregs are being investigated.

Although novel types of regulatory cells such as type 1 regulatory T (Tr1) cells and Th3 cells have been introduced recently, 'classical' Tregs refer to CD4^+CD25^+CD127^low T cells expressing intracellular transcription factor Foxp3 (55). About 5% to 10% proportions of peripheral CD4^+ T cells exist as Tregs constitutively in healthy humans and mice (56). These cells can be classified into 2 different subsets according to their sites (thymus and peripheral tissues) of development. Therefore, they are named as thymic Tregs and peripheral Tregs, respectively (55,57). Thymic Tregs tend to remain as suppressive cells with stable numbers whereas peripheral Tregs are induced from peripheral CD4^+ T cells under specific conditions by promoting Foxp3 expression. In addition, such peripheral Tregs can convert to effector T cells upon inflammatory stimulations such as IFN-γ and IL-17 (56).

Immunosuppressive mechanisms of Tregs

Tregs exert their immunosuppressive activities by both 'Ag-specific' and 'Ag-non-specific' pathways (Fig. 2). The 'Ag-specific' pathway is more dominant mechanism through TCR contacts with corresponding Ags expressed by other Ag-presenting cells (54). In such 'Ag-specific' mechanisms, Tregs can secret anti-inflammatory cytokines such as IL-10, IL-35, and TGF-β to suppress nearby effector cells (54,56). Tregs can also induce differentiation of 'tolerogenic' dendritic cells (DCs) from pro-inflammatory phenotypes by expressing CTLA-
4, consequently resulting in down-regulation of co-stimulatory molecules on DCs \( (8,56) \). Such ' tolerogenic' DCs can express IDO, an enzyme degrading tryptophan, and promote further expansion of Tregs \( (56) \). In addition, Tregs can directly induce apoptosis of effector cells by producing cell-lysis enzymes such as granzymes and perforins \( (58) \). Meanwhile, the 'Ag-non-specific' mechanism also exists. Its suppressive methods include ‘bystander immunosuppression’, in which Tregs can induce immunosuppression of immune cells close to them without direct ‘Ag-specific’ contacts \( (54,58,59) \). Ag-non-specific mechanisms enable application of polyclonally expanded Tregs without autoantigen specificity for treating autoimmune diseases.

Therapeutic approaches using Tregs include adoptive transfer after \textit{in vitro} expansion of naturally induced or artificially engineered Tregs, and \textit{in vivo} induction of Tregs by exogenous stimulations such as IL-2, a critical cytokine for maintenance and survival of Tregs. In this review, we will focus on the \textit{in vitro} expansion of naturally induced or artificially engineered Tregs.

**Polyclonal Tregs**

\textit{In vitro} expansion and administration of autologous Tregs have been attempted to suppress unintended inflammation by increasing numbers and functions of Tregs. \textit{In vitro} expansion of Tregs can be achieved by IL-2 stimulation combined with anti-CD3 and anti-CD28 Abs \( (60) \). Such expanded cells show broad spectra of TCR repertoire which can be naturally acquired. Therefore, they are called polyclonal Tregs. Administration of polyclonal Tregs in human patients has demonstrated safety and clinical improvement in chronic inflammatory conditions such as MS, type 1 DM, and organ transplantation \( (61-63) \). A phase I clinical trial for 12 patients with type 1 DM has reported that single or multiple infusions of autologous polyclonal Tregs are safe with ability to induce clinical remission in some patients \( (61) \). MS is an autoimmune disease mainly affecting the central nervous systems due to infiltration of autoimmun effector T cells. Autologous Tregs expanded \textit{ex vivo} can be administered both intravenously and intrathecally into patients with MS \( (62) \). Both routes of Treg injection have been found to be safe, with intrathecal injections showing higher efficacies than intravenous injections \( (62) \). Organ transplantation is also a field targeted by Treg-related therapy because graft failure as one of the most critical issues in this area is closely related with anti-graft T cell activities. Mechanisms of graft failure resemble those of autoimmune diseases. Enhanced Treg function or increased numbers of Treg can reduce risks of rejection, ultimately reaching discontinuation of immunosuppressive agents, which are potentially harmful after a long period of usages. In one study, Tregs acquired from patients themselves expanded and infused after liver transplantation \( (63) \). Both Treg-infused and liver-transplanted patients showed no serious safety issues with decreased anti-graft responses \( (63) \). Despite some positive results and ongoing clinical trials using polyclonal Tregs, several concerns were also raised by investigators. Because polyclonally expanded Tregs do not express specific TCRs for specific Ags in certain diseases, their immunosuppressive mechanisms mainly depend on an ‘Ag-non-specific’ manner known as ‘bystander immunosuppression’ \( (64) \). Therefore, extensive amounts of expansion and activation are needed before adoptive transfer to acquire sufficient clinical responses. Infusion of Tregs expanded by such non-specific and intense ways can raise potential risks for other detrimental complications such as malignancy and infection originated from unintended excessive immunosuppression of hosts.

**Ag-specific Tregs**

To overcome obstacles for adoptive transfer of polyclonal Tregs, alternative approaches have been attempted using ‘Ag-specific’ Tregs. Autoantigen-specific Tregs have been proven to
be more effective than polyclonal Tregs lacking Ag-specificity in various preclinical models of inflammatory diseases (65, 66). For instance, in a diabetic murine model, administration of Tregs specific for autoantigens in pancreas showed much higher efficacies than that of polyclonal Tregs (65). Therefore, such ‘Ag-specific’ Tregs are more widely investigated than ‘Ag-non-specific’ polyclonal Tregs nowadays. Generation of Ag-specific Tregs can be achieved by 2 separate methods (60): i) inserting Foxp3 into effector T cells which express autoantigen-specific TCRs using retroviral vectors or CRISPR/Cas9 system and then converting them into Tregs; ii) engineering Tregs to express chimeric Ag receptor (CAR) targeting specific autoantigens. Both methods have been more investigated for inflammatory diseases such as type 1 DM, MS, inflammatory bowel diseases, and graft-versus-host disease, than for rheumatologic conditions as with polyclonal Tregs (60). The first therapeutic attempt using TCR-engineered Tregs in humans was reported in Crohn’s disease by targeting ovalbumin, one of Ags known to activate Tregs in inflammatory bowel diseases (67). In that study, infusion of Tregs expressing TCR specific for ovalbumin was well tolerated. It showed clinical improvements measured by Crohn’s Disease Activity Index (67). Studies using CAR-engineered Tregs in autoimmune diseases were mostly in preclinical stages. There have been animal model studies for type I DM, MS, and ulcerative colitis by targeting insulin, myelin oligodendrocyte glycoprotein, and carcinoembryonic Ag, respectively, using CAR systems (68). All these preclinical studies demonstrated positive results (68). Although limited by numbers, several reports have shown successful transfer of Ag-specific Tregs in mice recapitulating RA, suggesting their therapeutic potentials in systemic autoimmune diseases of rheumatologic fields as in other diseases (69, 70). Beyond CAR-Treg therapies, one recent report has demonstrated that infusion of CAR-T cells targeting CD19-expressing cells after preconditioning for lymphodepletion can induce dramatic remission in a refractory SLE patient (71). This result suggests that the CAR system has potential to deplete specific autoreactive cell populations selectively in autoimmune diseases as it does in cancers.

Pros and cons of Ag-specific Treg therapies
Although Ag-specific Treg therapies are considered to be more appropriate and ideal for treating autoimmune diseases than polyclonal Treg therapies, both approaches including TCR-engineering and CAR-engineering have some issues to be addressed. TCR-engineered Tregs need MHC compatibility to detect autoantigens and be functioning. Meanwhile, although CAR-engineered Tregs do not need MHC compatibility, they require more than 100 targeted Ags to be activated. On the other hand, TCR only needs one matched peptide (72). Most importantly, identification of specific epitopes pathologically crucial in each autoimmune disease, potentially targeted by Tregs is an essential demand to generate both types of Ag-specific Tregs. Therefore, extensive investigations clarifying key autoantigens in pathogenesis of each autoimmune disease should be performed. In addition, the most appropriate structure of each engineering system and optimized generation protocols should be organized before their wide applications in the future.

MDSCs

Definitions and subgroups of MDSCs
MDSCs are a mixture of various immune cells derived from BMs with immune-suppressive functions (73). Since the first discovery in 1990s, MDSCs have been reported to be able to expand under specific conditions including cancer and various inflammatory diseases. Such chronic medical conditions compel myeloid progenitor cells to be persistently stimulated
by inflammatory signals. Soluble factors with pro-inflammatory properties, such as G-CSF, M-CSF, GM-CSF, IL-1β, and IL-6, can induce the generation and recruitment of MDSCs (73). Considering their potent immune-suppressive roles in in vitro studies, therapeutic approaches targeting MDSCs were firstly attempted in cancer treatment (74). Such attempts lead to later experimental application of MDSCs to prevent graft rejection in transplantation and treat various autoimmune diseases (74).

Based on their myeloid progenitors and morphological features in both human and mouse, MDSCs can be classified into 2 distinct groups: monocytic MDSCs (M-MDSCs) and granulocytic MDSCs (G-MDSCs) (73). In addition to these 2 major groups, a recent study has discovered early MDSCs lacking surface markers of mature immune cells in human without corresponding subgroups in mouse (75). Both major groups of MDSCs express CD11b as surface molecules. However, they can be separated by several phenotypical markers. Human M-MDSCs are known to express HLA-DR and CD14 whereas human G-MDSCs are characterized by expression of CD15 and CD66b (75). More recently, lectin-type oxidized LDL receptor 1 and S100A9 have been suggested as novel phenotype markers for human G-MDSCs and M-MDSCs, distinguishing them from classical neutrophils and monocytes, respectively (76,77). Similar to human MDSCs, all murine MDSCs express CD11b while expression of Ly6C and Ly6G (subunits of Gr1) is used to determine subtypes of MDSCs. Murine M-MDSCs express Ly6G<sup>-</sup>Ly6C<sup>high</sup> whereas G-MDSCs express Ly6G‘Ly6C<sup>low</sup> (78).

**Immuno-regulatory mechanisms of MDSCs**

MDSCs can exert potent immunosuppressive capacities by mainly targeting T cells through various cellular mechanisms (Fig. 3). First, MDSCs can produce ROS and NO, resulting in decreased TCR expression and increased TCR nitration, respectively, as well as increased

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**Figure 3.** Schematic immunosuppressive mechanisms of MDSCs. 1) MDSCs produce ROS and NO, and increase expression of ARG-1, which can exert immunosuppressive activities. 2) MDSCs deplete amino acids such as cysteine and L-arginine, essential for proliferation and activation of T cells. 3) MDSCs suppress expression of adhesion molecules (e.g., L-selectin) of effector cells. 4) MDSCs express CD39 and CD73, which can generate adenosine from extracellular ATP. 5) MDSCs express inhibitory molecules such as PD-L1, which is interacting with its corresponding ligand, PD-1 on effector T cells. All these mechanisms contribute to immunosuppressive functions of MDSCs. ARG-1, arginase-1.
expression of other immunosuppressive markers such as arginase-1 (79,80). Suppressive effects of ROS and NO on T cells are well-established in previous reports (81,82). Second, MDSCs can consume and deplete amino acids essential for T cell proliferation and activation. Cysteine and L-arginine are crucial amino acids for T cell functions. These molecules can be competitively transported into intracellular spaces of MDSCs or degraded by enzymes such as arginase-1 (83,84). Such effects of MDSCs can limit the availability of essential metabolites for T cell activation and proliferation. Third, MDSCs can inhibit homing of naïve T cells into targeted tissues by reducing adhesion molecules including L-selectin on cellular surfaces of immune cells through expression of L-selection-shedding enzymes such as metalloproteinase 17 (85). Fourth, MDSCs express CD39 and CD73 that can generate adenosine from extracellular ATP (86). Increased extracellular adenosines can interact with adenosine receptors on cellular membranes, leading to downregulation of intracellular pathways activating naïve T cells (86). Lastly, immune regulatory molecules such as PD-L1 are also presented by MDSCs. These molecules can interact with a corresponding protein such as PD-1 on T cells, consequently inducing apoptosis of T cells (87). In addition to direct inhibitory effects on effector T cells, MDSCs can also induce other immune cells with regulatory properties including M2 macrophages and Tregs by secreting cytokines such as IL-10 (88).

**Preclinical studies using MDSCs in autoimmune diseases**

Despite therapeutic potentials of MDSCs with various immune-regulatory effects on effector immune cells as described above, experimental studies investigating roles of MDSCs in autoimmune diseases have reported contradictory results. Th17 cells and IL-17 are important pro-inflammatory mediators to induce inflammatory arthritis in both human and mouse (89). Some studies using the CIA mouse model have demonstrated expanded MDSCs and increased Th17 cell populations with positive correlations (90). Depletion of MDSCs in this CIA mouse model decreased Th17 cells and severities of arthritis, suggesting pathologic roles of MDSCs in pre-clinical arthritis models (90). In contrast, other studies showed therapeutic effects in the same murine model (91). Although MDSCs are increased in CIA mice than in controls, infusion of expanded autologous MDSCs reduced Th17 cells and Th1 cells but increased Tregs, consequently attenuating inflammatory phenotypes (91). In that study, MDSCs lost their immune-regulatory functions under anti-IL-10 treatment or in IL-10 knock-out mice, suggesting that IL-10 could be one of anti-inflammatory mechanisms of MDSCs (91).

Similar to findings in the CIA mouse model, previous reports investigating functions of MDSCs in other systemic autoimmune diseases showed different results. One study reported that SLE patients presented more increased MDSC populations than healthy controls (92). The number of MDSCs is positively correlated with Th17 cell and arginase-1 activities and disease severities in that study (92). *In vitro* experiments using MDSCs acquired from SLE patients showed that MDSCs increased Th17 differentiation depending on arginase-1 production, suggesting pathologic roles of MDSCs via arginase-1 in SLE (92). Interestingly, because the previously introduced study using CIA mice has suggested that arginase-1 is one of mediators resulting in immunosuppressive functions of MDSCs (91), the role of arginases-1 might be crucial in both pro-inflammatory and anti-inflammatory functions of MDSCs. NOD mice resemble phenotypes of SJ S, which is a chronic inflammatory disease mainly affecting exocrine glands with autoimmunity similar to SLE (93). In this murine model, injection of autologous MDSCs resulted in aggravation of inflammation, suggesting pathologic functions of MDSCs in SJ S (94). Despite such results suggest detrimental roles of MDSCs in SLE and SJ S, other reports have shown therapeutic potentials of MDSCs. In lupus-prone mice, infusion of MDSCs improved lupus-like phenotypes with reduced serum
autoantibody levels and proteinuria (95). In addition to clinical symptoms of SLE, MDSCs also resulted in immune cell populations skewing to immune-regulatory phenotypes with increased regulatory B cells while decreasing Th17 cells and follicular helper T cells related to germinal center formation (95). Another study using lupus mice models has reported that the expression of PD-L1 in MDSCs is related to immune-regulatory potency of MDSCs (96). Immunosuppressive factors including PD-L1 are remarkably decreased in MDSCs acquired from MRL/lpr mice than in those from control mice, suggesting MDSCs in diseased hosts can be dysfunctional (96). Rather, PD-L1-positive MDSCs exert more potent immunosuppressive activities than PD-L1-negative MDSCs in lupus-prone murine models (96). Such approaches characterizing specific subsets of MDSCs with immune-regulatory properties might suggest some clues to mixed results of preclinical studies on autoimmune diseases.

**Challenges of MDSC application in autoimmune diseases**

Despite immune-regulatory effects of MDSCs proven in various experiments, clinical trials using MDSCs to treat autoimmune diseases have not been reported yet. This is because some important issues remain inconclusive before progressing to next steps. Discrepant results using MDSCs in preclinical studies mostly arise from heterogeneous methods in generating MDSCs. All variables such as sources (e.g., allogenic or autologous), timing of acquisition (e.g., disease state of hosts during autologous transfer), MDSC subtypes (e.g., G-MDSC or M-MDSC), and methods of inducing MDSC can influence and determine cellular characteristics of MDSCs. Therefore, optimal protocols for generating MDSCs to be transferred in autoimmune diseases should be organized first. In addition, characterization of specific subsets exerting the most potent immunosuppressive properties should also be performed while considering heterogeneous populations of overall MDSCs. Only consistent results acquired from preclinical studies using specified MDSCs manufactured under standard protocols can guarantee positive outcomes and safety in clinical trials of using MDSCs to treat autoimmune diseases.

**CONCLUSION**

As pathogenetic mechanisms of autoimmune diseases are getting unveiled, multifactorial approaches are being attempted to conquer pathologic conditions and reclaim immune tolerance in affected patients. Cell therapeutics using immune-regulatory cell populations are promising modalities that can contribute to the achievement of ultimate goals pursued by all investigators in immunologic and rheumatologic fields. We can easily expect that currently ongoing aggressive development of biotechnologies for generating and manipulating *in vitro* expanded cell therapeutics will accelerate clinical applications of these agents in wide spectrums of autoimmune diseases. Internationally consent protocols of each cell therapy about optimal manufacturing methods and proper regimens including sources, doses, and intervals will provide consistent efficacies and safeties in clinical trials in the future. There are still many challenges to overcome. However, huge efforts that are ongoing worldwide will lead to better positions of current cell therapies for treating autoimmune diseases.

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