Human mesenchymal stem cells suppress chronic airway inflammation in the murine ovalbumin asthma model

Tracey L. Bonfield,1 Mary Koloze,1 Donald P. Lennon,2 Brandon Zuchowski,1 Sung Eun Yang,2 and Arnold I. Caplan1

1Department of Pediatrics, and 2Skeletal Research Center, Department of Biology, Case Western Reserve University, Cleveland, Ohio

Submitted 29 May 2009; accepted in final form 3 September 2010

Bonfield TL, Koloze M, Lennon DP, Zuchowski B, Yang SE, Caplan AI. Human mesenchymal stem cells suppress chronic airway inflammation in the murine ovalbumin asthma model. Am J Physiol Lung Cell Mol Physiol 299:L760–L770, 2010. First published September 3, 2010; doi:10.1152/ajplung.00182.2009.—Allogeneic human mesenchymal stem cells (hMSCs) introduced intravenously can have profound anti-inflammatory activity resulting in suppression of graft vs. host disease as well as regenerative events in the case of stroke, infarct, spinal cord injury, meniscus regeneration, tendinitis, acute renal failure, and heart disease in human and animal models of these diseases. hMSCs produce bioactive factors that provide molecular cuing for: 1) immunosuppression of T cells; 2) antiscarring; 3) angiogenesis; 4) antiapoptosis; and 5) regeneration (i.e., mitotic for host-derived progenitor cells). Studies have shown that hMSCs have profound effects on the immune system and are well-tolerated and therapeutically active in immunocompetent rodent models of multiple sclerosis and stroke. Furthermore, intravenous administration of MSCs results in pulmonary localization. Asthma is a major debilitating pulmonary disease that impacts in excess of 150 million people in the world with uncontrolled asthma potentially leading to death. In addition, the socioeconomic impact of asthma-associated illnesses at the pediatric and adult level are in the millions of dollars in healthcare costs and lost days of work. hMSCs may provide a viable multi-action therapeutic for this inflammatory lung disease by secreting bioactive cytokines and proteins (12, 20, 32). It has been reported that allogeneic hMSCs appear to suppress graft vs. host disease (GvHD) (36) and Crohn’s disease as well as induce regenerative phenomena in the case of stroke, infarct, spinal cord injury, meniscus regeneration, tendinitis, acute renal failure, and heart disease in both human and animal models of disease (24). Furthermore, since hMSCs express no major histocompatibility complex (MHC) class II, they are a viable therapeutic across tissue typing (16, 35, 36). hMSCs produce large quantities of bioactive factors that provide molecular cuing for the pathway and activity status of the responding cells (13). These bioactive factors can be: 1) antiscarring; 2) angiogenic; 3) antiapoptotic; and 4) regenerative (i.e., mitotic for host-derived progenitor cells). As evidence of

ATHEMA IS A MAJOR DEBILITATING inflammatory lung disease with a tremendous socioeconomic impact in developing countries. Not only can uncontrolled asthma lead to excessive absenteeism from school and the workplace, but also it has major impact on healthcare costs and can result in death. Bronchial asthma has been characterized by chronic and allergic airway inflammation, which induces both cytological as well as histological changes in the airway structure over time (19). The pathogenic characteristics of allergic asthma are associated with airway inflammation and infiltration of mast cells, basophils, eosinophils, monocytes, and T helper type 2 lymphocytes along with the production of isotype-specific IgE (40). In the chronic stages of asthma, there is significant morbidity in lung structure leading to morphological destruction of lung tissue and airways. There are two basic kinds of medications for the treatment of asthma: 1) anti-inflammatory therapies used on a regular basis to prevent attacks, which include inhaled steroids, leukotriene inhibitors, anti-IgE therapy, and long-acting bronchodilators to help open airways (6); and 2) short-acting bronchodilators, which are rescue medications. Aerosolized corticosteroids are the standard anti-inflammatory medication for asthmatics and are generally effective for most, although there is a substantial number of asthmatics who remain symptomatic with this therapy (5). In addition, variability in immune challenge and response to therapy makes asthma a difficult disease to monitor and manage. New treatment options targeting the pathophysiological events that cause development and persistence of asthma are desired in patients with chronic asthmatic lung disease.

The pathogenesis of asthma can be classified as acute or chronic. Acute asthma is reflected in the immediate type of reactivity hallmarked by histamine release, airway reactivity, and bronchospasm (3, 41). Chronic asthma contains components of acute asthma but is prototyped by sustained inflammation and consistent uncontrolled lung remodeling in pursuit of lung repair (34). Lung remodeling results in tissue fibrosis, deposition of collagen, smooth muscle hypertrophy, and altered basal lung function (19). Treatment options described in the previous paragraph focus on minimizing both induced asthmatic exacerbation and the concurrent inflammation inherent in the response. Current therapeutics have the ability to minimize the inflammation in responsive individuals but do not have the capacity to decrease or reverse the ongoing remodeling process (5).

Human marrow-derived mesenchymal stem cells (hMSCs) are nonhematopoietic pericytes that have the ability to influence immune effector cell development, maturation, and function as well as alloreactive T cell responses, presumably through the production of bioactive cytokines and proteins (12, 20, 32). It has been reported that allogeneic hMSCs appear to suppress graft vs. host disease (GvHD) (36) and Crohn’s disease as well as induce regenerative phenomena in the case of stroke, infarct, spinal cord injury, meniscus regeneration, tendinitis, acute renal failure, and heart disease in both human and animal models of disease (24). Furthermore, since hMSCs express no major histocompatibility complex (MHC) class II, they are a viable therapeutic across tissue typing (16, 35, 36). hMSCs produce large quantities of bioactive factors that provide molecular cuing for the pathway and activity status of the responding cells (13). These bioactive factors can be: 1) antiscarring; 2) angiogenic; 3) antiapoptotic; and 4) regenerative (i.e., mitotic for host-derived progenitor cells). As evidence of
the profound effect of hMSCs on the immune system, our colleagues and others have reported that hMSCs are well-tolerated and therapeutically active in immunocompetent rodent models of multiple sclerosis (42, 43) and stroke (18, 36). Thus xenogeneic hMSCs repress host immunological surveillance in rodents. Furthermore, it has been shown that hMSCs given intravenously localize to the lung and thereby provide a local source of trophic factors in the pulmonary environment (30, 39).

Since it has been described that asthma is a disease of T cell hyperactivity and scarring, hMSCs may provide a viable multi- taction therapeutic either directly through cell contact or through the production of bioactive factors. The results of the studies reported here suggest the potential usefulness of hMSCs in the treatment of airway inflammation associated with severe uncontrolled chronic asthma.

MATERIALS AND METHODS

Mouse Model

All procedures involving mice were reviewed and approved by the Case Western Reserve University Institutional Animal Care and Use Committee (Case Western Reserve University Animal Assurance A3145-01 with IACUC 2009-012). BALB/c mice were purchased from The Jackson Laboratories (Bar Harbor, ME) and sensitized by intraperitoneal injections (100 μl) of 10 μg of ovalbumin emulsified in 1.5 mg of Al(OH)3. On day 14, mice were exposed to 1% wt/vol ovalbumin (9, 50) in PBS by intranasal challenge every other day for 4 wk. Sham sensitization and challenges were carried out with sterile PBS. During the last week of challenge (week 4), mice were given 1 × 106 hMSCs. Animals were harvested 7 days later. For each study, there were 7–10 animals in each group (ovalbumin-sensitized saline challenge with and without hMSCs, ovalbumin-sensitized ovalbumin challenge with and without hMSCs), which on death were separated into processing for inflammation (4–6 mice; see description below) or pathology (2–3 mice; see description below).

Marine Cell Source

Lung inflammation. Mice were injected subcutaneously with a lethal dose of ketamine (80 mg/kg) and xylazine (10 mg/kg). The thoracic cavity was opened, and the lungs were exposed. Bronchoalveolar lavage (BAL) was performed by inserting a cannula through a cut in the trachea into the bronchi and infusing 3 × 1-ml aliquots of warm PBS containing 0.2% lidocaine. The BAL fluid sample was recovered by aspirating the liquid with a syringe. Cells were separated from the lavage fluid, and differentials were evaluated with cytospins and Wright-Giemsa stains. Differential analysis included identifying macrophages, neutrophils, lymphocytes, eosinophils, or other cells such as basophils. The remaining cells not used for differential analysis were frozen for future analysis. Mean viability of the lavage cells used was >95% by trypan blue dye exclusion. For each of 8 different hMSC donors, 4–6 animals were evaluated for each group: saline challenge, saline challenge + hMSCs, saline challenge + bone marrow-derived macrophages (BMMs), ovalbumin challenge, ovalbumin challenge + hMSCs, and ovalbumin challenge + BMMs. BAL fluid was frozen at –80°C until assessment for cytokines.

Lung pathology. Concurrent animals for each of the 8 different hMSC donors were euthanized without BAL to preserve lung architecture. Paraformaldehyde-perfused lungs were sectioned at 4 μm and mounted for evaluation by hematoxylin and eosin (H&E) or trichrome stains. Sections were controlled for proximal airways for comparison purposes. For each experiment, 2–3 animals were evaluated for each group: saline challenge, saline challenge + hMSCs, saline challenge + BMMs, ovalbumin challenge, ovalbumin challenge + hMSCs, and ovalbumin challenge + BMMs. Quantitative measurements of inflammation in the tissue sections were made using Image-Pro Plus software (Media Cybernetics, Bethesda, MD). The inflammatory nuclei were identified based on pixel identification on histology sections post-H&E staining. Image analysis was extensively validated comparing differential vs. nuclei staining (data not shown). For each tissue section, 196 images were quantified using the scanning automation and the Image-Pro macro.

Systemic inflammation. Animals underwent cardiac puncture after completion of lavage for serum and plasma.

MSCs

Human marrow iliac aspires from healthy volunteers were used as the source of hMSCs as described elsewhere (38). Briefly, the marrow aspires were layered onto Percoll gradients, and the light cell fraction was retrieved, washed, and plated in DMEM containing 10% fetal bovine serum from highly assayed and selected sources (37, 38). Within 14 days, the colony-forming units that were selectively expanded to near confluence were then lifted from the culture dish with trypsin and replated at 1:3 to insure that they maintain active cell division. hMSCs were used at passage 2. The phenotype of the hMSCs was defined by evaluating 100 markers using fluorescent-activated cell sorting (FACS) as previously described (25, 28). The absence of CD34 and CD45 and the presence of SH2 (CD105), SH3/SH4 (CD73), cell surface protein Str-1, and others were used to identify hMSCs. Several in vitro assays for bone, cartilage, marrow stroma, and fat (26) and an in vivo assay (27) for the differentiation of bone in porous calcium phosphate ceramic cubes (3 mm) were used to test for the differentiation potential and purity of the hMSCs (38). In the experiments detailed here, hMSCs were harvested during log-growth at passage 2 and given in a single intravenous tail vein injection of 1 × 106 cells in 100 μl of sterile saline. hMSCs from 8 different donors were chosen based on their ceramic cube assay (23), which defines their multipotency. The controls included asthma studies with intravenous BMMs as well as the saline- and ovalbumin-challenged mice with and without hMSCs.

BMMs

BALB/c mice were used for BMM studies as previously described (8). These cells are syngeneic bone marrow-derived cells used as a control cell source for the hMSC-specific effect. In brief, the femur and tibia were removed and wiped with sterile alcohol. The bones were then put into RPMI containing antibiotics, and, using a sterile 1-ml syringe, fresh RPMI was pushed through the bone marrow cavity. The bone marrow cells were counted for viability (trypan blue exclusion) followed by culture for 7–10 days with L929 support medium.

Cytokine and Inflammatory Mediator Assessment

BAL and serum differentials were measured by cytospins and direct cell counts (17). BAL levels of cytokines were assessed by ELISAs and Luminex multiplex assays (R&D Systems, Minneapolis, MN). BAL fluid was centrifuged and stored at ~70°C until evaluation for cytokines IFN-γ, TNF-α, IL-4, IL-5, IL-10, IL-13, and inducible nitric oxide (NO) synthase (iNOS) as described in detail elsewhere (7). Total IgE was evaluated in the sera of the ovalbumin-sensitized, saline-challenged, and ovalbumin-challenged animals. The assay was performed as described by the manufacturers (BioLegend, San Diego, CA).

Statistics

Statistical analysis was performed using Prism software (San Jose, CA). Data are shown as means ± standard error of the mean unless indicated otherwise. Comparisons of survival at a specified time (e.g., 4 days) were made using Fisher exact test. Two-group comparisons
for continuous data were made using one-way ANOVA and Student’s t-test or the Kruskal-Wallis and Wilcoxon rank sum tests. Graphic representations show data within different groups and at different time points. Statistical significance pertains to an α-value of >95% using two-tailed analysis with P ≤ 0.05.

RESULTS

The Chronic Asthma Model

The initial studies included a validation of the ovalbumin model of asthma with a chronic time frame of challenge. BALB/c mice were sensitized with ovalbumin and then rested for 14 days. After the 14 days, the mice were challenged every other day for 4 wk to induce airway inflammation (Fig. 1). Chronic airway challenge with ovalbumin enhanced BAL total cell counts by 15 ± 3.2% (Fig. 1A; n = 4 studies, 4–6 animals/study; P = 0.05). The differential consisted of an increase in inflammatory profile (Fig. 1A), including lymphocytes (L; P ≤ 0.05), neutrophils (PMNs), and eosinophils (Eos; P ≤ 0.05). There was also a consistent decrease in macrophages (Mac; n = 4 studies, 4–6 animals/study; P < 0.05). From a histological standpoint, the chronic model had consistently fewer macrophages and lymphocytes with an increased deposition of collagen as determined by trichrome staining [Fig. 1B: saline challenge control vs. Fig. 1C: ovalbumin model (figures are representative of n = 8 different studies, 2–3 animals/study)]. The chronic model generated increased pulmonary inflammation and histology consistent with epithelial cell hyperplasia, goblet cell hyperplasia, increased mucus production, and extracellular matrix deposition (blue color with trichrome staining).

hMSCs in Murine Models

To determine the impact of hMSCs in the absence of established inflammation, hMSC were given to mice sensitized with ovalbumin and challenged with saline. This treatment assesses potential xenograft effects of hMSCs on murine lung tissue. Figure 2A shows the change in total cell count between ovalbumin-sensitized and saline-challenged models with and without intravenous exposure to hMSCs. The differential between the naïve controls and saline-challenged mice was minimal (data not shown). hMSCs did not statistically change the inflammatory phenotype of the saline-challenged controls, that is, no difference was observed in the BAL differential of the saline-challenged control, regardless of whether they were treated with hMSCs (Fig. 2B; n = 4 studies, 4–6 animals/study). Histologically, there was no significant difference between saline-challenged mice with or without hMSCs. Sham saline-treated mice from the chronic asthma model with (Fig. 2C) and without (Fig. 2D) hMSCs appeared similar with little or no airway thickening or mucus production (histology sections representative of n = 8 different studies, 2–3 animals/study).

hMSC Effect on Chronic Asthma Inflammation

Studies were designed to determine whether intravenously added hMSCs could alter the inflammatory profile of established inflammation associated with the chronic model of asthma. The specificity of the hMSC response was further studied by evaluating the BAL from ovalbumin-challenged mice treated with BMMs obtained from syngeneic mice. Mice were sensitized with ovalbumin and rested for 14 days, then challenged with ovalbumin every other day for 4 wk. After the 14 days, the mice were challenged every other day for 4 wk to induce airway inflammation (Fig. 1). Chronic airway challenge with ovalbumin enhanced BAL total cell counts by 15 ± 3.2% (Fig. 1A; n = 4 studies, 4–6 animals/study; P = 0.05). The differential consisted of an increase in inflammatory profile (Fig. 1A), including lymphocytes (L; P ≤ 0.05), neutrophils (PMNs), and eosinophils (Eos; P ≤ 0.05). There was also a consistent decrease in macrophages (Mac; n = 4 studies, 4–6 animals/study; P < 0.05). From a histological standpoint, the chronic model had consistently fewer macrophages and lymphocytes with an increased deposition of collagen as determined by trichrome staining [Fig. 1B: saline challenge control vs. Fig. 1C: ovalbumin model (figures are representative of n = 8 different studies, 2–3 animals/study)]. The chronic model generated increased pulmonary inflammation and histology consistent with epithelial cell hyperplasia, goblet cell hyperplasia, increased mucus production, and extracellular matrix deposition (blue color with trichrome staining).

**Fig. 1. Development of chronic model of murine asthma.** Mice were sensitized with ovalbumin (Ova), rested for 14 days, and then were challenged with Ova or saline every other day for 4 wk. Mice were killed 7 days later, and the lungs were processed for inflammation by bronchoalveolar lavage (BAL; A). Ovalbumin-challenged mice had a significant increase in lymphocytes (L; P ≤ 0.05) and eosinophils (Eos; n = 4, 4–6 animals for each study; P ≤ 0.05). The ovalbumin mice also had a significant decrease in macrophages (Mac; n = 4; P < 0.05). The remaining animals were processed for pathology without BAL (B, saline challenge; C, ovalbumin challenge; trichrome stain, ×40). Histology is representative of 8 different experiments [different batches of human mesenchymal stem cells (hMSCs)] with 2–3 mice in each group. Blue color shows the collagen/extracellular matrix deposition. PMN, polymorphonuclear neutrophils.
days. On day 14, mice were challenged every other day for 4 wk. During the last week, hMSCs or control cells BMM were administered to the animals intravenously followed by 3 more challenges with ovalbumin. Treatment with hMSCs resulted in decreased total cell count (Fig. 3A; \( n = 4 \), 4–6 animals/study; \( P = 0.05 \)). The differential showed a decrease in eosinophils (\( P = 0.05 \)) and macrophages (\( n = 4 \), 4–6 animals/study; \( P = 0.12 \); Fig. 4B) with an increase in neutrophils (Fig. 3A; \( n = 4 \), 4–6 animals/study; \( P = 0.02 \)) over the chronic response without hMSCs. Neither the total cell count (Fig. 3A) nor differential (data not shown) were changed by the BMM. Histologically, hMSC treatment resulted in a dramatic decrease in airway inflammation, goblet cell hyperplasia, and epithelial cell lining thickening (Fig. 4B) and collagen deposition (blue color with trichrome) compared with animals not treated with hMSCs (Fig. 4A). Treatment with BMM (Fig. 4C) did not result in improved histology. These images are representative of 8 different experiments (different batches of hMSCs) with 2–3 mice in each group.

Fig. 2. Xenographic effect of hMSCs on total cell recruitment, inflammation, and pathology. Animals were sensitized with ovalbumin and challenged with saline. The differential between these animals and naïve controls is negligible (data not shown). hMSC therapy did not significantly change the total cell count (A), inflammatory phenotype (B), or lung pathology (×40; C and D, untreated and hMSC-injected, respectively) of the saline control of the chronic murine model. Treating the saline-challenged mice with human hMSCs did not alter the lung inflammatory environment in the chronic murine model (\( n = 4 \) studies, 4–6 animals/study). Histology is representative of 8 different experiments (different batches of hMSCs) with 2–3 mice in each group.

Treatment of the Ovalbumin-Challenged Mice with hMSCs Results in Decreased Serum IgE

To begin to define the mechanisms by which the hMSCs may induce favorable changes in the ovalbumin challenge model, we measured circulating IgE. Systemic IgE levels in the saline-challenged mice were comparable with BALB/c circu-
hMSCs decrease lung inflammation in the chronic asthma lung model. hMSCs or bone marrow-derived macrophages (BMMs) were given by tail vein injection. Mice were evaluated after 7 days of challenge for total cell count (A) and inflammation (B). Treatment of the ovalbumin-challenged mice with hMSCs resulted in a significant decrease in total cell recruitment into the lung (A; n = 4; P < 0.05). This was not observed with the BMM treatment (n = 3 different preparations). The differential showed that treatment with hMSCs resulted in a modest decline in macrophages and significant decrease in eosinophils (n = 4; P = 0.05) but with a robust increase in neutrophils (B; n = 4; P = 0.05). There was no statistical change between ovalbumin-challenged mice and ovalbumin-challenged mice treated with BMM (data not shown).

Fig. 3. hMSCs decrease lung inflammation in the chronic asthma lung model. hMSCs decreased total cell count and inflammation. Mice were evaluated after 7 days of challenge for total cell count (A) and inflammation (B). Treatment of the ovalbumin-challenged mice with hMSCs resulted in a significant decrease in total cell recruitment into the lung (A; n = 4; P < 0.05). This was not observed with the BMM treatment (n = 3 different preparations). The differential showed that treatment with hMSCs resulted in a modest decline in macrophages and significant decrease in eosinophils (n = 4; P = 0.05) but with a robust increase in neutrophils (B; n = 4; P = 0.05). There was no statistical change between ovalbumin-challenged mice and ovalbumin-challenged mice treated with BMM (data not shown).

hMSCs and NO Production

NO is a product associated with asthmatic exacerbations. iNOS is the gene associated with NO production and is associated with asthmatic exacerbations. BAL cell pellets were harvested and processed for cDNA. Cell pellets were then evaluated for the expression of iNOS using real-time PCR. BAL cell pellets from saline challenge had iNOS expression (n = 4; P = 0.043) consistent with the ovalbumin model. hMSC treatment significantly decreased iNOS expression (P < 0.001), which corre-
lates with some resolution of lung pathology. To determine whether the impact of the hMSCs required cell-to-cell interaction or was generated by an hMSC-derived product, hMSC supernatant was cultured on the murine macrophage cell line (RAW 264.7), which was stimulated for 24 h with 0.01 g/ml LPS for 24 h with and without the addition of hMSC supernatants. At 24 h, the hMSC supernatant decreased iNOS gene expression by the macrophages (Fig. 7B; P ≤ 0.05; n = 3 supernatants).

**DISCUSSION**

Adult human marrow-derived MSCs have the unique capacity to support tissue regeneration and secrete soluble mediators that can immunomodulate inflammation. Intravenously introduced hMSCs localize in the lung before dispersing into the peripheral tissues and seemingly home to injured tissue (2, 36). Asthma is an inflammatory airway disease hallmarked by T cell hyperreactivity, scarring, and remodeling. Since hMSCs have the capacity to decrease and/or reverse scarring as well as suppress T cell activity, we investigated the potential of using hMSCs to decrease airway inflammation in the murine ovalbumin model of chronic asthma. Our data show that hMSCs are well-tolerated in immunocompetent murine models of asthma and that hMSC treatment can favorably change the outcome of asthma inflammation without pathology associated with cross-species application. The saline-challenged mice with and without hMSCs had no change in pathology associated with hMSCs, and there was a lack of induced acute phase reactants, TNF-α and IL-6, both systemically and locally in the lung. In addition, using syngeneic-derived BMMs, we demonstrate the specificity of the hMSC impact on decreasing inflammation associated with the ovalbumin model of chronic asthma. Furthermore, our data show that hMSCs

---

**Fig. 4.** The specificity of the hMSC resolution of lung pathology. The specificity of the hMSC response was further shown by evaluating the histology from ovalbumin-challenged mice treated with hMSC and BMM and not treated compared with the saline-challenged controls treated in the same manner. Three different hMSCs and BMM preparations were utilized for these studies with 2 mice each being evaluated for pathology without BAL to preserve architecture. A is the ovalbumin-sensitized, ovalbumin-challenged disease group (×40). B is ovalbumin-challenged group with hMSC therapy (×40), and C is ovalbumin-challenged group with BMM therapy (×40). These images are representative of n = 3 sets of hMSCs and BMM, with n = 3–4 animals for pathology for each study group of ovalbumin, ovalbumin + hMSC, and ovalbumin + BMM. Image analysis quantified inflammatory nuclei post-hematoxylin and eosin (H&E) staining (D) using color-based pixel analysis. Each section was analyzed for over 196 images using an automated microscope-driven software system (Image-Pro Plus 7.0). Each point is the mean analysis of each section evaluated (3 sections/slide). Ova challenge induced an increase in numbers of nuclei (P < 0.001), which is consistent with the histology images and increase in total cell count. The inflammation was effectively decreased by hMSC therapy but not bone marrow therapy.
given postinduction of airway disease dramatically decrease the pathology and airway inflammation associated with the ovalbumin model of chronic asthma, which may be related to modulating IL-5, IL-13, IgE, and iNOS expression. Furthermore, the impact of the hMSCs appears to be compartmentalized with IFN-γ levels decreasing in the lung while increasing in the serum.

Adult MSCs isolated from bone marrow reside as pericytes in situ and can differentiate in culture into a number of mesenchymal phenotypes including bone, cartilage, muscle, fat, and other connective tissues (20, 21, 27). hMSCs are capable of tissue replacement and/or altering their surrounding milieu and thus hold the potential to be used therapeutically as allogeneic sources of regeneration and repair in vivo (25). The therapeutic implication of hMSCs is based on the observation that culture-expanded hMSCs have no detectable MHC class II cell surface markers or costimulator molecules (28), which suggests that the hMSCs evade immune surveillance. In our study, intravenously introduced hMSCs did not change the cell recruitment or pathology in the controls used for our models of asthma. Importantly, the absence of graft effects was also verified pathologically, which suggests the absence of adverse GvHD in the saline-challenged chronic model.

Vasculature-associated stem cells are unique in having the capacity for self-renewal and differentiation. MSCs are also unique in their ability to conform to a specific identity in response to host cells, cytokines, and extracellular matrix (16). Studies have shown that MSCs persist for several months in a mouse model of osteogenesis imperfecta (31). However, the question remains as to whether the beneficial observations of using hMSCs are a result of repair and regeneration or due to the attenuation of the inflammatory response. In studies with the bleomycin model, murine MSC (mMSC) administration immediately after exposure to bleomycin in mice was associated with a significant reduction in inflammation and collagen deposition associated with the lung disease (44, 46). In these studies, the rates of engraftment were undetectable or at the limits of detection as defined by the authors. The implication is that the mechanism of the mMSC effect and improvement in the murine model of pulmonary fibrosis was not due to cell engraftment into the injured tissue but was potentially related to the secretion of MSC growth factors and cytokines that stimulate repair. The question remains as to the major site of action of mMSCs, whether it is in the injured tissue, the tissue-specific lymph nodes, or other interacting sites. The issue of cellular specificity is also a question for these types of studies. The problem is that no other cell type can serve as an effective control for these studies due to the unique phenotype and the type of model system we are using. Although complementary studies could be done using human vs. mouse fibroblasts, neither may really represent reasonable controls for nonterminally differentiated marrow-derived hMSCs. In fact, studies have defined hMSCs as pericytes, predifferentiated cells that provide the ability to conform to a specific identity in response to host cells, cytokines, and extracellular matrix (25). The therapeutic implication of hMSCs is based on their capacity for self-renewal and differentiation. MSCs are also unique in their ability to conform to a specific identity in response to host cells, cytokines, and extracellular matrix. The hMSCs are immunologically invisible to the mouse immune surveillance. In our study, intravenously introduced hMSCs did not change the cell recruitment or pathology in the saline-challenged chronic model.
measuring inflammation. Alternative sources of hMSCs, such as fat-derived vs. bone marrow-derived cells may make good controls, which is the consideration of future work but out of the scope of these studies.

Bronchial asthma is a chronic inflammatory disease of the airways and a significant health problem with major human and socioeconomic consequences (19, 29, 33). Clinical asthma is characterized by airway hyperactivity, mucus hypersecretion, fibrosis, and intermittent airway obstruction. In addition, asthma can be classified as either allergic or nonallergic with allergy being acknowledged as a major risk factor for the development of asthma (33). The pathogenic characteristics of allergic asthma are associated with airway inflammation and infiltration of mast cells, basophils, eosinophils, monocytes, and T helper type 2 lymphocytes along with the production of isotype-specific IgE (4). Bronchial asthma has also been characterized by chronic and allergic airway inflammation prototyped by the production of matrix metalloproteinases, which induces both cytological as well as histological changes in the airway structure over time (14). These changes have been called airway remodeling and include goblet cell hyperplasia, thickening and fibrosis of subepithelial basement membranes, and hyperplasia and hypertrophy of airway smooth muscle cells and bronchial glands (15). Concurrent with the ongoing airway remodeling, there is enhanced production of inflammatory mediators, growth factors, cytokines, extracellular matrix proteins, and reactive oxygen species (45). This airway remodeling can cause irreversible airway obstruction as well as an increase in airway hyperresponsiveness, which ultimately contributes to the severity of the asthma. In terms of a potential therapeutic, our data show that hMSCs are very promising because hMSCs resulted in a statistical decrease in BAL IFN-γ, IL-5, and IL-13 levels (A; n = 4, 4–6 animals/study; P = 0.05), hMSCs increased macrophage inflammatory protein-1α (MIP-1α; P < 0.05; n = 4, 4–6 animals/group) and keratinocyte-derived chemokine (KC; n = 4; P = 0.01). There was no change in MIP-1α, IL-6, or TNF-α, and IL-10 and IL-4 were not detectable in the BAL regardless of hMSC treatment. Cardiac puncture to obtain adequate serum samples was performed on the chronic asthma models after the treatment with hMSCs. The chronic asthma model had significantly elevated levels of systemic IL-1β (B; n = 8; P < 0.001), which was decreased significantly with hMSC treatment (n = 7; P = 0.05). Systemic IFN-γ concentrations increased in the chronic asthma model after MSC treatment (n = 8; P = 0.043). This was dependent on the presence of in vivo inflammation since saline animals given hMSCs had no detectable serum IFN-γ.

Fig. 6. hMSC attenuates inflammation in a tissue-specific manner. BAL fluid was obtained from the chronic asthma model with and without treatment with hMSCs and evaluated for IFN-γ, TNF-α, IL-4, IL-5, IL-10, and IL-13. The chronic models of asthma showed elevated IFN-γ (49 ± 3 pg/ml) relative to untreated mice (23 ± 7 pg/ml; n = 4; P = 0.03), consistent with the literature (48). IL-5 (13.5 ± 1.2 pg/ml) and IL-13 (63 ± 6.7 pg/ml) levels were also significantly elevated (n = 4, 4–6 animals/study; P ≤ 0.001) compared with baseline, which had no detectable levels of IL-5 or IL-13 (data not shown). Treatment of the animals with intravenous hMSCs resulted in a statistical decrease in BAL IFN-γ, IL-5, and IL-13 levels (A; n = 4, 4–6 animals/study; P = 0.05). hMSCs increased systemic tumor necrosis factor (TNF-α) (P < 0.05; n = 4, 4–6 animals/group and keratinocyte-derived chemokine (KC; n = 4; P = 0.01). There was no change in MIP-1α, IL-6, or TNF-α, and IL-10 and IL-4 were not detectable in the BAL regardless of hMSC treatment. Cardiac puncture to obtain adequate serum samples was performed on the chronic asthma models after the treatment with hMSCs. The chronic asthma model had significantly elevated levels of systemic IL-1β (B; n = 8; P < 0.001), which was decreased significantly with hMSC treatment (n = 7; P = 0.05). Systemic IFN-γ concentrations increased in the chronic asthma model after MSC treatment (n = 8; P = 0.043). This was dependent on the presence of in vivo inflammation since saline animals given hMSCs had no detectable serum IFN-γ.
impact of the hMSCs on the tissue milieu response. The cellular interaction, factors produced by the hMSCs, or the in our murine model of chronic asthma includes direct with the attenuation in inflammation and airway remodeling in the animals (data not shown). The mechanisms associated up to 4 wk in our booster model without any observable further, we have maintained animals post-hMSC therapy for hMSCs in murine models. To support the lack of GvHD the lack of acute GvHD in the xenographic therapy using /H9251 reactants including TNF-

airway disease. The decrease in these inflammatory media-
to contribute to the pathophysiology of chronic asthmatic IL-13, IgE, and iNOS, all of which have been documented to contribute to the pathophysiology of chronic asthmatic airway disease. The decrease in these inflammatory mediators was in the context of a lack of enhanced acute phase reactants including TNF-α and IL-6. This further supports the lack of acute GvHD in the xenographic therapy using hMSCs in murine models. To support the lack of GvHD further, we have maintained animals post-hMSC therapy for up to 4 wk in our booster model without any observable change in clinical scores, lung pathology, or adverse events in the animals (data not shown). The mechanisms associated with the attenuation in inflammation and airway remodeling in our murine model of chronic asthma includes direct cellular interaction, factors produced by the hMSCs, or the impact of the hMSCs on the tissue milieu response. The IL-13, IgE, and iNOS, all of which have been documented to contribute to the pathophysiology of chronic asthmatic airway disease. The decrease in these inflammatory mediators was in the context of a lack of enhanced acute phase reactants including TNF-α and IL-6. This further supports the lack of acute GvHD in the xenographic therapy using hMSCs in murine models. To support the lack of GvHD further, we have maintained animals post-hMSC therapy for up to 4 wk in our booster model without any observable change in clinical scores, lung pathology, or adverse events in the animals (data not shown). The mechanisms associated with the attenuation in inflammation and airway remodeling in our murine model of chronic asthma includes direct cellular interaction, factors produced by the hMSCs, or the impact of the hMSCs on the tissue milieu response. The

Fig. 7. hMSCs decreased inducible nitric oxide synthase (iNOS) expression in BAL cell pellets of ovalbumin-challenged mice. BAL cell pellets were obtained from mice sensitized with ovalbumin and challenged with either saline or ovalbumin in the presence and absence of hMSC therapy and evaluated for iNOS gene expression. BAL cell pellets from ovalbumin-challenged mice had significantly elevated iNOS gene expression (A; n = 6; P < 0.05) compared with saline-challenged animals (n = 6). iNOS gene expression was significantly decreased after hMSC therapy in the ovalbumin-challenged mice (n = 6; P < 0.05). In the 2nd part of these studies, hMSC supernatant was cultured on the murine macrophage cell line (RAW 264.7), which was stimulated for 24 h with 0.01 μg/ml LPS for 24 h with and without (US) the addition of hMSC supernatants. At 24 h, the hMSC supernatant decreased iNOS gene expression by the macrophages (B; P < 0.05; n = 3 different supernatants). Ct, threshold cycle.

ACKNOWLEDGMENTS

We thank David Carrino for helping in the preparation of this manuscript and Dr. Carolyn Kerscmar for initiating the interactions between T. L. Bonfield and A. I. Caplan culminating in this manuscript. We also thank Brian Jennings, Joseph Soltrberg, Christaan van Heekeren, Nicole Brown, and Sarah Frischmann for the assistance with the animal model and the image analysis.

GRANTS

The funding for this research was graciously provided by the David and Virginia Baldwin Fund, the Center for Stem Cell and Regenerative Medicine, and the Case Western Reserve Vision Fund.

DISCLOSURES

No conflicts of interest, financial or otherwise, are declared by the author(s).
REFERENCES


