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Review Article

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Recent Advances in Cell Therapeutics for Systemic Autoimmune Diseases

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Conflict of Interest
The authors declare no potential conflicts of interest.

Abbreviations
AD, adipose tissue; BM, bone marrow; CAR, chimeric Ag receptor; CIA, collagen-induced arthritis; DC, dendritic cells; DM, diabetes mellitus; G-MDSC, granulocytic myeloid-derived suppressor cell; IDO, indoleamine-2,3-dioxygenase; MDSC, myeloid-derived

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

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suppressor cell; M-MDSC, monocytic myeloid-derived suppressor cell; MS, multiple sclerosis; MSC, mesenchymal stromal cell; NO, nitric oxide; NOD, non-obese diabetes; RA, rheumatoid arthritis; SjS, Sjogren's syndrome; SLE, systemic lupus erythematosus; SSc, systemic sclerosis; UC, umbilical cord.

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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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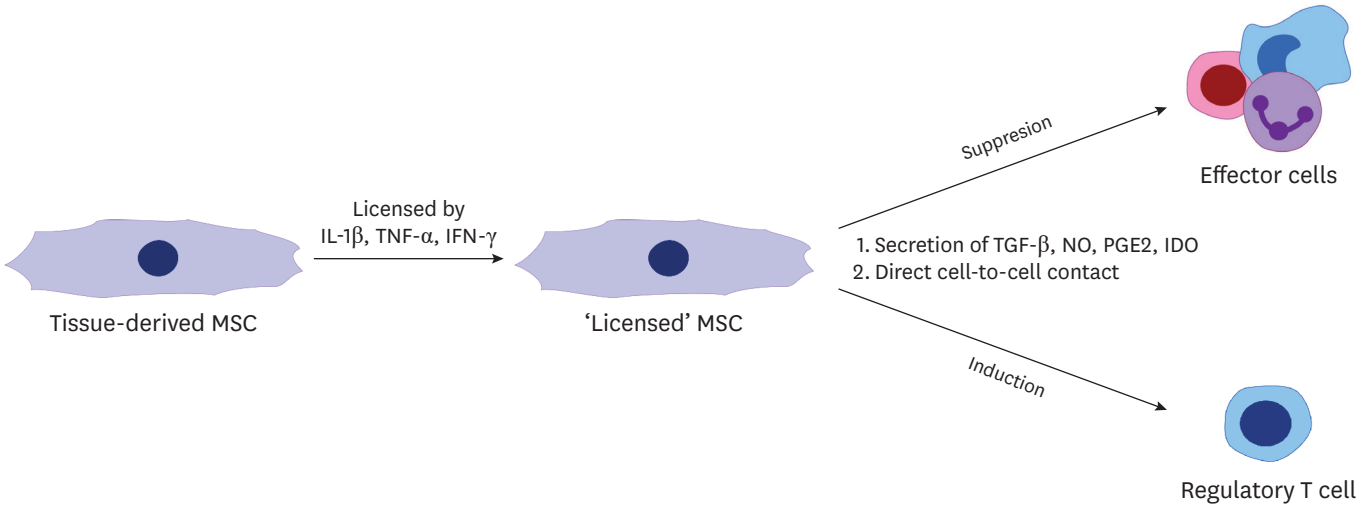

Figure 1. Schematic immunosuppressive mechanisms of MDSCs. Tissue-derived MDSCs (e.g., from bone marrows, umbilical cords, and adipose tissues) are licensed by inflammatory stimulation such as IL-1β, TNF-α, and IFN-γ to exert more potent immune-regulatory properties. MDSCs can suppress effector immune cells through secretion of TGF-β, NO, prostaglandin E2, and IDO, and direct cell-to-cell contacts. Furthermore, they also induce differentiation of Tregs.

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

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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

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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-

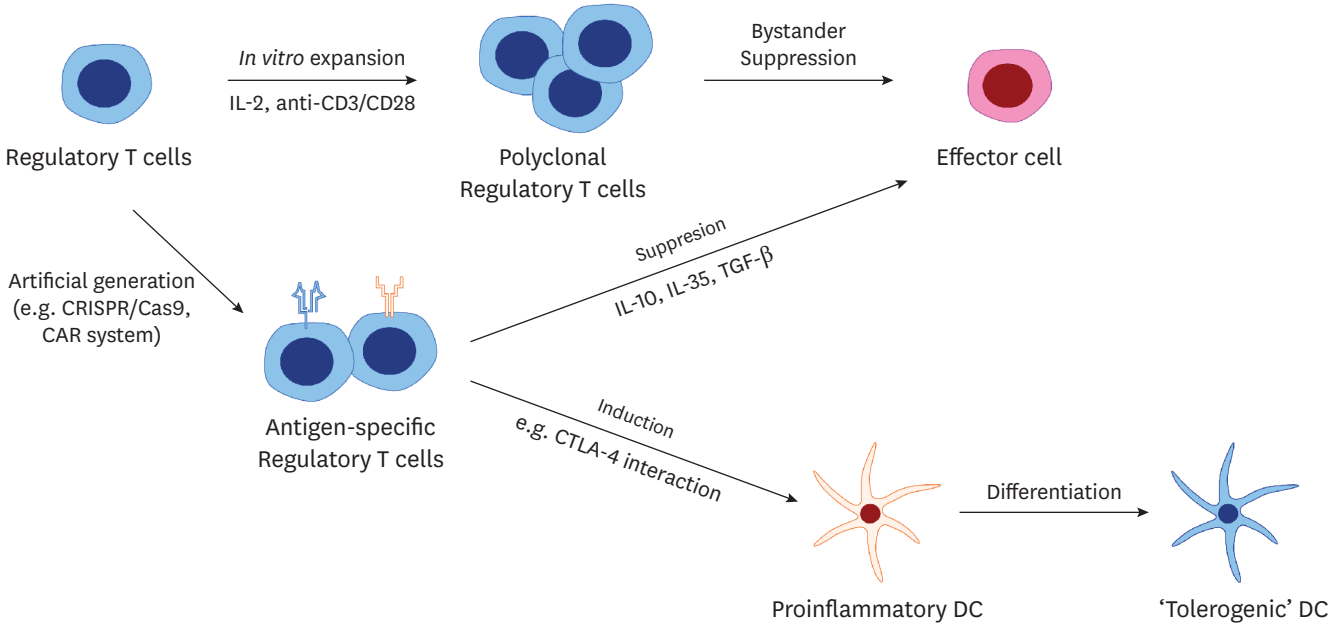

Figure 2. Types of Treg therapies and their immunosuppressive mechanisms. Two types of Tregs are generated by *in vitro* expansion. Polyclonal Tregs can be made under IL-2 and anti-CD3/CD28 Ab stimulation. Polyclonal Tregs exert diverse TCR repertoire and suppress effector T cells by the 'Ag-nonspecific' pathway called 'bystander immunosuppression'. Ag-specific Tregs are made by representative 2 methods: the CRISPR/Cas9 system and the CAR system. These Tregs secret IL-10, IL-35, and TGF-β to suppress effector T cells, and promote differentiation of 'tolerogenic' DC through expression of CTLA-4.

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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 *in vitro* expansion of naturally induced or artificially engineered Tregs, and *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 *in vitro* expansion of naturally induced or artificially engineered Tregs.

Polyclonal Tregs

In vitro expansion and administration of autologous Tregs have been attempted to suppress unintended inflammation by increasing numbers and functions of Tregs. *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 I 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 autoimmune effector T cells. Autologous Tregs expanded *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 $Ly6G^{-}Ly6C^{high}$ whereas G-MDSCs express $Ly6G^{+}Ly6C^{low}$ (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

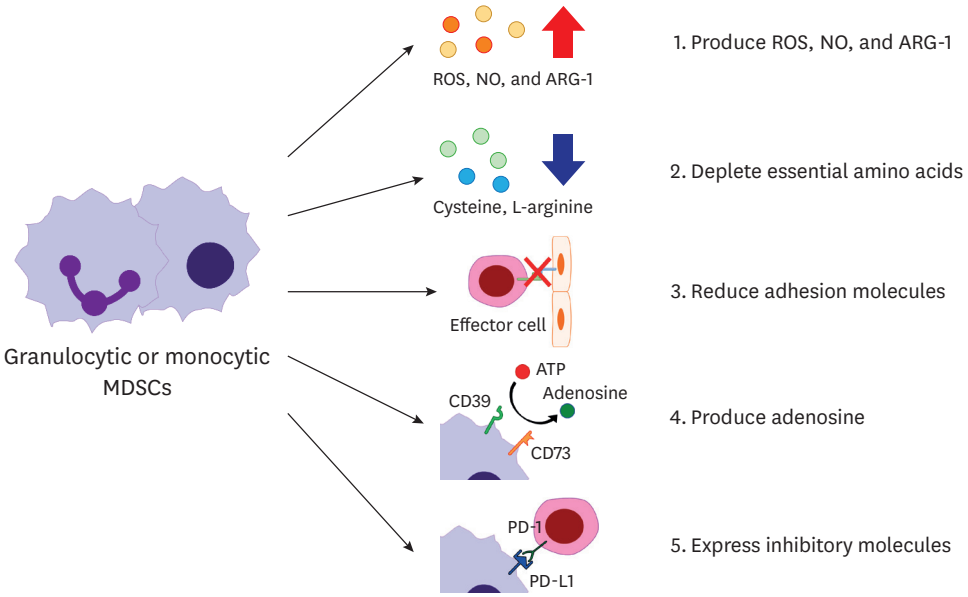

Figure 3. Schematic immunosuppressive mechanisms of MDSCs. 1) MDSCs produce ROS and NO, and increase expression of ARG-1, which can exert immunosuppressive activities. 2) MDSCs deplete amino acids such as cysteine and L-arginine, essential for proliferation and activation of T cells. 3) MDSCs suppress 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.

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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 naive 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 MDSCs 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 SjS, 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 SjS (94). Despite such results suggest detrimental roles of MDSCs in SLE and SjS, 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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REFERENCES

1. Marrack P, Kappler J, Kotzin BL. Autoimmune disease: why and where it occurs. *Nat Med* 2001;7:899-905.
PUBMED | CROSSREF
2. Cronstein BN, Aune TM. Methotrexate and its mechanisms of action in inflammatory arthritis. *Nat Rev Rheumatol* 2020;16:145-154.
PUBMED | CROSSREF
3. Hardy RS, Raza K, Cooper MS. Therapeutic glucocorticoids: mechanisms of actions in rheumatic diseases. *Nat Rev Rheumatol* 2020;16:133-144.
PUBMED | CROSSREF
4. Nikiphorou E, Buch MH, Hyrich KL. Biologics registers in RA: methodological aspects, current role and future applications. *Nat Rev Rheumatol* 2017;13:503-510.
PUBMED | CROSSREF
5. Rendas-Baum R, Wallenstein GV, Koncz T, Kosinski M, Yang M, Bradley J, Zwillich SH. Evaluating the efficacy of sequential biologic therapies for rheumatoid arthritis patients with an inadequate response to tumor necrosis factor-α inhibitors. *Arthritis Res Ther* 2011;13:R25.
PUBMED | CROSSREF
6. Kuijper TM, Lamers-Karnebeek FB, Jacobs JW, Hazes JM, Luime JJ. Flare rate in patients with rheumatoid arthritis in low disease activity or remission when tapering or stopping synthetic or biologic DMARD: a systematic review. *J Rheumatol* 2015;42:2012-2022.
PUBMED | CROSSREF
7. Murphy G, Isenberg DA. New therapies for systemic lupus erythematosus - past imperfect, future tense. *Nat Rev Rheumatol* 2019;15:403-412.
PUBMED | CROSSREF
8. Mosanya CH, Isaacs JD. Tolerising cellular therapies: what is their promise for autoimmune disease? *Ann Rheum Dis* 2019;78:297-310.
PUBMED | CROSSREF
9. Dominici M, Le Blanc K, Mueller I, Slaper-Cortenbach I, Marini F, Krause D, Deans R, Keating A, Prockop D, Horwitz E. Minimal criteria for defining multipotent mesenchymal stromal cells. The International Society for Cellular Therapy position statement. *Cytotherapy* 2006;8:315-317.
PUBMED | CROSSREF
10. Wang Y, Chen X, Cao W, Shi Y. Plasticity of mesenchymal stem cells in immunomodulation: pathological and therapeutic implications. *Nat Immunol* 2014;15:1009-1016.
PUBMED | CROSSREF
11. Friedenstein AJ, Gorskaja JF, Kulagina NN. Fibroblast precursors in normal and irradiated mouse hematopoietic organs. *Exp Hematol* 1976;4:267-274.
PUBMED
12. El-Jawhari JJ, El-Sherbiny Y, McGonagle D, Jones E. Multipotent mesenchymal stromal cells in rheumatoid arthritis and systemic lupus erythematosus; from a leading role in pathogenesis to potential therapeutic saviors? *Front Immunol* 2021;12:643170.
PUBMED | CROSSREF
13. Kyurkchiev D, Bochev I, Ivanova-Todorova E, Mourdjeva M, Oreshkova T, Belemezova K, Kyurkchiev S. Secretion of immunoregulatory cytokines by mesenchymal stem cells. *World J Stem Cells* 2014;6:552-570.
PUBMED | CROSSREF
14. Jiang D, Muschhammer J, Qi Y, Kügler A, de Vries JC, Saffarzadeh M, Sindrilaru A, Beken SV, Wlaschek M, Kluth MA, et al. Suppression of neutrophil-mediated tissue damage-a novel skill of mesenchymal stem cells. *Stem Cells* 2016;34:2393-2406.
PUBMED | CROSSREF
15. Ren G, Zhao X, Zhang L, Zhang J, L'Huillier A, Ling W, Roberts AI, Le AD, Shi S, Shao C, et al. Inflammatory cytokine-induced intercellular adhesion molecule-1 and vascular cell adhesion molecule-1 in mesenchymal stem cells are critical for immunosuppression. *J Immunol* 2010;184:2321-2328.
PUBMED | CROSSREF
16. Ryan ST, Hosseini-Beheshti E, Afrose D, Ding X, Xia B, Grau GE, Little CB, McClements L, Li JJ. Extracellular vesicles from mesenchymal stromal cells for the treatment of inflammation-related conditions. *Int J Mol Sci* 2021;22:3023.
PUBMED | CROSSREF
17. Maccario R, Podestà M, Moretta A, Cometa A, Comoli P, Montagna D, Daudt L, Ibatici A, Piaggio G, Pozzi S, et al. Interaction of human mesenchymal stem cells with cells involved in alloantigen-specific immune

response favors the differentiation of CD4+ T-cell subsets expressing a regulatory/suppressive phenotype. *Haematologica* 2005;90:516-525.
PUBMED

18. Corcione A, Benvenuto F, Ferretti E, Giunti D, Cappiello V, Cazzanti F, Risso M, Gualandi F, Mancardi GL, Pistoia V, et al. Human mesenchymal stem cells modulate B-cell functions. *Blood* 2006;107:367-372.
PUBMED | CROSSREF
19. Le Blanc K, Tammik C, Rosendahl K, Zetterberg E, Ringdén O. HLA expression and immunologic properties of differentiated and undifferentiated mesenchymal stem cells. *Exp Hematol* 2003;31:890-896.
PUBMED | CROSSREF
20. Majumdar MK, Keane-Moore M, Buyaner D, Hardy WB, Moorman MA, McIntosh KR, Mosca JD. Characterization and functionality of cell surface molecules on human mesenchymal stem cells. *J Biomed Sci* 2003;10:228-241.
PUBMED | CROSSREF
21. Haynesworth SE, Goshima J, Goldberg VM, Caplan AI. Characterization of cells with osteogenic potential from human marrow. *Bone* 1992;13:81-88.
PUBMED | CROSSREF
22. In 't Anker PS, Scherjon SA, Kleijburg-van der Keur C, de Groot-Swings GM, Claas FH, Fibbe WE, Kanhai HH. Isolation of mesenchymal stem cells of fetal or maternal origin from human placenta. *Stem Cells* 2004;22:1338-1345.
PUBMED | CROSSREF
23. Zuk PA, Zhu M, Mizuno H, Huang J, Futrell JW, Katz AJ, Benhaim P, Lorenz HP, Hedrick MH. Multilineage cells from human adipose tissue: implications for cell-based therapies. *Tissue Eng* 2001;7:211-228.
PUBMED | CROSSREF
24. Kim HJ, Shin S, Jeong SY, Lim SU, Lee DW, Kwon YK, Kang J, Kim SW, Jung CK, Lee C, et al. Nasal turbinate mesenchymal stromal cells preserve characteristics of their neural crest origin and exert distinct paracrine activity. *J Clin Med* 2021;10:1792.
PUBMED | CROSSREF
25. Ménard C, Tarte K. Immunoregulatory properties of clinical grade mesenchymal stromal cells: evidence, uncertainties, and clinical application. *Stem Cell Res Ther* 2013;4:64.
PUBMED | CROSSREF
26. Djouad F, Fritz V, Apparailly F, Louis-Plence P, Bony C, Sany J, Jorgensen C, Noël D. Reversal of the immunosuppressive properties of mesenchymal stem cells by tumor necrosis factor alpha in collagen-induced arthritis. *Arthritis Rheum* 2005;52:1595-1603.
PUBMED | CROSSREF
27. Augello A, Tasso R, Negrini SM, Cancedda R, Pennesi G. Cell therapy using allogeneic bone marrow mesenchymal stem cells prevents tissue damage in collagen-induced arthritis. *Arthritis Rheum* 2007;56:1175-1186.
PUBMED | CROSSREF
28. González MA, Gonzalez-Rey E, Rico L, Büscher D, Delgado M. Treatment of experimental arthritis by inducing immune tolerance with human adipose-derived mesenchymal stem cells. *Arthritis Rheum* 2009;60:1006-1019.
PUBMED | CROSSREF
29. Park EH, Lim HS, Lee S, Roh K, Seo KW, Kang KS, Shin K. Intravenous infusion of umbilical cord blood-derived mesenchymal stem cells in rheumatoid arthritis: a phase Ia clinical trial. *Stem Cells Transl Med* 2018;7:636-642.
PUBMED | CROSSREF
30. Wang L, Wang L, Cong X, Liu G, Zhou J, Bai B, Li Y, Bai W, Li M, Ji H, et al. Human umbilical cord mesenchymal stem cell therapy for patients with active rheumatoid arthritis: safety and efficacy. *Stem Cells Dev* 2013;22:3192-3202.
PUBMED | CROSSREF
31. Álvaro-Gracia JM, Jover JA, García-Vicuña R, Carreño L, Alonso A, Marsal S, Blanco F, Martínez-Taboada VM, Taylor P, Martín-Martín C, et al. Intravenous administration of expanded allogeneic adipose-derived mesenchymal stem cells in refractory rheumatoid arthritis (Cx611): results of a multicentre, dose escalation, randomised, single-blind, placebo-controlled phase Ib/IIa clinical trial. *Ann Rheum Dis* 2017;76:196-202.
PUBMED | CROSSREF
32. Ghoryani M, Shariati-Sarabi Z, Tavakkol-Afshari J, Mohammadi M. The sufficient immunoregulatory effect of autologous bone marrow-derived mesenchymal stem cell transplantation on regulatory T cells in patients with refractory rheumatoid arthritis. *J Immunol Res* 2020;2020:3562753.
PUBMED | CROSSREF

33. Zhou K, Zhang H, Jin O, Feng X, Yao G, Hou Y, Sun L. Transplantation of human bone marrow mesenchymal stem cell ameliorates the autoimmune pathogenesis in MRL/lpr mice. *Cell Mol Immunol* 2008;5:417-424.
PUBMED | CROSSREF

34. Choi EW, Shin IS, Park SY, Park JH, Kim JS, Yoon EJ, Kang SK, Ra JC, Hong SH. Reversal of serologic, immunologic, and histologic dysfunction in mice with systemic lupus erythematosus by long-term serial adipose tissue-derived mesenchymal stem cell transplantation. *Arthritis Rheum* 2012;64:243-253.
PUBMED | CROSSREF

35. Gu Z, Akiyama K, Ma X, Zhang H, Feng X, Yao G, Hou Y, Lu L, Gilkeson GS, Silver RM, et al. Transplantation of umbilical cord mesenchymal stem cells alleviates lupus nephritis in MRL/lpr mice. *Lupus* 2010;19:1502-1514.
PUBMED | CROSSREF

36. Sun L, Akiyama K, Zhang H, Yamaza T, Hou Y, Zhao S, Xu T, Le A, Shi S. Mesenchymal stem cell transplantation reverses multiorgan dysfunction in systemic lupus erythematosus mice and humans. *Stem Cells* 2009;27:1421-1432.
PUBMED | CROSSREF

37. Liang J, Zhang H, Hua B, Wang H, Lu L, Shi S, Hou Y, Zeng X, Gilkeson GS, Sun L. Allogenic mesenchymal stem cells transplantation in refractory systemic lupus erythematosus: a pilot clinical study. *Ann Rheum Dis* 2010;69:1423-1429.
PUBMED | CROSSREF

38. Sun L, Wang D, Liang J, Zhang H, Feng X, Wang H, Hua B, Liu B, Ye S, Hu X, et al. Umbilical cord mesenchymal stem cell transplantation in severe and refractory systemic lupus erythematosus. *Arthritis Rheum* 2010;62:2467-2475.
PUBMED | CROSSREF

39. Li X, Wang D, Liang J, Zhang H, Sun L. Mesenchymal SCT ameliorates refractory cytopenia in patients with systemic lupus erythematosus. *Bone Marrow Transplant* 2013;48:544-550.
PUBMED | CROSSREF

40. Wang D, Zhang H, Liang J, Li X, Feng X, Wang H, Hua B, Liu B, Lu L, Gilkeson GS, et al. Allogeneic mesenchymal stem cell transplantation in severe and refractory systemic lupus erythematosus: 4 years of experience. *Cell Transplant* 2013;22:2267-2277.
PUBMED | CROSSREF

41. Gu F, Wang D, Zhang H, Feng X, Gilkeson GS, Shi S, Sun L. Allogeneic mesenchymal stem cell transplantation for lupus nephritis patients refractory to conventional therapy. *Clin Rheumatol* 2014;33:1611-1619.
PUBMED | CROSSREF

42. Carrion F, Nova E, Ruiz C, Diaz F, Inostroza C, Rojo D, Mönckeberg G, Figueroa FE. Autologous mesenchymal stem cell treatment increased T regulatory cells with no effect on disease activity in two systemic lupus erythematosus patients. *Lupus* 2010;19:317-322.
PUBMED | CROSSREF

43. Liang J, Zhang H, Kong W, Deng W, Wang D, Feng X, Zhao C, Hua B, Wang H, Sun L. Safety analysis in patients with autoimmune disease receiving allogeneic mesenchymal stem cells infusion: a long-term retrospective study. *Stem Cell Res Ther* 2018;9:312.
PUBMED | CROSSREF

44. Farge D, Loisel S, Lansiaux P, Tarte K. Mesenchymal stromal cells for systemic sclerosis treatment. *Autoimmun Rev* 2021;20:102755.
PUBMED | CROSSREF

45. Guiducci S, Porta F, Saccardi R, Guidi S, Ibba-Manneschi L, Manetti M, Mazzanti B, Dal Pozzo S, Milia AF, Bellando-Randone S, et al. Autologous mesenchymal stem cells foster revascularization of ischemic limbs in systemic sclerosis: a case report. *Ann Intern Med* 2010;153:650-654.
PUBMED | CROSSREF

46. Ishigatsubo Y, Ihata A, Kobayashi H, Hama M, Kirino Y, Ueda A, Takeno M, Shirai A, Ohno S. Therapeutic angiogenesis in patients with systemic sclerosis by autologous transplantation of bone-marrow-derived cells. *Mod Rheumatol* 2010;20:263-272.
PUBMED | CROSSREF

47. Christopeit M, Schendel M, Föll J, Müller LP, Keysser G, Behre G. Marked improvement of severe progressive systemic sclerosis after transplantation of mesenchymal stem cells from an allogeneic haploidentical-related donor mediated by ligation of CD137L. *Leukemia* 2008;22:1062-1064.
PUBMED | CROSSREF

48. Keyszer G, Christopeit M, Fick S, Schendel M, Taute BM, Behre G, Müller LP, Schmoll HJ. Treatment of severe progressive systemic sclerosis with transplantation of mesenchymal stromal cells from allogeneic related donors: report of five cases. *Arthritis Rheum* 2011;63:2540-2542.
PUBMED | CROSSREF

49. Chihaby N, Orliaguet M, Le Pottier L, Pers JO, Boisramé S. Treatment of Sjögren's syndrome with mesenchymal stem cells: a systematic review. *Int J Mol Sci* 2021;22:10474.
PUBMED | CROSSREF

50. Xu J, Wang D, Liu D, Fan Z, Zhang H, Liu O, Ding G, Gao R, Zhang C, Ding Y, et al. Allogeneic mesenchymal stem cell treatment alleviates experimental and clinical Sjögren syndrome. *Blood* 2012;120:3142-3151.
PUBMED | CROSSREF

51. Lukomska B, Stanaszek L, Zuba-Surma E, Legosz P, Sarzynska S, Drela K. Challenges and controversies in human mesenchymal stem cell therapy. *Stem Cells Int* 2019;2019:9628536.
PUBMED | CROSSREF

52. Liu M, Lei H, Dong P, Fu X, Yang Z, Yang Y, Ma J, Liu X, Cao Y, Xiao R. Adipose-derived mesenchymal stem cells from the elderly exhibit decreased migration and differentiation abilities with senescent properties. *Cell Transplant* 2017;26:1505-1519.
PUBMED | CROSSREF

53. Joswig AJ, Mitchell A, Cummings KJ, Levine GJ, Gregory CA, Smith R 3rd, Watts AE. Repeated intra-articular injection of allogeneic mesenchymal stem cells causes an adverse response compared to autologous cells in the equine model. *Stem Cell Res Ther* 2017;8:42.
PUBMED | CROSSREF

54. Vignali DA, Collison LW, Workman CJ. How regulatory T cells work. *Nat Rev Immunol* 2008;8:523-532.
PUBMED | CROSSREF

55. Dominguez-Villar M, Hafler DA. Regulatory T cells in autoimmune disease. *Nat Immunol* 2018;19:665-673.
PUBMED | CROSSREF

56. Sharabi A, Tsokos MG, Ding Y, Malek TR, Klatzmann D, Tsokos GC. Regulatory T cells in the treatment of disease. *Nat Rev Drug Discov* 2018;17:823-844.
PUBMED | CROSSREF

57. Jordan MS, Boesteanu A, Reed AJ, Petrone AL, Holenbeck AE, Lerman MA, Naji A, Caton AJ. Thymic selection of CD4+CD25+ regulatory T cells induced by an agonist self-peptide. *Nat Immunol* 2001;2:301-306.
PUBMED | CROSSREF

58. Sojka DK, Huang YH, Fowell DJ. Mechanisms of regulatory T-cell suppression - a diverse arsenal for a moving target. *Immunology* 2008;124:13-22.
PUBMED | CROSSREF

59. Sakaguchi S, Wing K, Onishi Y, Prieto-Martin P, Yamaguchi T. Regulatory T cells: how do they suppress immune responses? *Int Immunol* 2009;21:1105-1111.
PUBMED | CROSSREF

60. Selck C, Dominguez-Villar M. Antigen-specific regulatory T cell therapy in autoimmune diseases and transplantation. *Front Immunol* 2021;12:661875.
PUBMED | CROSSREF

61. Marek-Trzonkowska N, Mysliwiec M, Dobyszuk A, Grabowska M, Techmanska I, Juscinska J, Wujtewicz MA, Witkowski P, Mlynarski W, Balcerska A, et al. Administration of CD4+CD25highCD127- regulatory T cells preserves β-cell function in type 1 diabetes in children. *Diabetes Care* 2012;35:1817-1820.
PUBMED | CROSSREF

62. Chwojnicki K, Iwaszkiewicz-Grześ D, Jankowska A, Zieliński M, Łowiec P, Gliwiński M, Grzywińska M, Kowalczyk K, Konarzewska A, Glasner P, et al. Administration of $CD4^{+}CD25^{high}CD127^{-}FoxP3^{+}$ regulatory T cells for relapsing-remitting multiple sclerosis: a phase 1 study. *BioDrugs* 2021;35:47-60.
PUBMED | CROSSREF

63. Sánchez-Fueyo A, Whitehouse G, Grageda N, Cramp ME, Lim TY, Romano M, Thirkell S, Lowe K, Fry L, Heward J, et al. Applicability, safety, and biological activity of regulatory T cell therapy in liver transplantation. *Am J Transplant* 2020;20:1125-1136.
PUBMED | CROSSREF

64. Thornton AM, Shevach EM. Suppressor effector function of CD4+CD25+ immunoregulatory T cells is antigen nonspecific. *J Immunol* 2000;164:183-190.
PUBMED | CROSSREF

65. Tang Q, Henriksen KJ, Bi M, Finger EB, Szot G, Ye J, Masteller EL, McDevitt H, Bonyhadi M, Bluestone JA. *In vitro*-expanded antigen-specific regulatory T cells suppress autoimmune diabetes. *J Exp Med* 2004;199:1455-1465.
PUBMED | CROSSREF

66. Stephens LA, Malpass KH, Anderton SM. Curing CNS autoimmune disease with myelin-reactive Foxp3+ Treg. *Eur J Immunol* 2009;39:1108-1117.
PUBMED | CROSSREF

67. Desreumaux P, Foussat A, Allez M, Beaugerie L, Hébuterne X, Bouhnik Y, Nachury M, Brun V, Bastian H, Belmonte N, et al. Safety and efficacy of antigen-specific regulatory T-cell therapy for patients with refractory Crohn's disease. *Gastroenterology* 2012;143:1207-1217.e2.
PUBMED | CROSSREF

68. Sadeqi Nezhad M, Seifalian A, Bagheri N, Yaghoubi S, Karimi MH, Adbollahpour-Alitappeh M. Chimeric antigen receptor based therapy as a potential approach in autoimmune diseases: how close are we to the treatment? *Front Immunol* 2020;11:603237.
PUBMED | CROSSREF

69. Wright GP, Notley CA, Xue SA, Bendle GM, Holler A, Schumacher TN, Ehrenstein MR, Stauss HJ. Adoptive therapy with redirected primary regulatory T cells results in antigen-specific suppression of arthritis. *Proc Natl Acad Sci U S A* 2009;106:19078-19083.
PUBMED | CROSSREF

70. Fujio K, Okamoto A, Araki Y, Shoda H, Tahara H, Tsuno NH, Takahashi K, Kitamura T, Yamamoto K. Gene therapy of arthritis with TCR isolated from the inflamed paw. *J Immunol* 2006;177:8140-8147.
PUBMED | CROSSREF

71. Mougiakakos D, Krönke G, Völkl S, Kretschmann S, Aigner M, Kharboutli S, Böltz S, Manger B, Mackensen A, Schett G. CD19-targeted CAR T cells in refractory systemic lupus erythematosus. *N Engl J Med* 2021;385:567-569.
PUBMED | CROSSREF

72. Rana J, Biswas M. Regulatory T cell therapy: current and future design perspectives. *Cell Immunol* 2020;356:104193.
PUBMED | CROSSREF

73. Veglia F, Sanseviero E, Gabrilovich DI. Myeloid-derived suppressor cells in the era of increasing myeloid cell diversity. *Nat Rev Immunol* 2021;21:485-498.
PUBMED | CROSSREF

74. Zhang J, Hodges A, Chen SH, Pan PY. Myeloid-derived suppressor cells as cellular immunotherapy in transplantation and autoimmune diseases. *Cell Immunol* 2021;362:104300.
PUBMED | CROSSREF

75. Bronte V, Brandau S, Chen SH, Colombo MP, Frey AB, Greten TF, Mandruzzato S, Murray PJ, Ochoa A, Ostrand-Rosenberg S, et al. Recommendations for myeloid-derived suppressor cell nomenclature and characterization standards. *Nat Commun* 2016;7:12150.
PUBMED | CROSSREF

76. Zhao F, Hoechst B, Duffy A, Gamrekelashvili J, Fioravanti S, Manns MP, Greten TF, Korangy F. S100A9 a new marker for monocytic human myeloid-derived suppressor cells. *Immunology* 2012;136:176-183.
PUBMED | CROSSREF

77. Condamine T, Dominguez GA, Youn JI, Kossenkov AV, Mony S, Alicea-Torres K, Tcyganov E, Hashimoto A, Nefedova Y, Lin C, et al. Lectin-type oxidized LDL receptor-1 distinguishes population of human polymorphonuclear myeloid-derived suppressor cells in cancer patients. *Sci Immunol* 2016;1:aaf8943.
PUBMED | CROSSREF

78. Youn JI, Nagaraj S, Collazo M, Gabrilovich DI. Subsets of myeloid-derived suppressor cells in tumor-bearing mice. *J Immunol* 2008;181:5791-5802.
PUBMED | CROSSREF

79. Kusmartsev S, Nefedova Y, Yoder D, Gabrilovich DI. Antigen-specific inhibition of CD8+ T cell response by immature myeloid cells in cancer is mediated by reactive oxygen species. *J Immunol* 2004;172:989-999.
PUBMED | CROSSREF

80. Raber PL, Thevenot P, Sierra R, Wyczechowska D, Halle D, Ramirez ME, Ochoa AC, Fletcher M, Velasco C, Wilk A, et al. Subpopulations of myeloid-derived suppressor cells impair T cell responses through independent nitric oxide-related pathways. *Int J Cancer* 2014;134:2853-2864.
PUBMED | CROSSREF

81. Bingisser RM, Tilbrook PA, Holt PG, Kees UR. Macrophage-derived nitric oxide regulates T cell activation via reversible disruption of the Jak3/STAT5 signaling pathway. *J Immunol* 1998;160:5729-5734.
PUBMED

82. Yarosz EL, Chang CH. The role of reactive oxygen species in regulating T cell-mediated immunity and disease. *Immune Netw* 2018;18:e14.
PUBMED | CROSSREF

83. Rodriguez PC, Quiceno DG, Zabaleta J, Ortiz B, Zea AH, Piazuelo MB, Delgado A, Correa P, Brayer J, Sotomayor EM, et al. Arginase I production in the tumor microenvironment by mature myeloid cells inhibits T-cell receptor expression and antigen-specific T-cell responses. *Cancer Res* 2004;64:5839-5849.
PUBMED | CROSSREF

84. Srivastava MK, Sinha P, Clements VK, Rodriguez P, Ostrand-Rosenberg S. Myeloid-derived suppressor cells inhibit T-cell activation by depleting cystine and cysteine. *Cancer Res* 2010;70:68-77.
PUBMED | CROSSREF

85. Hanson EM, Clements VK, Sinha P, Ilkovitch D, Ostrand-Rosenberg S. Myeloid-derived suppressor cells down-regulate L-selectin expression on CD4+ and CD8+ T cells. *J Immunol* 2009;183:937-944.
PUBMED | CROSSREF

86. Yang Y, Li C, Liu T, Dai X, Bazhin AV. Myeloid-derived suppressor cells in tumors: from mechanisms to antigen specificity and microenvironmental regulation. *Front Immunol* 2020;11:1371.
PUBMED | CROSSREF

87. Juneja VR, McGuire KA, Manguso RT, LaFleur MW, Collins N, Haining WN, Freeman GJ, Sharpe AH. PD-L1 on tumor cells is sufficient for immune evasion in immunogenic tumors and inhibits CD8 T cell cytotoxicity. *J Exp Med* 2017;214:895-904.
PUBMED | CROSSREF

88. Groth C, Hu X, Weber R, Fleming V, Altevogt P, Utikal J, Umansky V. Immunosuppression mediated by myeloid-derived suppressor cells (MDSCs) during tumour progression. *Br J Cancer* 2019;120:16-25.
PUBMED | CROSSREF

89. Leipe J, Grunke M, Dechant C, Reindl C, Kerzendorf U, Schulze-Koops H, Skapenko A. Role of Th17 cells in human autoimmune arthritis. *Arthritis Rheum* 2010;62:2876-2885.
PUBMED | CROSSREF

90. Zhang H, Wang S, Huang Y, Wang H, Zhao J, Gaskin F, Yang N, Fu SM. Myeloid-derived suppressor cells are proinflammatory and regulate collagen-induced arthritis through manipulating Th17 cell differentiation. *Clin Immunol* 2015;157:175-186.
PUBMED | CROSSREF

91. Park MJ, Lee SH, Kim EK, Lee EJ, Baek JA, Park SH, Kwok SK, Cho ML. Interleukin-10 produced by myeloid-derived suppressor cells is critical for the induction of Tregs and attenuation of rheumatoid inflammation in mice. *Sci Rep* 2018;8:3753.
PUBMED | CROSSREF

92. Wu H, Zhen Y, Ma Z, Li H, Yu J, Xu ZG, Wang XY, Yi H, Yang YG. Arginase-1-dependent promotion of TH17 differentiation and disease progression by MDSCs in systemic lupus erythematosus. *Sci Transl Med* 2016;8:331ra40.
PUBMED | CROSSREF

93. Park YS, Gauna AE, Cha S. Mouse models of primary Sjogren's syndrome. *Curr Pharm Des* 2015;21:2350-2364.
PUBMED | CROSSREF

94. Qi J, Li D, Shi G, Zhang X, Pan Y, Dou H, Yao G, Hou Y. Myeloid-derived suppressor cells exacerbate Sjögren's syndrome by inhibiting Th2 immune responses. *Mol Immunol* 2018;101:251-258.
PUBMED | CROSSREF

95. Park MJ, Lee SH, Kim EK, Lee EJ, Park SH, Kwok SK, Cho ML. Myeloid-derived suppressor cells induce the expansion of regulatory B cells and ameliorate autoimmunity in the sanroque mouse model of systemic lupus erythematosus. *Arthritis Rheumatol* 2016;68:2717-2727.
PUBMED | CROSSREF

96. Park MJ, Baek JA, Choi JW, Jang SG, Kim DS, Park SH, Cho ML, Kwok SK. Programmed death-ligand 1 expression potentiates the immune modulatory function of myeloid-derived suppressor cells in systemic lupus erythematosus. *Front Immunol* 2021;12:606024.
PUBMED | CROSSREF