

Mesenchymal stem cells in the treatment of inflammatory and autoimmune diseases in experimental animal models

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Abstract

Multipotent mesenchymal stromal cells [also known as

mesenchymal stem cells (MSCs)] are currently being studied as a cell-based treatment for inflammatory disorders. Experimental animal models of human immune-mediated diseases have been instrumental in establishing their immunosuppressive properties. In this review, we summarize recent studies examining the effectiveness of MSCs as immunotherapy in several widely-studied animal models, including type 1 diabetes, experimental autoimmune arthritis, experimental autoimmune encephalomyelitis, inflammatory bowel disease, graft-*vs*-host disease, and systemic lupus erythematosus. In addition, we discuss mechanisms identified by which MSCs mediate immune suppression in specific disease models, and potential sources of functional variability of MSCs between studies.

Key words: Mesenchymal stromal cells; Mesenchymal stem cells; Autoimmunity; Animal models; Inflammation; Immunotherapy

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Core tip: In this review, we summarize recent studies examining the effectiveness of mesenchymal stromal cells (MSCs) as immunotherapy in several widely-studied animal models, including type 1 diabetes, experimental autoimmune arthritis, experimental autoimmune encephalomyelitis, inflammatory bowel disease, graft-*vs*-host disease, and systemic lupus erythematosus. In addition, we discuss mechanisms identified by which MSCs mediate immune suppression *in vivo* and potential sources of functional variability of MSCs between studies.

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INTRODUCTION

Multipotent mesenchymal stromal cells [also known as mesenchymal stem cells (MSCs)] are currently being studied as a cell-based treatment for a variety of human diseases. MSCs initially received attention for their somewhat restricted multipotency, as MSCs can differentiate *in vitro* into cells of the mesoderm lineage such as adipocytes, chondrocytes, and osteocytes, but not cells of other lineages such as hematopoietic cells^[1]. In addition to multipotency, MSCs have demonstrated capacity for immunosuppression, suggesting that MSCs may be useful for the treatment of immune-mediated disorders^[2]. Importantly, several attributes of MSCs contribute to their practicality as a source for cell-based therapies. First, MSCs have a wide anatomical distribution and can be isolated from several human tissues such as bone marrow, adipose tissue, and dental pulp^[3-6]. Second, although MSCs are relatively rare within these tissues, their great capacity for self-renewal allows for the efficient expansion of these cells *in vitro* using simple cell culture methods^[7]. Thirdly, MSCs are non-immunogenic due to their low expression of antigen-presenting molecules^[8]. This allows MSCs to avoid generating an allogeneic response when transferred into an un-matched recipient, and therefore donor and recipients do not need to be well-matched for histocompatibility. Due in part to the efficiency with which MSCs can be prepared for clinical use, they have received much attention from the medical research community and their utility for use in regenerative medicine and immunotherapy has been tested in more than 100 clinical trials worldwide^[9].

Despite this intense interest, important questions regarding MSCs remain unanswered. Notably, clinical trials testing the effectiveness of MSC-based immunotherapy in treating inflammatory disorders and autoimmune diseases have produced mixed results^[10,11]. In some trials administration of MSCs helped to alleviate symptoms, while in others no effects relative to placebo were detected. These discrepant results may be due to variability in the immunosuppressive capacity among MSCs obtained from different tissue sources or expanded under different conditions *in vitro* prior to their injection into subjects^[12]. Understanding the source of this functional variability and finding ways to predict or alter the activity of a given lot of MSCs is an essential step toward achieving consistent clinical results from MSC-based therapies. Additionally, while multiple mechanisms have been identified as the means by which MSCs can mediate immune suppression, those most important for suppression *in vivo* are not well-characterized. Indeed the mechanisms most important for clinical efficacy may differ between diseases, and identifying those which are most important for a given disease will allow for more effective MSC-based therapies, and potentially give important insights into the disease itself.

Pre-clinical animal models of human diseases

have already been instrumental in the study of MSCs, and are likely to be useful tools for answering these remaining questions. Mouse studies were first used to demonstrate that MSCs possessed immunosuppressive function *in vivo*, and subsequently have helped to identify some mechanisms used by MSCs to mediate these effects. Importantly, MSC-mediated immune suppression is cross reactive between species in many animal models, allowing the effectiveness of human MSCs in suppressing inflammatory disorders to be evaluated experimentally in animal models. In this review article, we summarize what is currently known about the therapeutic effectiveness of MSCs *in vivo* in several widely-studied animal models of immune-mediated diseases. Additionally, we discuss mechanisms identified by which MSCs mediate immune suppression in specific disease models, and highlight important areas of research in the general field of MSC-related immunotherapy research. To avoid confusion, this review focuses exclusively on results obtained from animal studies, with an emphasis on MSC-mediated effects that have been demonstrated *in vivo*. A thorough and informative review of clinical studies using MSC-based cellular therapies has recently been published elsewhere^[12].

ANIMAL MODELS AND TREATMENT WITH MSC

Type 1 diabetes

Type 1 diabetes (T1D) is the result of an immune-mediated response against insulin-producing pancreatic β -cells^[13]. The autoimmune attack is largely mediated by T cells and in most patients will result in the complete loss of β -cell function. Individuals with T1D are therefore unable to produce insulin in response to increasing blood glucose levels and dependent upon exogenous insulin treatment for the remainder of their lives. Even with insulin replacement therapy, individuals with T1D are at risk of long-term complication arising from improperly-regulated blood glucose levels, such as retinopathy, kidney damage, and heart disease. Several animal models of T1D exist that are used to study both the immunological pathogenesis of the disease as well as the long-term consequences of β -cell loss^[14]. The non-obese diabetic (NOD) mouse strain is used as an animal model for spontaneous autoimmune diabetes, and disease in these animals appears to share many features with T1D in humans. Alternatively, autoimmune diabetes can be induced in several strains of mice and rats by treatment with a compound toxic to β -cells. Iterative treatment with low doses of streptozotocin (STZ) damages β -cells, and this damage attracts immune cells to the islet leading to insulinitis and eventually immune-mediated β -cell destruction. In all models of T1D disease progression can be monitored by measuring glucose levels in the blood or urine, with increases in glucose levels being associated with

progressive loss of β -cell function. Disease severity between groups can also be compared by histological examination of the pancreas of experimental animals for immune cell infiltration.

MSC-based treatment has been largely successful in treating the symptoms of autoimmune diabetes in animal models. In NOD mice, treatment with MSCs has been reported to protect mice if administered before disease onset^[15,16], and to reverse disease when administered after the onset of hyperglycemia^[17]. Interestingly, MSCs derived from NOD mice were unable to suppress disease in recipients, but those derived from BALB/c mice or the non-obese diabetes resistant strain were able to do so, suggesting that MSCs in the NOD mouse may have a defect in their ability to suppress immune responses^[16]. In these studies, MSC treatment was associated with a reduction in the frequency of inflammatory CD4⁺ T cells^[16,17] and an increase in the frequency of regulatory T cells^[15-17]. Similar results have been reported using an STZ-induced model in C57BL/6 mice, with syngeneic bone marrow-derived MSCs again reverting hyperglycemic animals to normal blood glucose levels^[18]. In a rat model of STZ-induced T1D, autologous bone marrow-derived MSCs treatment led to increased insulin secretion and sustained normoglycemia, with a shift in T cell cytokine production toward that of T_H2 cells^[19]. There is also evidence that MSC treatment can aid in tissue repair after the onset of disease, as human-derived MSCs homed to the pancreatic islets and led to an increase in islet frequency and insulin production in NOD/scid mice treated with STZ^[20]. Mouse-derived MSCs displayed similar behavior, homing to both the pancreas and kidney when injected into STZ-treated mice, and were associated with an increase in renal function relative to diabetic mice not receiving MSCs^[18]. The mechanisms used by MSCs to mediate these anti-inflammatory and tissue repair effects *in vivo* have yet to be determined.

The transplantation of allogeneic islets has been suggested as a treatment for patients with T1D, but the long term engraftment of these transplanted tissues has been difficult to achieve^[21]. Here the immunosuppressive capacity of MSCs may prove useful for preventing the rejection of transplanted islets as well as mediating islet repair immediately after transplantation. In STZ-induced diabetes models in rats^[22] and mice^[23], the long-term survival of islet allografts was significantly enhanced by co-transfer with MSCs. Notably, in a model of non-human primates (cynomolgus monkey), co-transplant of allogeneic bone marrow-derived MSCs intraportally with islets significantly enhanced islet engraftment and function, which was associated with increased number of regulatory T cells^[24]. In rats, MSC co-transfer led to a reduction in T_H1-associated cytokines and an increase in IL-10-producing regulatory T cells^[22]. In contrast, mouse MSCs reportedly mediated their immunosuppressive effects by the production of metalloproteinases that cleave the alpha chain of the IL-2 receptor (CD25)

from the surface of activated T cells, leaving these T cells hyporesponsive to IL-2^[23]. Collectively, MSC-based therapy has performed well in treating mouse models of T1D, both in suppressing the autoimmune attack of endogenous β -cells and in improving the maintenance of allogeneic islet allografts.

Experimental autoimmune arthritis

Rheumatoid arthritis (RA) is a common autoimmune disease characterized by chronic joint inflammation. It is thought that the disease is initiated when autoreactive T cells infiltrate the synovial tissue and secrete cytokines and chemokines, recruiting other immune cells to the joint^[25]. The resulting inflammatory milieu progressively damages the joint and surrounding tissues, a process that can be extremely painful and debilitating. The most commonly used animal model for the study of RA is the collagen-induced arthritis (CIA) model^[26]. In this model, a susceptible strain of mouse is injected subcutaneously at the base of the tail with an emulsion of complete Freund's adjuvant and type II collagen. This injection produces a potent T cell-mediated immune response to type II collagen that eventually leads to inflammation in the joints of the paws. In some cases a second booster immunization is given several weeks after the initial injection to increase disease incidence. The severity of disease can be quantified by scoring each paw on a scale from 0 (no inflammation) to 3 (severe inflammation), or by directly measuring any increase in paw width using a caliper. CIA shares many similarities with RA such as the presence of autoantibodies, the involvement of T_H1 and T_H17 cells in disease progression, and a strong link between disease susceptibility and the genomic region encoding for class II MHC molecules^[26].

Several groups have studied the efficacy of MSC immunotherapy in mouse models for RA and have generally found that MSCs can reduce both the incidence and severity of disease. In the CIA mouse model, intraperitoneal injection of allogeneic bone marrow-derived MSCs at the time of initial immunization significantly reduced incidence of disease, and administration of MSCs after disease onset had a therapeutic effect as well^[27]. A study using adipose-derived MSCs reported similar results, as mice receiving multiple doses of MSCs were less likely to show signs of joint inflammation and overall had a milder form of arthritis than those animals not receiving MSCs^[28]. Notably, this study also found that both human and murine MSCs could suppress autoimmunity in the CIA model. These results, along with another study showing that MSCs derived from rat bone marrow are effective as well^[29], suggests that at least some immunosuppressive mechanisms employed by MSCs are cross-reactive between species. Human MSCs derived from umbilical blood suppress CIA as well^[30]. Additionally, murine MSCs injected directly into inflamed joints reduced inflammation in an antigen-induced arthritis model^[31]. These results are in contrast with an earlier report which instead

found that administering MSCs after disease onset exacerbated disease rather than ameliorating it^[32]. It should be noted, however, that these experiments used an immortalized MSC-derived cell line rather than conventional MSCs. Additionally, this study and another using conventional murine MSCs found that despite their failure to suppress CIA *in vivo*, MSCs did suppress immune responses *in vitro*^[32,33].

The mechanisms through which MSCs inhibit experimental arthritis are still in question. Several groups have reported a decrease in levels of pro-inflammatory cytokine such as TNF α ^[27,29,30] or IL-6^[30], and increases in the anti-inflammatory cytokine IL-10^[29,30]. MSCs may also modulate the nature of the T cell response in CIA by reducing the frequency of T_H1 and T_H17 cells^[28,30], and/or increasing the frequency of T_H2 cells^[30,34]. MSCs appear to promote regulatory T cells as well, as several studies have observed an increase in regulatory T cell frequency after administration of MSCs^[27,28,30]. While T cells from MSC-treated mice are generally hyporesponsive, MSC treatment does not appear to effect T cell priming, suggesting that the immunosuppressive effects of MSCs occur after the autoimmune response is initiated^[30]. MSCs from mice deficient in IL-6 or iNOS production have partially impaired immunosuppressive function^[34]. IL-6-deficient MSCs released significantly lower levels of prostaglandin E2, suggesting that autocrine IL-6 may induce the production of anti-inflammatory lipid mediators by MSCs^[34]. Although MSC treatment clearly modulates the T cell response in CIA, it is currently not known if this effect is directly attributable to MSCs or rather an indirect effect of MSCs acting on other cells of the immune system.

Graft-versus-host disease

Allogeneic hematopoietic stem cell transplantation is used for the treatment of blood-derived malignancies and immune deficiencies^[35]. Graft-versus-host disease (GVHD), a major complication that occurs after transplantation, is the result of donor-derived immune cells mounting an alloreactive response against the recipient's tissues or organs. GVHD occurs in up to 50% of transplant recipients, and therefore is a significant source of the mortality associated with these therapies^[36]. Animal models are widely used to study the GVHD response by depleting endogenous hematopoietic cells in recipient animals by radiation or chemotherapy and reconstituting the immune system in these animals with allogeneic bone marrow cells^[37]. The progression of GVHD in animal models is frequently assessed by weight loss, survival, and histological examination of organs and tissues commonly affected by GVHD. It should be noted that experimental GVHD models can vary greatly, both in the details of the protocol used and in the degree of MHC mismatch between donor and recipient strains^[37].

The effects of MSC-based therapies in these animal

models have been widely-studied, with most reports observing that MSC infusion was effective in treating or delaying experimental GVHD. An early study reported that transfer of bone marrow cells from MRL/lpr mice into irradiated recipients resulted in a GVHD-like wasting disease, but recipient animals survived much longer when the bone marrow transfer was accompanied by a bone graft (presumably containing MSCs)^[38]. In a MHC-mismatched model (bone marrow from C3H/He into irradiated BALB/c mice), a single dose of bone marrow-derived MSCs at the time of bone marrow infusion allowed recipient mice to survive much longer than those receiving only bone marrow cells^[39]. Experiments using these same strains found that an immortalized MSC cell line could suppress GVHD in this model as well^[40]. MSCs derived from adipose tissue were also able to potentially extend the life of animals in a haploidentical GVHD model^[41]. It should be noted that these authors found that MSC treatment early in the course of disease was effective, but when MSCs were administered later little benefit was observed, suggesting that the timing of MSC treatments are critical in GVHD models^[41]. T cell-derived IFN γ may be an important factor as well, as MSCs treated with IFN γ prior to infusion are reportedly superior to untreated MSCs in increasing survival after bone marrow transplant^[42]. These authors also found that MSCs were unable to enhance survival if recipients received a transplant containing IFN γ -deficient T cells, suggesting that activation-induced production of inflammatory cytokines may be required for maximal immunosuppression by MSCs *in vivo*^[42].

A xenogeneic model has also been used to investigate the effects of human-derived MSCs on the progression of GVHD^[43-45]. In this model, human peripheral blood mononuclear cells (PBMCs) are infused into irradiated NSG mice^[46], where they proliferate and initiate a GVHD-like response against mouse tissues in the recipient. Three weekly doses of umbilical cord-derived human MSC starting at the time of PBMC infusion completely prevented GVHD and strongly reduced T cell proliferation *in vivo*^[43]. Timing was again critical, as treatment started several weeks after PBMC infusion failed to prevent GVHD^[43]. In other studies using a similar xenogeneic model, bone marrow-derived human MSCs^[44] or cord blood-derived MSCs^[45] were able to suppress GVHD as well. Treating MSCs with IFN γ prior to infusion enhanced the immunosuppressive capacity of MSCs, as IFN γ -treated MSC suppressed GVHD even when far fewer cells were administered^[44].

Other reports failed to observe any positive effect of MSC treatment in GVHD^[47-51]. Although these studies failed to observe *in vivo* effects, most reported that MSC were immunosuppressive *in vitro*^[47,48,50,51], and one found that animals receiving MSCs had reduced GVHD-mediated tissue pathology despite a lack of survival enhancement^[49]. These seemingly conflicting outcomes are potentially the result of variation in experimental

design as well as in the immunosuppressive capabilities of MSCs. As mentioned above, several studies found that the timing of MSC infusion was crucial for GVHD suppression^[41,43]. MSCs in general appear to intrinsically vary in their immunosuppressive capabilities depending upon the source animal, tissue of origin, isolation methods, passage number, and route of infusion. Variation can exist between MSC lots even when these variables are held constant, as demonstrated by a report showing that MSC lines isolated from the same mouse strain in the same laboratory can have drastically different immunosuppressive capabilities *in vivo*^[52]. Interestingly, these authors found no difference in the *in vitro* immunosuppressive capabilities of these independent MSC lines despite the difference in their effectiveness *in vivo*, suggesting that *in vitro* capabilities do not necessarily reflect *in vivo* function^[52]. The specific GVHD model chosen can also have an impact on experimental results, as murine MSCs are reportedly effective in preventing GVHD in a sibling transfer murine GVHD model, but can only delay disease when donors and recipients are MHC-mismatched^[53]. While most studies find that MSCs are capable of suppressing GVHD in animal models, it is essential to better understand the source of these discrepant results and find ways to predict if a given lot of MSCs will have immunosuppressive function *in vivo*. Elucidating the mechanisms MSCs use to mediate immune suppression in GVHD models should also be the focus of future work. Several groups have reported that T cells are inhibited in animals receiving MSCs, but the molecular mediators responsible for this phenomenon are largely unknown.

Experimental autoimmune encephalomyelitis

Multiple sclerosis (MS) is a disorder of the central nervous system (CNS) characterized by the progressive demyelination of nerves in the spinal cord and brain^[54]. The resulting loss of neurological function can lead to a wide variety of symptoms including pain, loss of sensation, muscle spasms, loss of coordination, vision problems, ataxia, difficulty of thinking, and emotional distress. The cause of demyelination in MS is thought to be an inflammation of the CNS caused by infiltrating immune cells^[54]. Autoimmunity in the CD4⁺ T cell compartment appears to play a central role in the pathogenesis of MS based upon evidence from animal studies and genetic association studies in humans identifying haplotypes of the MHC region associated with both susceptibility and protection from disease^[55]. The most commonly-studied animal model for MS is experimental autoimmune encephalomyelitis (EAE), an induced model for CNS inflammation^[56,57]. In these models, mice are immunized to induce an immune response to CNS associated antigens such as myelin oligodendrocyte glycoprotein (MOG) or myelin proteolipid protein (PLP). Alternatively, EAE can be introduced by the adoptive transfer of activated

myelin-specific CD4⁺ T cells. EAE can follow distinct clinical courses depending upon the mouse strain used and the specific antigen against which the immune response is raised, each reflecting different aspects of MS in humans. Immunizing C57BL/6 mice against MOG results in a progressive disease course, whereas SJL mice immunized against PLP display a relapsing/remitting form of the disease^[56]. The first sign of disease is usually a limp tail followed by weakness or paralysis in the hind limbs as demyelination progresses. Disease severity is often represented by a paralysis scoring system where individual animals are scored based upon the level of paralysis observed. Histological examination of the spinal cord for immune cell infiltration can also be used to give a quantification of disease severity.

The impact of MSC-based therapy in EAE has been extensively studied, with most of studies reporting a potent immunosuppressive effect. Mice receiving syngeneic bone marrow-derived MSCs showed a significantly milder disease course than that of untreated animals in a progressive EAE model^[58]. This effect was evident when MSCs were administered either before disease onset or at the peak of disease severity, but not when administered later in the course of disease. This result suggests that the improvement in neurological function mediated by MSCs is due to their immunosuppressive functions rather than their multipotency. MSCs were effective in suppressing EAE in a relapsing/remitting model as well^[59]. Allogeneic MSCs suppressed EAE with an efficiency similar to that of syngeneic MSCs^[60]. Human MSCs are effective in EAE models as well, as MSCs from both bone marrow^[61,62] and adipose tissue^[63] have been reported to suppress either progressive or relapsing/remitting forms of disease.

As in other T cell-mediated autoimmune mouse models, questions still remain as to the mechanisms of immune suppression mediated by MSCs in EAE. A reduction in the frequency of T cells secreting inflammatory cytokines accompanies the reduction in disease activity^[59,64], and T cells in mice receiving MSCs appear to be hyporesponsive to antigenic stimulation or anergic^[58,59]. Murine MSCs appear to mediate immune suppression at least in part by a novel pathway inhibiting the CCL2 chemokine axis^[64]. MSCs secrete several matrix metalloproteinases that can cleave MSC-derived CCL2, resulting in a isoform that inhibits rather than activates CCR2-expressing immune cells^[65]. This mechanism plays a key role in MSC-mediated inhibition of EAE, as MSCs from CCL2^{-/-} mice are unable to suppress disease^[64]. While this mechanism is clearly important in the EAE model, its role in other T cell-mediated autoimmune animal models remains to be tested. CCL2 signaling is especially important for infiltration of the CNS by inflammatory T cells, but MSCs may rely on other immunosuppressive mechanisms in other disease models where the CCL2/CCR2 signaling

axis is less involved in disease pathogenesis^[66,67]. Finally, a defect in MSC function may be important in the pathogenesis of EAE, as MSCs derived from mice with ongoing disease are unable to suppress disease upon transfer to autologous recipients^[68].

Inflammatory bowel disease

Inflammatory bowel disease (IBD) in humans refers to a family of disorders characterized by destructive inflammation of the colon or small intestine^[69]. Damage caused by chronic inflammation leads to a host of gastrointestinal symptoms such as severe cramps and abdominal pain, diarrhea, and weight loss. In severe cases affected parts of the intestines are removed surgically, sometimes requiring a permanent colostomy. While the causes of IBD are not well understood, CD4⁺ T cells are thought to play a key role in the disease^[70]. The most commonly studied animal models of IBD involve administering an agent that causes chemical damage to the intestine such as dextran sulfate sodium (DSS) added to drinking water or intrarectally-administered trinitrobenzene sulfonic acid (TNBS)^[71]. The intestinal injury allows immune cells of the lamina propria to come in contact with luminal contents such as enteric bacteria, triggering an inflammatory cascade that bears some resemblance to human IBD. Severity of disease can vary depending upon the strain of mouse used as well as the route and frequency of administration of the chemical used. The disease course can be monitored by progressive weight loss or the appearance of diarrhea and/or bloody stools, or assessed post mortem by measuring colon length and histological examination looking for damage and immune cell infiltration^[71].

Therapies using MSCs have been largely successful in treating disease in animal models of IBD. In a DSS-induced acute colitis model, adipose-derived MSCs suppressed most measurable disease outcomes and improved survival^[72]. This effect was observed when MSCs derived from either human or mouse tissues were used, and MSCs from allogeneic donors were as effective in treating disease as those from syngeneic donors^[72]. Similar results were obtained when colitis was induced by intrarectal administration of TNBS^[73]. Human MSCs derived from bone marrow, gingiva or cord blood were also effective in treating DSS-induced colitis in mice^[74,75]. MSCs from rats are capable of suppressing experimental colitis in both rats^[76] and mice^[77].

MSCs appear to suppress disease in IBD models through multiple mechanisms. Several studies have reported that MSC infusion increased the frequency of regulatory T cells accompanied by a reduction in T cells secreting inflammatory cytokines^[72,73,78]. In the DSS-induced colitis model, MSCs from mice deficient in Fas ligand (FasL) were unable to suppress disease^[77,78]. FasL and its receptor Fas are both essential molecules for maintaining T cell homeostasis, and signaling

downstream of Fas leads to the rapid induction of apoptosis in susceptible cells, including activated effector T cells^[79]. During the course of experimental colitis MSCs can induce FasL-mediated apoptosis in T cells, and this increase in the frequency of apoptotic cells indirectly leads to an increase in regulatory T cells, as macrophages engulfing the apoptotic T cells increase their production of TGFβ^[78]. Interestingly, MSCs from Fas-deficient animals were also unable to suppress colitis despite being able to induce T cell apoptosis *in vitro*. The loss of Fas in MSCs disrupted their ability to produce CCL2, suggesting that non-apoptotic Fas signaling is required for CCL2 secretion in MSCs^[78]. Therefore Fas-deficient MSCs are incapable of attracting T cells into close proximity for FasL-mediated killing^[78]. In this study, CCL2 secreted by MSCs acted as a chemoattractant, while in the EAE model MSCs were reported to secrete an isoform of CCL2 that antagonized CCR2 signaling and inhibited CCL2-mediated chemotaxis^[64]. How MSCs can use two forms of the same chemokine with opposing activities to mediate immunosuppression is currently unknown, as are the conditions that cause MSCs to produce one form of CCL2 over the other.

Systemic lupus erythematosus

Systemic lupus erythematosus (SLE) is a complex autoimmune disease that causes progressive and profound damage to a variety of organs and tissues^[80]. A hallmark of SLE is the presence of antibodies against nuclear antigens such as DNA or chromatin-associated proteins^[80]. Intranuclear antigens are rarely found in the extracellular space under normal conditions as dying cells are quickly cleared by phagocytic cells before intracellular contents are released. In patients with SLE, however, it is thought that an increase in the rate of cell death and/or defects in the mechanisms responsible for the clearance of dying cells results in the release of intranuclear contents. Once exposed, free chromatin is bound by autoantibodies resulting in large immune complexes. These immune complexes accumulate in certain places in the body and induce inflammation at these sites. Immune complexes frequently accumulate in the blood vessels surrounding glomeruli in the kidney in patients with SLE, leading to extensive tissue damage and loss of renal function^[80]. Other complications arising from SLE include increased risk for atherosclerosis, neurological problems, and inflammation of the heart or lungs.

The most commonly-studied animal models for SLE are mouse strains in which a lupus-like disease arises spontaneously. The model thought to best approximate the disease course seen in humans with SLE uses the first generation progeny of a breeding pair consisting of a New Zealand Black (NZB) mouse and a New Zealand White (NZW) mouse^[81]. The resulting hybrid mice (NZB/NZW F₁) develop anti-nuclear and anti-DNA antibodies along with a form of glomerulonephritis that resembles

that seen in patients with SLE. Another model uses mice with a mutation in the gene encoding Fas (lpr) on the MRL strain background. The immune cells of MRL/lpr mice cannot undergo Fas-mediated apoptosis, resulting in a pronounced lymphoproliferative disorder that leads to the development of anti-nuclear antibodies and subsequent glomerulonephritis^[81]. In both of these spontaneous models of SLE, progression of the disease can be tracked by assessing renal function and testing for the presence of anti-nuclear antibodies in the serum.

The effectiveness of MSC-based therapy in the treatment of animal models of SLE is still in question. Initial studies in the MRL/lpr model reported positive results. The infusion of allogeneic bone marrow-derived MSCs reduced serum levels of anti-DNA antibodies and improved renal function^[82]. Clinical improvement was accompanied by an increase in the frequency of regulatory T cells and a reduction of IL-17-producing CD4⁺ T cells^[82]. MSCs from human tissues suppressed disease in the MRL/lpr model as well, as infusion of human MSCs derived from either bone marrow^[83] or umbilical cord blood^[84] led to a reduction in anti-dsDNA antibodies, proteinuria, and renal pathology. Results obtained in the NZB/NZW F₁ model are more ambiguous, however. Human MSCs from umbilical cord blood had only a moderate effect on disease parameters and animal survival, despite markedly reducing serum levels of pro-inflammatory cytokines (IL-2, TNF α , and IL-12) and increasing anti-inflammatory cytokine concentrations^[85]. Similar results were obtained when MSCs derived from human embryonic stem cells were used, although only animal survival was considered in this study^[86]. In a study using MSCs from murine bone marrow, however, allogeneic MSCs unexpectedly exacerbated the disease, increasing titers of anti-DNA antibodies and deposition of immune complexes in the kidney^[87]. The MSCs used in this study were able to suppress T cell proliferation *in vitro*, suggesting that grossly dysfunctional MSCs are not to blame for this result^[87]. Considering these discrepant observations, it is premature to draw any conclusions regarding the effectiveness of MSCs in treating animal models of SLE.

Experimental autoimmune uveitis

Autoimmune uveitis is a group of heterogeneous and complex human eye diseases that are collectively a major source of vision impairment and blindness^[88]. Genetic and experimental studies suggest that autoimmune uveitis is mediated by a response against retina-associated antigens, with T_H1 and T_H17 cells playing an especially crucial role^[89,90]. Experimental autoimmune uveitis (EAU), the most commonly-studied animal model for human uveitis, can be induced in susceptible animals by immunization with retina antigens or transfer of retina-specific T_H1 or T_H17 cells^[91]. Most studies of MSC-based treatment in EAU have observed a potent immunosuppressive effect. In a mouse model

of EAU induced by immunization with a self uveitogenic peptide derived from the intraphotoreceptor binding protein, treatment with a single intraperitoneal injection of syngeneic bone marrow-derived MSCs was able to significantly attenuate EAU^[92]. Human-derived MSCs were effective in preventing EAU in this mouse model as well^[93]. EAU can be induced in rats using a similar protocol, and bone marrow-derived MSCs from both syngeneic and allogeneic donors were able to suppress EAU in recipient animals^[94,95].

While MSC treatment appears to be effective in EAU, little is currently known about how MSCs mediate disease suppression. Most studies report a decrease in production of pro-inflammatory cytokines accompanied by an increase in regulatory T cell frequency^[92-95]. T cells from MSC-treated rats showed reduced IFN γ and IL-2 production relative to those from untreated animals, along with a moderate increase in production of IL-10 and TGF β ^[95]. Additionally, MSC treatment reduced T_H17 cell frequency in the eye and the spleen while increasing the frequency of regulatory T cells in both locations^[95]. Whether MSCs modulate the T cell response in EAU directly or indirectly has not yet been conclusively demonstrated. Importantly, the mechanisms used to mediate immunosuppression may differ between species, as murine and rat MSCs induced regulatory T cells^[92,94,95], but human MSCs induced IL-10-producing B cells while having little effect on regulatory T cell frequency^[93].

UNANSWERED QUESTIONS: FUTURE PERSPECTIVES

MSCs are currently being studied for use in cellular immunotherapy, but the performance of MSC-based therapies in clinical trials has been inconsistent. In mouse studies MSCs have more frequently shown an immunosuppressive effect (Table 1), although several studies found that treatment with MSCs either had no effect on disease or worsened disease outcomes^[32,33,47-51,87]. These exceptions are informative and, considering the general bias against publishing negative outcomes, suggest that MSC performance in mouse models may be just as inconsistent as it has been in the treatment of human disease. Understanding the sources of this inconsistency is an essential step in improving MSC-based therapies, and using mouse models provides a practical means of doing so.

The variable outcomes seen in these studies could come from many different sources. First, the functional characteristics of MSCs may differ based upon the species or tissue or origin. Among mouse studies there currently exists no evidence that MSCs from a particular tissue are consistently more effective in suppressing immune responses, and MSCs from human, mouse, and rat tissues have all effectively treated disease in mouse models. Instead, intrinsic variation in MSC

Table 1 Immunomodulatory effect of mesenchymal stromal cells in animal disease models

Disease model	Anti-inflammatory MSC effects	Negative/neutral studies?
Type 1 diabetes	↑ Regulatory T cells (15-17, 19) ↓ Inflammatory T cells (16, 17) T _H 1 → T _H 2 (16, 19) ↑ Tissue repair (18-20)	None reported
Pancreatic islet transplantation	↑ Islet survival (22-24) ↑ Regulatory T cells (22, 24) ↓ T _H 1 cytokines (22) ↓ T cell responsiveness (23)	None reported
Experimental autoimmune arthritis	↓ Inflammatory cytokines (27, 29-31) ↑ Regulatory T cells (27, 28, 30, 34) ↓ T cell responsiveness (27) ↑ IL-10 (28-30) ↓ T _H 1/T _H 17 cells (28, 30) ↑ T _H 2 cells (30, 34)	No suppression (31-33)
Graft-vs-host disease	↓ Auto-antibodies (38) ↓ Inflammatory cytokines (39, 42, 44, 53) ↑ Regulatory T cells (40) ↓ T cell proliferation (44) ↓ T _H 1 cells (49)	Prevented disease, but no effect after disease onset (43, 47) No suppression (48, 50, 51) Reduced pathology but no effect on survival (49)
Experimental autoimmune encephalomyelitis	↓ T cell responsiveness (58) ↓ CNS infiltration (59-61, 64, 68) ↓ Auto-antibodies (59) ↓ Inflammatory cytokines (60, 64, 68) T _H 1 → T _H 2 (61) ↓ T _H 17 cells (64)	None reported
Inflammatory bowel disease	↓ Inflammatory T cells (72, 73, 78) ↓ Inflammatory cytokines (72-76) ↑ Anti-inflammatory cytokines (72-75, 78) ↓ Intestinal CD4 ⁺ T cell infiltration (72-74) ↑ Regulatory T cells (72-75, 78) ↓ Growth factor expression (76) ↑ FasL-mediated T cell apoptosis (78) ↓ T cell responsiveness (73)	None reported
Systemic lupus erythematosus	↓ Anti-DNA antibodies (82-85) ↑ Regulatory T cells (82) ↓ T cell frequency (83) ↓ T _H 17 cells (82) ↓ Plasma cells (82) ↓ Inflammatory cytokines (85) ↑ Anti-inflammatory cytokines (85)	Worsened disease, increased auto-antibodies (87)
Experimental autoimmune uveitis	↓ Inflammatory cytokines (93-95) ↑ IL-10 (93, 94) ↑ Regulatory T cells (92-95) ↓ Inflammatory T cells (95) ↓ T _H 1 cells (93) ↓ T _H 17 cells (93, 95) ↑ IL-10-producing B cells (93)	Not effective late in disease course, but effective before and shortly after disease onset (94)

MSC: Mesenchymal stromal cell.

lots may be the source of functional differences, as differences in immunosuppressive function can be seen even between MSC lots derived independently from the same mouse tissue in the same laboratory^[52]. This type of variation is likely to arise stochastically and therefore identifying biomarkers associated with *in vivo* MSC function may be useful in identifying particular lots of MSCs that are more likely to be effective *in vivo*. Mouse models provide a practical means of performing these experiments as human MSCs have proven effective in treating disease in many of these models.

While many immunosuppressive mechanisms have

been reported from *in vitro* studies of MSC function, far fewer have been identified using *in vivo* experiments^[96]. Several elegant studies reviewed here have shown that a particular mechanism is important for *in vivo* suppression in a particular disease model, but these mechanisms have yet to be confirmed in additional models^[23,34,65,77,78]. Indeed it may be the case that different immunosuppressive mechanisms are important in different disease models, and that MSC lots effective at suppressing one immune-mediated disorder are not necessarily effective in other models. Further work is required to establish if certain immunosuppressive

mechanisms are essential in multiple disease models, or if MSC immunosuppression is mediated by unique mechanisms depending upon the inflammatory context.

Collectively, the studies reviewed above demonstrate that MSC-based therapies are generally effective in treating animal models of several inflammatory conditions. Although most studies observed an immunosuppressive effect of MSC treatment, a few did not—mirroring the overall effectiveness seen in clinical trials of human MSC treatments. Understanding the sources of these inconsistent results may lead to means of improving the reliability of MSC-based therapies. Mouse models of immune-mediated disease provide a practical means of studying the variability of MSC immunosuppressive function and identifying general and model-specific mechanisms of immune suppression essential for effective MSC-based immunotherapy.

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REFERENCES

- Pittenger MF**, Mackay AM, Beck SC, Jaiswal RK, Douglas R, Mosca JD, Moorman MA, Simonetti DW, Craig S, Marshak DR. Multilineage potential of adult human mesenchymal stem cells. *Science* 1999; **284**: 143-147 [PMID: 10102814 DOI: 10.1126/science.284.5411.143]
- English K**. Mechanisms of mesenchymal stromal cell immunomodulation. *Immunol Cell Biol* 2013; **91**: 19-26 [PMID: 23090487 DOI: 10.1038/icb.2012.56]
- Hoogduijn MJ**, Crop MJ, Peeters AM, Van Osch GJ, Balk AH, Ijzermans JN, Weimar W, Baan CC. Human heart, spleen, and perirenal fat-derived mesenchymal stem cells have immunomodulatory capacities. *Stem Cells Dev* 2007; **16**: 597-604 [PMID: 17784833 DOI: 10.1089/scd.2006.0110]
- De Ugarte DA**, Morizono K, Elbarbary A, Alfonso Z, Zuk PA, Zhu M, Dragoo JL, Ashjian P, Thomas B, Benhaim P, Chen I, Fraser J, Hedrick MH. Comparison of multi-lineage cells from human adipose tissue and bone marrow. *Cells Tissues Organs* 2003; **174**: 101-109 [PMID: 12835573 DOI: 10.1159/000071150]
- Gronthos S**, Mankani M, Brahimi J, Robey PG, Shi S. Postnatal human dental pulp stem cells (DPSCs) in vitro and in vivo. *Proc Natl Acad Sci USA* 2000; **97**: 13625-13630 [PMID: 11087820 DOI: 10.1073/pnas.240309797]
- Jiang Y**, Jahagirdar BN, Reinhardt RL, Schwartz RE, Keene CD, Ortiz-Gonzalez XR, Reyes M, Lenvik T, Lund T, Blackstad M, Du J, Aldrich S, Lisberg A, Low WC, Largaespada DA, Verfaillie CM. Pluripotency of mesenchymal stem cells derived from adult marrow. *Nature* 2002; **418**: 41-49 [PMID: 12077603 DOI: 10.1038/nature00870]
- Bruder SP**, Jaiswal N, Haynesworth SE. Growth kinetics, self-renewal, and the osteogenic potential of purified human mesenchymal stem cells during extensive subcultivation and following cryopreservation. *J Cell Biochem* 1997; **64**: 278-294 [PMID: 9027588 DOI: 10.1002/(SICI)1097-4644(199702)64:2<278::AID-JCB11>3.0.CO;2-F]
- Prockop DJ**. Repair of tissues by adult stem/progenitor cells (MSCs): controversies, myths, and changing paradigms. *Mol Ther* 2009; **17**: 939-946 [PMID: 19337235 DOI: 10.1038/mt.2009.62]
- Frenette PS**, Pinho S, Lucas D, Scheiermann C. Mesenchymal stem cell: keystone of the hematopoietic stem cell niche and a stepping-stone for regenerative medicine. *Annu Rev Immunol* 2013; **31**: 285-316 [PMID: 23298209 DOI: 10.1146/annurev-immunol-032712-095919]
- Griffin MD**, Elliman SJ, Cahill E, English K, Ceredig R, Ritter T. Concise review: adult mesenchymal stromal cell therapy for inflammatory diseases: how well are we joining the dots? *Stem Cells* 2013; **31**: 2033-2041 [PMID: 23766124 DOI: 10.1002/stem.1452]
- Tyndall A**. Successes and failures of stem cell transplantation in autoimmune diseases. *Hematology Am Soc Hematol Educ Program* 2011; **2011**: 280-284 [PMID: 22160046 DOI: 10.1182/asheducation-2011.1.280]
- Sharma RR**, Pollock K, Hubel A, McKenna D. Mesenchymal stem or stromal cells: a review of clinical applications and manufacturing practices. *Transfusion* 2014; **54**: 1418-1437 [PMID: 24898458 DOI: 10.1111/trf.12421]
- van Belle TL**, Coppieters KT, von Herrath MG. Type 1 diabetes: etiology, immunology, and therapeutic strategies. *Physiol Rev* 2011; **91**: 79-118 [PMID: 21248163 DOI: 10.1152/physrev.00003.2010]
- Leiter EH**, Schile A. Genetic and Pharmacologic Models for Type 1 Diabetes. *Curr Protoc Mouse Biol* 2013; **3**: 9-19 [PMID: 24592352 DOI: 10.1002/9780470942390.mo120154]
- Madec AM**, Mallone R, Afonso G, Abou Mrad E, Mesnier A, Eljaafari A, Thivolet C. Mesenchymal stem cells protect NOD mice from diabetes by inducing regulatory T cells. *Diabetologia* 2009; **52**: 1391-1399 [PMID: 19421731 DOI: 10.1007/s00125-009-1374-z]
- Fiorina P**, Jurewicz M, Augello A, Vergani A, Dada S, La Rosa S, Selig M, Godwin J, Law K, Placidi C, Smith RN, Capella C, Rodig S, Adra CN, Atkinson M, Sayegh MH, Abdi R. Immunomodulatory function of bone marrow-derived mesenchymal stem cells in experimental autoimmune type 1 diabetes. *J Immunol* 2009; **183**: 993-1004 [PMID: 19561093 DOI: 10.4049/jimmunol.0900803]
- Bassi ÈJ**, Moraes-Vieira PM, Moreira-Sá CS, Almeida DC, Vieira LM, Cunha CS, Hiyane MI, Basso AS, Pacheco-Silva A, Câmara NO. Immune regulatory properties of allogeneic adipose-derived mesenchymal stem cells in the treatment of experimental autoimmune diabetes. *Diabetes* 2012; **61**: 2534-2545 [PMID: 22688334 DOI: 10.2337/db11-0844]
- Ezquer FE**, Ezquer ME, Parrau DB, Carpio D, Yañez AJ, Conget PA. Systemic administration of multipotent mesenchymal stromal cells reverts hyperglycemia and prevents nephropathy in type 1 diabetic mice. *Biol Blood Marrow Transplant* 2008; **14**: 631-640 [PMID: 18489988 DOI: 10.1016/j.bbmt.2008.01.006]
- Boumaza I**, Srinivasan S, Witt WT, Feghali-Bostwick C, Dai Y, Garcia-Ocana A, Feili-Hariri M. Autologous bone marrow-derived rat mesenchymal stem cells promote PDX-1 and insulin expression in the islets, alter T cell cytokine pattern and preserve regulatory T cells in the periphery and induce sustained normoglycemia. *J Autoimmun* 2009; **32**: 33-42 [PMID: 19062254 DOI: 10.1016/j.jaut.2008.10.004]
- Lee RH**, Seo MJ, Reger RL, Spees JL, Pulin AA, Olson SD, Prockop DJ. Multipotent stromal cells from human marrow home to and promote repair of pancreatic islets and renal glomeruli in diabetic NOD/scid mice. *Proc Natl Acad Sci USA* 2006; **103**: 17438-17443 [PMID: 17088535 DOI: 10.1073/pnas.0608249103]
- Chhabra P**, Brayman KL. Current status of immunomodulatory and cellular therapies in preclinical and clinical islet transplantation. *J Transplant* 2011; **2011**: 637692 [PMID: 22046502 DOI: 10.1155/2011/637692]
- Solari MG**, Srinivasan S, Boumaza I, Unadkat J, Harb G, Garcia-Ocana A, Feili-Hariri M. Marginal mass islet transplantation with autologous mesenchymal stem cells promotes long-term islet allograft survival and sustained normoglycemia. *J Autoimmun* 2009; **32**: 116-124 [PMID: 19217258 DOI: 10.1016/j.jaut.2009.01.003]
- Ding Y**, Xu D, Feng G, Bushell A, Muschel RJ, Wood KJ. Mesenchymal stem cells prevent the rejection of fully allogeneic islet grafts by the immunosuppressive activity of matrix metalloproteinase-2 and -9. *Diabetes* 2009; **58**: 1797-1806 [PMID: 19509016 DOI: 10.2337/db09-0317]
- Berman DM**, Willman MA, Han D, Kleiner G, Kenyon NM,

- Cabrera O, Karl JA, Wiseman RW, O'Connor DH, Bartholomew AM, Kenyon NS. Mesenchymal stem cells enhance allogeneic islet engraftment in nonhuman primates. *Diabetes* 2010; **59**: 2558-2568 [PMID: 20622174 DOI: 10.2337/db10-0136]
- 25 **Holmdahl R**, Malmström V, Burkhardt H. Autoimmune priming, tissue attack and chronic inflammation - the three stages of rheumatoid arthritis. *Eur J Immunol* 2014; **44**: 1593-1599 [PMID: 24737176 DOI: 10.1002/eji.201444486]
- 26 **Brand DD**, Latham KA, Rosloniec EF. Collagen-induced arthritis. *Nat Protoc* 2007; **2**: 1269-1275 [PMID: 17546023 DOI: 10.1038/nprot.2007.173]
- 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 [PMID: 17393437 DOI: 10.1002/art.22511]
- 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 [PMID: 19333946 DOI: 10.1002/art.24405]
- 29 **Mao F**, Xu WR, Qian H, Zhu W, Yan YM, Shao QX, Xu HX. Immunosuppressive effects of mesenchymal stem cells in collagen-induced mouse arthritis. *Inflamm Res* 2010; **59**: 219-225 [PMID: 19763787 DOI: 10.1007/s00011-009-0090-y]
- 30 **Liu Y**, Mu R, Wang S, Long L, Liu X, Li R, Sun J, Guo J, Zhang X, Guo J, Yu P, Li C, Liu X, Huang Z, Wang D, Li H, Gu Z, Liu B, Li Z. Therapeutic potential of human umbilical cord mesenchymal stem cells in the treatment of rheumatoid arthritis. *Arthritis Res Ther* 2010; **12**: R210 [PMID: 21080925 DOI: 10.1186/ar3187]
- 31 **Kehe O**, Cartwright A, Askari A, El Haj AJ, Middleton J. Intra-articular injection of mesenchymal stem cells leads to reduced inflammation and cartilage damage in murine antigen-induced arthritis. *J Transl Med* 2014; **12**: 157 [PMID: 24893776 DOI: 10.1186/1479-5876-12-157]
- 32 **Djouad F**, Fritz V, Apparailly F, Louis-Pence 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 [PMID: 15880818 DOI: 10.1002/art.21012]
- 33 **Schurgers E**, Kelchtermans H, Mitera T, Geboes L, Matthys P. Discrepancy between the in vitro and in vivo effects of murine mesenchymal stem cells on T-cell proliferation and collagen-induced arthritis. *Arthritis Res Ther* 2010; **12**: R31 [PMID: 20175883 DOI: 10.1186/ar2939]
- 34 **Bouffi C**, Bony C, Courties G, Jorgensen C, Noël D. IL-6-dependent PGE2 secretion by mesenchymal stem cells inhibits local inflammation in experimental arthritis. *PLoS One* 2010; **5**: e14247 [PMID: 21151872 DOI: 10.1371/journal.pone.0014247]
- 35 **Coplan EA**. Hematopoietic stem-cell transplantation. *N Engl J Med* 2006; **354**: 1813-1826 [PMID: 16641398 DOI: 10.1056/NEJMra052638]
- 36 **Socié G**, Ritz J. Current issues in chronic graft-versus-host disease. *Blood* 2014; **124**: 374-384 [PMID: 24914139 DOI: 10.1182/blood-2014-01-514752]
- 37 **Schroeder MA**, DiPersio JF. Mouse models of graft-versus-host disease: advances and limitations. *Dis Model Mech* 2011; **4**: 318-333 [PMID: 21558065 DOI: 10.1242/dmm.006668]
- 38 **Miyashima S**, Nagata N, Nakagawa T, Hosaka N, Takeuchi K, Ogawa R, Ikehara S. Prevention of lpr-graft-versus-host disease and transfer of autoimmune diseases in normal C57BL/6 mice by transplantation of bone marrow cells plus bones (stromal cells) from MRL/lpr mice. *J Immunol* 1996; **156**: 79-84 [PMID: 8598497]
- 39 **Chung NG**, Jeong DC, Park SJ, Choi BO, Cho B, Kim HK, Chun CS, Won JH, Han CW. Cotransplantation of marrow stromal cells may prevent lethal graft-versus-host disease in major histocompatibility complex mismatched murine hematopoietic stem cell transplantation. *Int J Hematol* 2004; **80**: 370-376 [PMID: 15615264 DOI: 10.1532/IJH97.A30409]
- 40 **Joo SY**, Cho KA, Jung YJ, Kim HS, Park SY, Choi YB, Hong KM, Woo SY, Seoh JY, Cho SJ, Ryu KH. Mesenchymal stromal cells inhibit graft-versus-host disease of mice in a dose-dependent manner. *Cytotherapy* 2010; **12**: 361-370 [PMID: 20078382 DOI: 10.3109/14653240903502712]
- 41 **Yañez R**, Lamana ML, García-Castro J, Colmenero I, Ramírez M, Bueren JA. Adipose tissue-derived mesenchymal stem cells have in vivo immunosuppressive properties applicable for the control of the graft-versus-host disease. *Stem Cells* 2006; **24**: 2582-2591 [PMID: 16873762 DOI: 10.1634/stemcells.2006-0228]
- 42 **Polchert D**, Sobinsky J, Douglas G, Kidd M, Moadsiri A, Reina E, Genrich K, Mehrotra S, Setty S, Smith B, Bartholomew A. IFN-gamma activation of mesenchymal stem cells for treatment and prevention of graft versus host disease. *Eur J Immunol* 2008; **38**: 1745-1755 [PMID: 18493986 DOI: 10.1002/eji.200738129]
- 43 **Tisato V**, Naresh K, Girdlestone J, Navarrete C, Dazzi F. Mesenchymal stem cells of cord blood origin are effective at preventing but not treating graft-versus-host disease. *Leukemia* 2007; **21**: 1992-1999 [PMID: 17625609 DOI: 10.1038/sj.leu.2404847]
- 44 **Tobin LM**, Healy ME, English K, Mahon BP. Human mesenchymal stem cells suppress donor CD4(+) T cell proliferation and reduce pathology in a humanized mouse model of acute graft-versus-host disease. *Clin Exp Immunol* 2013; **172**: 333-348 [PMID: 23574329 DOI: 10.1111/cei.12056]
- 45 **Gregoire-Gauthier J**, Selleri S, Fontaine F, Dieng MM, Patey N, Despars G, Beauséjour CM, Haddad E. Therapeutic efficacy of cord blood-derived mesenchymal stromal cells for the prevention of acute graft-versus-host disease in a xenogenic mouse model. *Stem Cells Dev* 2012; **21**: 1616-1626 [PMID: 21910645 DOI: 10.1089/scd.2011.0413]
- 46 **Brehm MA**, Wiles MV, Greiner DL, Shultz LD. Generation of improved humanized mouse models for human infectious diseases. *J Immunol Methods* 2014; **410**: 3-17 [PMID: 24607601 DOI: 10.1016/j.jim.2014.02.011]
- 47 **Badillo AT**, Peranteau WH, Heaton TE, Quinn C, Flake AW. Murine bone marrow derived stromal progenitor cells fail to prevent or treat acute graft-versus-host disease. *Br J Haematol* 2008; **141**: 224-234 [PMID: 18318768 DOI: 10.1111/j.1365-2141.2008.07040.x]
- 48 **Jeon MS**, Lim HJ, Yi TG, Im MW, Yoo HS, Choi JH, Choi EY, Song SU. Xenoreactivity of human clonal mesenchymal stem cells in a major histocompatibility complex-matched allogeneic graft-versus-host disease mouse model. *Cell Immunol* 2010; **261**: 57-63 [PMID: 20004369 DOI: 10.1016/j.cellimm.2009.11.001]
- 49 **Lu X**, Liu T, Gu L, Huang C, Zhu H, Meng W, Xi Y, Li S, Liu Y. Immunomodulatory effects of mesenchymal stem cells involved in favoring type 2 T cell subsets. *Transpl Immunol* 2009; **22**: 55-61 [PMID: 19695330 DOI: 10.1016/j.trim.2009.08.002]
- 50 **Min CK**, Kim BG, Park G, Cho B, Oh IH. IL-10-transduced bone marrow mesenchymal stem cells can attenuate the severity of acute graft-versus-host disease after experimental allogeneic stem cell transplantation. *Bone Marrow Transplant* 2007; **39**: 637-645 [PMID: 17369865 DOI: 10.1038/sj.bmt.1705644]
- 51 **Sudres M**, Norol F, Trenado A, Grégoire S, Charlotte F, Levacher B, Lataillade JJ, Bourin P, Holy X, Vernant JP, Klatzmann D, Cohen JL. Bone marrow mesenchymal stem cells suppress lymphocyte proliferation in vitro but fail to prevent graft-versus-host disease in mice. *J Immunol* 2006; **176**: 7761-7767 [PMID: 16751424 DOI: 10.4049/jimmunol.176.12.7761]
- 52 **Yoo HS**, Yi T, Cho YK, Kim WC, Song SU, Jeon MS. Mesenchymal Stem Cell Lines Isolated by Different Isolation Methods Show Variations in the Regulation of Graft-versus-host Disease. *Immune Netw* 2013; **13**: 133-140 [PMID: 24009540 DOI: 10.4110/in.2013.13.4.133]
- 53 **Christensen ME**, Turner BE, Sinfield LJ, Kollar K, Cullup H, Waterhouse NJ, Hart DN, Atkinson K, Rice AM. Mesenchymal stromal cells transiently alter the inflammatory milieu post-transplant to delay graft-versus-host disease. *Haematologica* 2010; **95**: 2102-2110 [PMID: 20801899 DOI: 10.3324/haematol.2010.028910]
- 54 **Nylander A**, Hafler DA. Multiple sclerosis. *J Clin Invest* 2012; **122**:

- 1180-1188 [PMID: 22466660 DOI: 10.1172/JCI58649]
- 55 **Sawcer S**, Franklin RJ, Ban M. Multiple sclerosis genetics. *Lancet Neurol* 2014; **13**: 700-709 [PMID: 24852507 DOI: 10.1016/S1474-4422(14)70041-9]
- 56 **Rao P**, Segal BM. Experimental autoimmune encephalomyelitis. *Methods Mol Biol* 2012; **900**: 363-380 [PMID: 22933079 DOI: 10.1007/978-1-60761-720-4_18]
- 57 **Constantinescu CS**, Farooqi N, O'Brien K, Gran B. Experimental autoimmune encephalomyelitis (EAE) as a model for multiple sclerosis (MS). *Br J Pharmacol* 2011; **164**: 1079-1106 [PMID: 21371012 DOI: 10.1111/j.1476-5381.2011.01302.x]
- 58 **Zappia E**, Casazza S, Pedemonte E, Benvenuto F, Bonanni I, Gerdoni E, Giunti D, Ceravolo A, Cazzanti F, Frassoni F, Mancardi G, Uccelli A. Mesenchymal stem cells ameliorate experimental autoimmune encephalomyelitis inducing T-cell anergy. *Blood* 2005; **106**: 1755-1761 [PMID: 15905186 DOI: 10.1182/blood-2005-04-1496]
- 59 **Gerdoni E**, Gallo B, Casazza S, Musio S, Bonanni I, Pedemonte E, Mantegazza R, Frassoni F, Mancardi G, Pedotti R, Uccelli A. Mesenchymal stem cells effectively modulate pathogenic immune response in experimental autoimmune encephalomyelitis. *Ann Neurol* 2007; **61**: 219-227 [PMID: 17387730 DOI: 10.1002/ana.21076]
- 60 **Rafei M**, Birman E, Forner K, Galipeau J. Allogeneic mesenchymal stem cells for treatment of experimental autoimmune encephalomyelitis. *Mol Ther* 2009; **17**: 1799-1803 [PMID: 19602999 DOI: 10.1038/mt.2009.157]
- 61 **Bai L**, Lennon DP, Eaton V, Maier K, Caplan AI, Miller SD, Miller RH. Human bone marrow-derived mesenchymal stem cells induce Th2-polarized immune response and promote endogenous repair in animal models of multiple sclerosis. *Glia* 2009; **57**: 1192-1203 [PMID: 19191336 DOI: 10.1002/glia.20841]
- 62 **Gordon D**, Pavlovskaya G, Glover CP, Uney JB, Wraith D, Scolding NJ. Human mesenchymal stem cells abrogate experimental allergic encephalomyelitis after intraperitoneal injection, and with sparse CNS infiltration. *Neurosci Lett* 2008; **448**: 71-73 [PMID: 18950680 DOI: 10.1016/j.neulet.2008.10.040]
- 63 **Payne NL**, Dantanarayana A, Sun G, Moussa L, Caine S, McDonald C, Herszfeld D, Bernard CC, Siatskas C. Early intervention with gene-modified mesenchymal stem cells overexpressing interleukin-4 enhances anti-inflammatory responses and functional recovery in experimental autoimmune demyelination. *Cell Adh Migr* 2009; **6**: 179-189 [PMID: 22568986 DOI: 10.4161/cam.20341]
- 64 **Rafei M**, Campeau PM, Aguilar-Mahecha A, Buchanan M, Williams P, Birman E, Yuan S, Young YK, Boivin MN, Forner K, Basik M, Galipeau J. Mesenchymal stromal cells ameliorate experimental autoimmune encephalomyelitis by inhibiting CD4 Th17 T cells in a CC chemokine ligand 2-dependent manner. *J Immunol* 2009; **182**: 5994-6002 [PMID: 19414750 DOI: 10.4049/jimmunol.0803962]
- 65 **Rafei M**, Hsieh J, Fortier S, Li M, Yuan S, Birman E, Forner K, Boivin MN, Doody K, Tremblay M, Annabi B, Galipeau J. Mesenchymal stromal cell-derived CCL2 suppresses plasma cell immunoglobulin production via STAT3 inactivation and PAX5 induction. *Blood* 2008; **112**: 4991-4998 [PMID: 18812467 DOI: 10.1182/blood-2008-07-166892]
- 66 **Huang DR**, Wang J, Kivisakk P, Rollins BJ, Ransohoff RM. Absence of monocyte chemoattractant protein 1 in mice leads to decreased local macrophage recruitment and antigen-specific T helper cell type 1 immune response in experimental autoimmune encephalomyelitis. *J Exp Med* 2001; **193**: 713-726 [PMID: 11257138 DOI: 10.1084/jem.193.6.713]
- 67 **Jee Y**, Yoon WK, Okura Y, Tanuma N, Matsumoto Y. Upregulation of monocyte chemoattractant protein-1 and CC chemokine receptor 2 in the central nervous system is closely associated with relapse of autoimmune encephalomyelitis in Lewis rats. *J Neuroimmunol* 2002; **128**: 49-57 [PMID: 12098510 DOI: 10.1016/S0165-5728(02)00147-9]
- 68 **Zhang X**, Bowles AC, Semon JA, Scruggs BA, Zhang S, Strong AL, Gimble JM, Bunnell BA. Transplantation of autologous adipose stem cells lacks therapeutic efficacy in the experimental autoimmune encephalomyelitis model. *PLoS One* 2014; **9**: e85007 [PMID: 24465465 DOI: 10.1371/journal.pone.0085007]
- 69 **Abraham C**, Cho JH. Inflammatory bowel disease. *N Engl J Med* 2009; **361**: 2066-2078 [PMID: 19923578 DOI: 10.1056/NEJMra0804647]
- 70 **Corridoni D**, Arseneau KO, Cominelli F. Inflammatory bowel disease. *Immunol Lett* 2014; **161**: 231-235 [PMID: 24938525 DOI: 10.1016/j.imlet.2014.04.004]
- 71 **Low D**, Nguyen DD, Mizoguchi E. Animal models of ulcerative colitis and their application in drug research. *Drug Des Devel Ther* 2013; **7**: 1341-1357 [PMID: 24250223 DOI: 10.2147/DDDT.S40107]
- 72 **Gonzalez-Rey E**, Anderson P, González MA, Rico L, Büscher D, Delgado M. Human adult stem cells derived from adipose tissue protect against experimental colitis and sepsis. *Gut* 2009; **58**: 929-939 [PMID: 19136511 DOI: 10.1136/gut.2008.168534]
- 73 **González MA**, Gonzalez-Rey E, Rico L, Büscher D, Delgado M. Adipose-derived mesenchymal stem cells alleviate experimental colitis by inhibiting inflammatory and autoimmune responses. *Gastroenterology* 2009; **136**: 978-989 [PMID: 19135996 DOI: 10.1053/j.gastro.2008.11.041]
- 74 **Zhang Q**, Shi S, Liu Y, Uyanne J, Shi Y, Shi S, Le AD. Mesenchymal stem cells derived from human gingiva are capable of immunomodulatory functions and ameliorate inflammation-related tissue destruction in experimental colitis. *J Immunol* 2009; **183**: 7787-7798 [PMID: 19923445 DOI: 10.4049/jimmunol.0902318]
- 75 **Kim HS**, Shin TH, Lee BC, Yu KR, Seo Y, Lee S, Seo MS, Hong IS, Choi SW, Seo KW, Núñez G, Park JH, Kang KS. Human umbilical cord blood mesenchymal stem cells reduce colitis in mice by activating NOD2 signaling to COX2. *Gastroenterology* 2013; **145**: 1392-1403.e1-8 [PMID: 23973922 DOI: 10.1053/j.gastro.2013.08.033]
- 76 **Tanaka F**, Tominaga K, Ochi M, Tanigawa T, Watanabe T, Fujiwara Y, Ohta K, Oshitani N, Higuchi K, Arakawa T. Exogenous administration of mesenchymal stem cells ameliorates dextran sulfate sodium-induced colitis via anti-inflammatory action in damaged tissue in rats. *Life Sci* 2008; **83**: 771-779 [PMID: 18950645 DOI: 10.1016/j.lfs.2008.09.016]
- 77 **Zhao Y**, Wang L, Jin Y, Shi S. Fas ligand regulates the immunomodulatory properties of dental pulp stem cells. *J Dent Res* 2012; **91**: 948-954 [PMID: 22904205 DOI: 10.1177/0022034512458690]
- 78 **Akiyama K**, Chen C, Wang D, Xu X, Qu C, Yamaza T, Cai T, Chen W, Sun L, Shi S. Mesenchymal-stem-cell-induced immunoregulation involves FAS-ligand-/FAS-mediated T cell apoptosis. *Cell Stem Cell* 2012; **10**: 544-555 [PMID: 22542159 DOI: 10.1016/j.stem.2012.03.007]
- 79 **Green DR**, Droin N, Pinkoski M. Activation-induced cell death in T cells. *Immunol Rev* 2003; **193**: 70-81 [PMID: 12752672 DOI: 10.1034/j.1600-065X.2003.00051.x]
- 80 **Tsokos GC**. Systemic lupus erythematosus. *N Engl J Med* 2011; **365**: 2110-2121 [PMID: 22129255 DOI: 10.1056/NEJMra1100359]
- 81 **Peng SL**. Experimental use of mouse models of systemic lupus erythematosus. *Methods Mol Biol* 2012; **900**: 135-168 [PMID: 22933068 DOI: 10.1007/978-1-60761-720-4_7]
- 82 **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 [PMID: 19489103 DOI: 10.1002/stem.68]
- 83 **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 [PMID: 19118507 DOI: 10.1038/cmi.2008.52]
- 84 **Gu Z**, Akiyama K, Ma X, Zhang H, Feng X, Yao G, Hou Y, Lu L, Gilkeson GS, Silver RM, Zeng X, Shi S, Sun L. Transplantation of umbilical cord mesenchymal stem cells alleviates lupus nephritis in

- MRL/lpr mice. *Lupus* 2010; **19**: 1502-1514 [PMID: 20647254 DOI: 10.1177/0961203310373782]
- 85 **Chang JW**, Hung SP, Wu HH, Wu WM, Yang AH, Tsai HL, Yang LY, Lee OK. Therapeutic effects of umbilical cord blood-derived mesenchymal stem cell transplantation in experimental lupus nephritis. *Cell Transplant* 2011; **20**: 245-257 [PMID: 20719085 DOI: 10.3727/096368910X520056]
- 86 **Kimbrel EA**, Kouris NA, Yavarian GJ, Chu J, Qin Y, Chan A, Singh RP, McCurdy D, Gordon L, Levinson RD, Lanza R. Mesenchymal stem cell population derived from human pluripotent stem cells displays potent immunomodulatory and therapeutic properties. *Stem Cells Dev* 2014; **23**: 1611-1624 [PMID: 24650034 DOI: 10.1089/scd.2013.0554]
- 87 **Youd M**, Blickarz C, Woodworth L, Touzjian T, Edling A, Tedstone J, Ruzek M, Tubo R, Kaplan J, Lodie T. Allogeneic mesenchymal stem cells do not protect NZBxNZW F1 mice from developing lupus disease. *Clin Exp Immunol* 2010; **161**: 176-186 [PMID: 20456409 DOI: 10.1111/j.1365-2249.2010.04158.x]
- 88 **Selmi C**. Diagnosis and classification of autoimmune uveitis. *Autoimmun Rev* 2014; **13**: 591-594 [PMID: 24424168 DOI: 10.1016/j.autrev.2014.01.006]
- 89 **Luger D**, Silver PB, Tang J, Cua D, Chen Z, Iwakura Y, Bowman EP, Sgambellone NM, Chan CC, Caspi RR. Either a Th17 or a Th1 effector response can drive autoimmunity: conditions of disease induction affect dominant effector category. *J Exp Med* 2008; **205**: 799-810 [PMID: 18391061 DOI: 10.1084/jem.20071258]
- 90 **Yang MM**, Lai TY, Luk FO, Pang CP. The roles of genetic factors in uveitis and their clinical significance. *Retina* 2014; **34**: 1-11 [PMID: 23903794 DOI: 10.1097/IAE.0b013e31829f7415]
- 91 **Caspi RR**. Understanding autoimmunity in the eye: from animal models to novel therapies. *Discov Med* 2014; **17**: 155-162 [PMID: 24641958]
- 92 **Tasso R**, Ilengo C, Quarto R, Cancedda R, Caspi RR, Pennesi G. Mesenchymal stem cells induce functionally active T-regulatory lymphocytes in a paracrine fashion and ameliorate experimental autoimmune uveitis. *Invest Ophthalmol Vis Sci* 2012; **53**: 786-793 [PMID: 22232435 DOI: 10.1167/iops.11-8211]
- 93 **Oh JY**, Kim TW, Jeong HJ, Lee HJ, Ryu JS, Wee WR, Heo JW, Kim MK. Intraperitoneal infusion of mesenchymal stem/stromal cells prevents experimental autoimmune uveitis in mice. *Mediators Inflamm* 2014; **2014**: 624640 [PMID: 25136147 DOI: 10.1155/2014/624640]
- 94 **Zhang X**, Ren X, Li G, Jiao C, Zhang L, Zhao S, Wang J, Han ZC, Li X. Mesenchymal stem cells ameliorate experimental autoimmune uveoretinitis by comprehensive modulation of systemic autoimmunity. *Invest Ophthalmol Vis Sci* 2011; **52**: 3143-3152 [PMID: 21296818 DOI: 10.1167/iops.10-6334]
- 95 **Li G**, Yuan L, Ren X, Nian H, Zhang L, Han ZC, Li X, Zhang X. The effect of mesenchymal stem cells on dynamic changes of T cell subsets in experimental autoimmune uveoretinitis. *Clin Exp Immunol* 2013; **173**: 28-37 [PMID: 23607419 DOI: 10.1111/cei.12080]
- 96 **Ma S**, Xie N, Li W, Yuan B, Shi Y, Wang Y. Immunobiology of mesenchymal stem cells. *Cell Death Differ* 2014; **21**: 216-225 [PMID: 24185619 DOI: 10.1038/cdd.2013.158]

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