Hematopoetic and mesenchymal stem cell transplantation in the treatment of refractory systemic lupus erythematosus — Where are we now?

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Abstract  Cell based therapies are of increasing interest in the treatment of systemic lupus due to their potential for long term suppression or cure of disease. Two methods for stem cell transplantation are currently being investigated/performed for treatment of lupus. Autologous hematopoetic stem cell transplantation is used in patients refractory to standard therapy. The morbidity and mortality of the procedure limit its use to select patients. Results indicate 50% long term disease free survival. The technical difficulty of the procedure requires it to be performed only in experienced centers. Mesenchymal stem cell transplants are a new emerging therapy for the treatment of lupus. Studies in murine models of lupus provide evidence of efficacy with safety. Limited uncontrolled trials in humans provide evidence of efficacy as well. Controlled trials are needed to assess the efficacy of both these therapies compared to standard therapy.

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1. Introduction

Systemic lupus erythematosus (SLE) is a devastating autoimmune disease that targets multiple organ systems. SLE is characterized by autoantibody production, immune complex (IC) formation, chronic inflammation and end organ damage. Although the etiology of this disease is not completely understood, a great deal of evidence suggests that persistent immune cell activation due to inadequate immune surveillance/suppression is associated with the development of SLE [1,2]. Currently, treatment options for SLE are limited with notable side effects, thus significant effort is being expended in order to develop more effective therapeutic strategies [3]. Most treatment options for autoimmune diseases, including SLE, have a broad impact on the immune system and are not targeted to specific immune dysfunctions [4]. These types of therapeutics can interfere with the necessary functions of the immune system resulting in infections and malignancies, thus development of more specifically targeted therapies are of particular interest [3,4].

Studies in lupus prone mouse strains confirmed that systemic lupus is primarily a bone marrow derived disease as reconstituting a normal mouse with lupus derived bone marrow led to disease, while reconstituting a lupus mouse with marrow from a non-lupus strain resulted in prevention/resolution of disease [5,6]. Subsequently, in lupus patients receiving allogeneic bone marrow transplants for hematologic malignancies, lupus disease remission was often noted [7]. These findings in mice and humans suggested that stem cell or bone marrow abnormalities underlie lupus pathogenesis and that bone marrow/stem cell transplantation may serve as a new therapeutic approach in lupus. Recently, there is increasing interest in a different type of stem cell transplant in autoimmune diseases, including lupus. This approach uses mesenchymal stem cells rather than hematopoietic stem cells. Early uncontrolled studies suggest that this approach is also a promising therapeutic area for investigation in lupus [8]. This review will summarize the published literature to date on the success of bone marrow/stem cell transplant approaches for the treatment of lupus.

2. Hematopoietic bone marrow/stem cell transplantation

As noted above, there are a number of case reports of lupus patients and patients with other autoimmune diseases receiving allogeneic or autologous bone marrow transplants for treatment of malignancies [7]. These reports noted that often disease was put into remission for extended periods indicating a therapeutic benefit of the procedure. The immediate mortality rates and side effects of allogeneic and autologous bone marrow transplants, however, rendered allogeneic/autologous bone marrow transplantation’s risk to benefit ratio too high for consideration as therapy.

The advent of bone marrow stem cell transplants, with less treatment related mortality and side effects, provided a new avenue for therapeutic consideration in lupus patients [9]. In attempts to make the procedure even safer, investigators began using non-myeloablative conditioning regimens to lower the risk of infection and treatment related morbidity/mortality [10–12]. Reports in the literature indicate that over 200 patients received autologous stem cell transplants as therapy for their lupus [12]. In all cases, patients were refractory to aggressive standard of care measures. The response rate is similar between the reports with the majority of the patients coming from the European Bone Marrow Transplant group (n=85) and a single center experience at Northwestern United States (n=50) [12]. The conditioning regimen used at the different institutions varied, though cyclophosphamide and anti-thymocyte globulin were the most common agents used. The results were similar as far as efficacy is concerned between the institutions with approximately 50% achieving 5 year relapse free survival, with many of all medications [10–12]. In contrast, overall mortality varied significantly from 16 to 29% considering only studies with 10 or more patients. Transplant related mortality varied from 4 to 25% [10–12].

The treatment effect of the procedure is not from the stem cells, but the conditioning regimen that “resets” the bone marrow. Eliminating autoreactive lymphocytes with reconstitution of the immune system with naïve cells, allows the immune system to start over with a delay or prolonged interval to reemergence of autoimmunity [12]. The stem cells decrease the time of marrow recovery and infection risk.

A problem with autologous stem cell transplantation is patient selection. Due to the high risk of the procedure itself, only patients that have failed all conventional therapy are candidates. However, to survive the procedure, patients cannot have significant end organ damage. This difficulty in identifying the right patients for this procedure led to the failure of an NIH funded trial of autologous stem cell transplantation in lupus to enroll sufficient patients [12].

In summary, autologous bone marrow stem cell transplantation following non-myeloablative conditioning results in prolonged remissions in 50% of patients. Although procedure mortality rates remain significant, in selected patients it is a viable alternative. It must be emphasized, however, that the procedure should only be done in institutions with significant experience in performing the procedure, in consultation with experts in the management of severe lupus. Hopefully, newer conditioning regimens will be developed that will further lower the procedure mortality rate making it a treatment option for more patients with less severe disease.

3. Mesenchymal stem cell transplantation

A population of non-hematopoietic stem cells, mesenchymal stem cells (MSC), are of increasing interest as a therapeutic option for many autoimmunity diseases. MSCs are a multi-potent stem cell population that can differentiate into osteoblasts, chondrocytes, and adipocytes [13]. A desirable property of MSCs for therapeutic use is that they are a readily available stem cell population, as they can be derived from various sources such as bone marrow, umbilical cords, adipose tissue, and dental pulp [14]. In addition to the ready availability of MSCs, they have many known immuno-modulatory properties that would be predicted to be effective in developing a cellular therapy for SLE, along with other autoimmune diseases.
3.1. Immune cell suppression by MSCs

It is clear that MSCs are effective immunosuppressive cells, however, the mechanisms by which MSCs are able to elicit such effective immunosuppression are unclear, though they involve both the innate and adaptive arms of the immune system. Previous studies revealed suppressive functions of MSCs that include inhibiting the activity of T cells, B cells, natural killer (NK) cells, and antigen presenting cells (APC) [15]. MSCs may modulate the immune system by a variety of methods including, but not limited to, the secretion of anti-inflammatory cytokines, expansion of regulatory T cells, and down regulation of co-stimulatory molecules on APCs [16]. The dissection of these mechanisms by which MSCs suppress disease provides valuable insight into how this stem cell subset can be best utilized in cell therapy as well as insight into the pathogenesis of disease.

Although MSCs are able to suppress most cells of the immune system they are unable to do so without proper activation. Activation of MSCs occurs when they are within the appropriate pro-inflammatory cytokine microenvironment. Upon transfer, MSCs home to sites of inflammation (Fig. 1). MSCs express a wide variety of chemokine receptors such as CCR1, CCR3, CCR7, CCR8, CCR10, CCR11, CXCR3, CXCR4, and CXCR6 [17]. Furthermore, these chemokine receptors have ligands that are specific for certain tissues. Not all MSCs in a population will possess the same combination of chemokine receptors [18]. This heterogeneity of the MSC population is what gives MSCs the potential to home to diverse sites to perform their immune modulatory functions [19,20]. Concerns about trapping of MSCs in remote organs post transplantation occurred when studies in mice showed accumulation of cells in the lung [21]. Human MSCs, however, seem to act differently than those of mouse in that they are not retained in the lung permanently [22]. Human MSCs when transplanted into mice are trapped initially in the lung. Yet, soon after, MSCs were cleared and found in other organs. In fact, MSCs can be found up to a year post transfer in the skin, lung, kidney, liver, and thymus when these organs are analyzed via RT-PCR [23].

Once MSCs have homed to an inflamed tissue, they encounter the microenvironment that is essential to the development of their suppressive properties. Cytokines recognized to enhance the immuno-modulatory properties of MSCs include IFNγ, IL1β, and TNFα [24]. These cytokines are prevalent in areas of heightened inflammation where MSCs home upon transplantation. Numerous groups have shown that IFNγ is required to initiate the suppressive efficacy of MSCs [25,26]. Without the IFNγ induced activation of MSCs, they were less efficient suppressors of immune responses in graft versus host disease (GVHD) [25]. Furthermore, when MSCs were transferred into mice deficient in IFNγ the MSCs were unable to suppress GVHD [25]. Although IFNγ clearly is required for the heightened suppressive functions of MSCs, other pro-inflammatory cytokines have an additive effect on MSCs. MSCs activated with TNFα or IL1β, in addition to IFNγ, were significantly more immune suppressive than MSCs cultured with any one cytokine [24]. Furthermore, upon activation, MSCs produce various chemokines to attract immune cells. Among these chemokines are CXCL9 and CXCL10. Both of these chemokines are attractants for T cells and will draw these cells to the MSCs [24]. Once T cells are within range, MSCs can then suppress these activated cells. Mixed cytokine microenvironments are common at sites of inflammation, thus MSCs, once homed to the area of interest, will likely experience the appropriate cytokine milieu to become activated for suppression. As aforementioned MSCs may home to, and accumulate in, organs that are not the target of disease. Thus along with the secretion of chemo-attractants MSCs can also produce anti-inflammatory cytokines by which they can elicit their suppressive effects from a...
distance. TNF-α stimulated gene/protein 6 (TSG-6) is one of these suppressive cytokines. In vivo studies have shown the production of TSG-6 by MSCs that have accumulated in the lung has been sufficient to provide anti-inflammatory effects in the murine heart and cornea injury models [27,28].

Upon homing and activation, MSCs suppress the activation and proliferation of various immune cells. The interaction of MSCs and other immune cells is not a one way street however, as the immune cells also interact and influence the properties of the MSCs. The interactions of MSCs and T cells are the best defined mechanism by which MSCs influence the immune system. T cells are nearly ubiquitous at sites of inflammation and, in the instance of a type 1 inflammatory response, significant amounts of IFN-γ are made by Th1 cells [29]. Due to their ability to produce IFN-γ, Th1 cells play an important role in the activation of MSCs. Once activated, MSCs can effectively suppress proliferation of T cells in mixed lymphocyte reactions, when added at the initiation of culture or when added to an ongoing cell culture [30]. MSCs achieve T cell suppression by both direct and indirect methods. MSCs can produce various inhibitory cytokines or signaling molecules capable of inhibiting T cell responses. MSCs produce IL10, an anti-inflammatory cytokine, and induce regulatory APCs that assist in the inhibition of T cell activation [30]. A vital immunosuppressive protein produced by MSCs is inducible nitric oxide synthase (iNOS). iNOS facilitates the production of NO, which in turn, suppresses the activation of T cells putting them in a reversible anergic state [24]. Prostaglandin E2 (PGE2) and indoleamine 2,3-dioxygenase (IDO) are proteins also produced by MSCs that elicit an inhibitory effect on T cells as well as other immune players. MSCs readily produce these proteins upon activation and they have a synergistic effect on the suppression of T cells [31–33]. MSCs also promote the expansion of regulatory T cells (Tregs) [34]. A mechanism by which MSCs induce Tregs is by the productions of IDO [35]. The expansion of the Treg population, along with the production of the T cell inhibitory proteins, are methods by which MSCs are able to effectively mold a homeostatic immune environment through T cell regulation.

B cells play an important role in autoimmune diseases. In diseases such as lupus, B cells produce autoantibodies to an array of nuclear antigens, present antigen to T cells, secrete key cytokines and help to establish ectopic lymphoid activity, thus exacerbating disease [36]. MSCs impact B cells by inhibiting proliferation of B cells. Moreover, B cell differentiation and their chemotactic properties are also suppressed when cultured in the presence of activated MSCs [37]. Recent studies into the mechanism by which MSCs dampen B cell immune responses showed that the suppression is primarily achieved by soluble factors produced by MSCs. Contradicting the in vitro conclusions of MSCs’ suppressive influence on B cells are in vivo studies showing the inability of MSCs to decrease auto-antibody production and expansion of plasma cells [37,38]. Although this in vivo expansion of B cells could potentially exacerbate disease in lupus prone mice, significant histological improvements suggest that MSCs are still functional in ameliorating disease [37].

Peripheral tolerance, which is often broken in autoimmune diseases, is facilitated by APCs. Professional APCs include three main cell types: dendritic cells (DC), macrophages, and B cells. All of these cell types uptake and present antigen along with proper co-stimulation for T cells. T cells can then elicit an adaptive immune response. In lupus, presentation of self-antigens occurs via APCs resulting in a self-directed immune response by T cells [39]. MSCs interfere with antigen presentation of DCs, which are major activators of T cells [39]. Antigen presentation is hindered by MSCs by preventing the TNF-α induced maturation of DC. In addition to interference with antigen presentation, MSCs can also prevent the migration of DC. Chemokine receptor CCR7, whose ligand is present in the lymph nodes, and the epithelial anchoring protein E-cadherin play an important role in the migration of DCs to the lymph node. Hindrance of DC migration to lymph nodes is due to decreased expression of CCR7 and increased expression of E-cadherin, essentially stalling the DC from reaching the lymph node for proper T cell antigen presentation [39]. Without antigen presentation/co-stimulation to activate the T cell, an adaptive T cell immune response will not occur. In autoimmunity, this type of immune control can inhibit auto-reactive T cells from being activated towards a self-antigen.

MSCs have unique attributes beyond their suppressive abilities that make them attractive for cellular therapy. To begin, MSCs are immune privileged by avoiding, at least at first, allo-reaction [40]. Therefore the hurdle of finding an MHC compatible donor is not a concern in MSC transplantation. Unlike hematopoetic stem cells (HSC), MSCs can double 40–50 times allowing them to be cultured and expanded to numbers compatible donor is not a concern in MSC transplantation. MSCs are immune privileged by avoiding, at least at first, allo-reaction [40]. Therefore the hurdle of finding an MHC compatible donor is not a concern in MSC transplantation. Unlike hematopoetic stem cells (HSC), MSCs can double 40–50 times allowing them to be cultured and expanded to numbers suitable for transplantation from small initial cell numbers [41]. Lastly, MSCs home to areas of tissue injury [42]. These areas are the zones in which excessive inflammation is occurring. Upon transplantation, MSCs will home towards areas of inflammation, become activated in the pro-inflammatory environment, and exert their suppressive effects on the surrounding host immune cells.

3.2. MSC and lupus

The systemic autoimmunity seen in SLE is a combination of regulatory mechanisms gone awry, leading to a disease that has numerous components contributing to disease manifestations. Because of this multi-cell dysregulation, SLE treatments are difficult to develop because they require broad dampening of immune responsiveness often leading to undesirable side effects. New therapeutics that inhibits autoimmune cells, while allowing the rest of the immune system to still function, would be a major step forward. This potential of targeted cellular therapy is the attraction of MSCs for cellular therapy. MSCs have the in vitro and in vivo ability to promote immuno-modulatory effects without the infectious consequences often seen with extensive immune suppression. Because of MSC’s multi-cell suppressive capabilities, they have the potential to be a good cellular therapy for SLE.

To date, numerous studies have examined the effects of MSC transplantation on SLE and other autoimmune diseases. In murine collagen-induced arthritis, MSCs have shown conflicting results regarding their efficacy in disease prevention. Several studies showcase the effectiveness of MSC after the establishment of arthritic disease [43,44]. However, other studies with the CIA model have shown that no immunosuppressive effects are seen with MSC injection [45,46]. Regardless of the varied results seen in the CIA model of autoimmune disease, both murine and human in vivo studies suggested that
MSCs have the potential to be a beneficial treatment option for SLE. Allogeneic non-lupus prone MSC transplantation into murine models of lupus prolonged life and decreased disease severity, marked by reduction of serologic markers, glomerular immune complex deposition, and lymphocyte infiltration [37,47]. The profound impact of murine MSCs on SLE leads to the examination of the effects of human MSC (hMSC) transplantation into these same murine models. Upon transplantation into lupus prone mice, hMSC were also able to decrease disease severity [48,49]. Mice receiving hMSC also experienced reduced proteinuria, renal injury, anti-dsDNA antibodies, and pro-inflammatory cytokines. These mice also experienced an increase in their peripheral Treg population. Collectively, these murine studies suggested MSC transplantation to be a potential treatment in SLE patients.

In vitro studies with hMSC showed that they are effective in immune suppression. However, studies examining MSCs from SLE patients have suggested that they are not as effective as MSCs from normal patients [34]. SLE MSCs have various abnormalities such as slower growth in culture and early signs of senescence suggesting that they are not as likely to be efficacious as MSCs from healthy individuals [50,51]. Two female lupus patients receiving autologous MSCs experienced an increase in circulating Tregs and decreased peripheral blood lymphocyte function [52]. However, these two patients did not experience any beneficial effects on disease severity. These results imply that SLE-MSCs may lack in suppressive function in comparison to MSCs from non-lupus patients.

On the basis of the positive outcomes seen in SLE mice upon MSC transplantation, studies began investigating the effects of allogeneic MSC transplantation on patients with treatment refractory SLE. One study treated four cyclophosphamide (CTX)/glucocorticoid treatment-refractory SLE patients with allogeneic bone marrow derived MSCs (BM-MSC) and found that all patients experienced 12–18 months of disease remission and improved SLEDAI score with no serious adverse events from the treatment. Reduction of disease was marked by improved serologic markers and renal function [8]. A larger study group of 15 patients with SLE refractory to treatment received allogeneic BM-MSC transplants [53]. All patients experienced clinical improvement following treatment with decreases in proteinuria and anti-dsDNA levels. Both of these studies demonstrated the potential beneficial results of BM-MSC transplantation in human SLE. In all these patients, however, they continued on standard therapy with the possibility that the response seen was due to a delayed response to standard treatment rather than due to the MSCs.

Umbilical cord derived MSC (UC-MSC) also demonstrated a significant therapeutic effect in animal studies in lupus. To examine the effectiveness of UC-MSC in human disease, 16 patients with refractory SLE received UC-MSC transplantation [54]. Results from this study showed that UC-MSCs were successful in reducing SLE disease severity with improved disease activity scores. Patients also had significant improvements in serum titers of ANA and anti-dsDNA, increased serum albumin, and complement C3. In addition to the clinical improvement of disease, patients experienced an increase in peripheral Tregs along with a restoration of the Th1/Th2 cytokine balance.

Although these uncontrolled studies of MSCs in the treatment of lupus have shown promise, MSC have also been used in two large randomized controlled trials for GVHD and Crohn’s disease. 55 patients with steroid-resistant acute GVHD received bone marrow derived MSC from various donors. No side effects were seen during or immediately after the infusion of the allogeneic MSC infusion. 30 patients of the 55 had a complete response upon MSC infusion while another 9 showed improvement [55]. Another study of 10 Crohn's disease patients who were refractory to current medical treatments received autologous MSC. Their autologous MSCs were equally suppressive when compared to healthy donor MSCs. Regardless of in vitro suppressive capacity, results from this study were not significant enough to draw conclusions on efficacy of MSCs in Crohn's disease. 2 patients saw clinical improvement of disease while 3 patients had to undergo surgery due to worsened disease post infusion [56]. Results of these trials suggest the safety of using MSC from both autologous and allogeneic donors from possible treatment.

Notably, none of the MSC transplant studies to date in humans have conclusively shown treatment related side effects. This indicates that MSC transplantation may be a beneficial option for SLE, especially in patients refractory to current available treatments. None of these studies, however, were blinded or controlled and all patients continued to receive standard therapy. Studies in animal models suggest that side effects are plausible due to the multipotency of the MSCs. Concerns regarding their safety include transformation into osteoclasts in inappropriate locations, malignancy conversion, etc. Thus, though the data for MSC transplantation in lupus is hopeful, proof of efficacy remains to be proven in controlled trials comparing standard therapy versus the addition of MSC. Another concern is the possible tumor enhancing properties of MSCs. The tumor microenvironment often has a similar cytokine profile to that of an area of tissue damage. This cytokine milieu can attract MSCs to this area where they can elicit tumor enhancing effects [57]. In vitro work has shown that MSCs primed with IFN-γ can induce the death of various tumor cell lines. However, MSCs with or without IFN-γ priming accelerated tumor growth in vivo when co-transferred with tumor cell lines into nude mice [26]. These results suggest that MSC therapy would have to be reserved for patients that fit a specific list of criteria, including no history of tumors.

4. Future directions

It is clear that MSC transplantation has the potential for substantial therapeutic effects on patients with SLE. However, the mechanisms by which MSCs are eliciting their suppressive effects in vivo are still not completely understood. MSCs from various sources all appear to have immunomodulatory abilities albeit to varying degrees. Further examination of MSCs must be done to discern the potency of MSCs from these different sources. In addition to various sources, investigation of MSCs from healthy and diseased individuals must also be completed to determine if suppressive efficacy is hindered when the MSCs are from an autoimmune donor. Although various studies support the efficacy of autoimmune MSCs [56,58], conflicting results require more studies to establish whether SLE disease activity contributes to the reduction of suppressive capacity that has been noted in SLE-MSC. The source and donor of...
MSCs will likely prove to be of importance as future studies regarding MSC transplantation are performed. MSCs require a particular microenvironment in order to achieve maximum suppressive function. The appropriate activation microenvironment of MSC requires additional clarification since the environment that is essential to activate MSCs is also the pro-inflammatory environment that leads to the pathogenesis of autoimmune diseases. Questions arise such as to what point MSC activation should occur to promote their immuno-suppressive behavior once transplanted. Furthermore, the patient’s microenvironment may need to be assessed before transplantation to predict whether MSCs will retain suppressive function. Prediction of what patients will respond best to MSC treatment in addition to the initial efficacy of the MSCs is also something to be examined. An in vitro MSC marker correlating to the in vivo functionality of the cells is of particular need. Developing a read out to indicate in vitro the MSCs in vivo suppressive abilities will assist in determining what source/donor would be optimal for transplantation into a particular patient. Although further studies are necessary, current evidence suggests that MSCs will become a successful option for the treatment of SLE. Autologous hematopoietic stem cell transplantation remains a viable option for patients with severe disease refractory to standard therapy. Careful patient selection and performance only in experienced centers are crucial for proper use of this therapy.

Conflict of interest statement

The authors declare that there are no conflicts of interest.

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