Mesenchymal Stem Cells Modulate Lung Injury

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Mesenchymal stem cells (MSCs) have been shown to differentiate into a variety of mesenchymal cell types, including fibroblasts, myofibroblasts, osteoblasts, chondroblasts, adipocytes, and myoblasts, as well as epithelial cells. It has been shown that these cells can be recovered from bone marrow as well as umbilical cord blood, and they can be propagated, stored, and administered to animals and patients in clinical trials. It is clear that the cells engraft in the lung, and several laboratories have demonstrated an ameliorating effect in models of acute injury caused by LPS and in chronic lung injury induced by bleomycin and asbestos. However, it is not at all clear under what conditions these cells must be applied to provide an advantage and when using these cells might cause exacerbation of the lung injury. This brief review focuses on the biology of MSCs *in vitro*, how the cells have been used in some animal models, and the potential for their use in therapeutic strategies for diseases as diverse as lung cancer and interstitial fibrosis.

Keywords: mesenchymal stem cells; lung injury; growth factors

Mesenchymal stem cells or marrow stromal cells are being studied with increasing intensity as their potential grows for use as a therapeutic tool (1). These cells, commonly referred to as adult mesenchymal stem cells (MSCs) or progenitor cells, have been collected from the bone marrow of several species and from human umbilical cord blood (1). An attractive feature of these cells is their capacity to differentiate into a number of mature cell types, including fibroblasts, myofibroblasts, osteoblasts, chondroblasts, adipocytes, myoblasts, and epithelial cells (2, 3). The prevailing opinion is that these cells ameliorate lung injury when applied as a therapeutic approach in animal models (4, 5). There are exceptions to this view (6), and this raises questions as to how the cells should be used and the fundamental mechanisms through which MSCs modulate surrounding tissues. A central concept of the Aspen Lung Conference (where this paper was originally presented) is "The Environment and the Lung." There is little doubt that stem cell therapy can eventually impact on environmental lung diseases inasmuch as agents such as smoke, allergens, infectious organisms, and inhaled organic particles and fibers all cause significant lung afflictions. It will be essential to learn as much as possible about the biology of stem cells and their potential for use in therapeutic approaches. In this review, we discuss some of the current literature on mesenchymal stem cell biology, present potential clinical applications, and provide some recent findings from our laboratory.

MESENCHYMAL STEM CELLS

MSCs were originally identified more than 120 years ago as a component population among the predominant hematopoietic cells of the bone marrow (1). The latter obviously provide new red blood cells as well as the monocytes and lymphocytes that respond in acute and chronic diseases. These cells typically survive for only a few days to weeks, and must be replaced by a series of progenitor cells in the bone marrow. However, there also is a population of stem cells in the bone marrow that provides nonhematopoietic cells, the so-called MSCs (1–3). A German scientist reported in 1867 (reviewed in Reference 1) that when he injected a dye into the veins of animals undergoing wound repair, inflammatory and fibroblast-like cells containing the dye appeared in the wounds. The conclusion was that these cells populated the bone marrow and responded to injury. While this was the first known mention of an apparent stromal cell in the bone marrow, Friedenstein, more than 100 years later (7), showed that the bone marrow contains cells that can differentiate into a variety of mesenchymal cell types as listed above. This investigator cultured whole bone marrow from rats and discovered spindle-shaped cells that could be maintained through multiple passages and multiplied rapidly under the appropriate conditions (7). These cells then were shown to differentiate along the mesenchymal cell lines indicated above and could double 20 to 30 times. These adult mesenchymal stem cells have subsequently been derived and cultured from mice, rabbits, and humans (1–3), all with very similar biological properties. We have shown (*see below*) that mouse bone marrow and human umbilical cord blood MSCs exhibit essentially identical responses to growth factors such as platelet-derived growth factor (PDGF) and transforming growth factor β (TGF- β_1).

Many of the characteristics and requirements of these cells have been determined over the past few decades. Cultured MSCs produce an extracellular matrix with fibronectin and several collagen proteins (1). Several laboratories have developed lists of cytokines and growth factors produced by MSCs (8). These include interleukins, colony stimulating factor, and other well-defined biological molecules (8). A very interesting aspect of MSCs is the environment they impart for the successful long-term culture of hematopoietic cells (1). When whole bone marrow is cultured, an adherent cell population with multiple characteristics of MSCs forms contacts with the hematopoietic stem cells (HSCs) and appears to actually shroud the HSCs (reviewed in Reference 1). After 20 weeks of culture under these conditions, the HSCs can go on to differentiate into a variety of blood cell types, but it remains unclear if the "feeder layer" of mesenchymal-like cells are in fact MSCs and can differentiate to mesenchymal cell types. The results from these kinds of experiments support the view that MSCs, as inhabitants of normal bone marrow, provide a number of potent cytokines and growth factors that support the growth and differentiation of hematopoietic cells. Whether or not the MSCs migrate from the bone marrow and

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provide a source of cells that can modulate lung injury and differentiate into a variety of lung cell types is an important and growing field of study (1–3, 9).

EXPERIMENTAL MODELS

Lipopolysaccharide (LPS) has been used for decades as a means of inducing acute lung injury (ALI) (10). ALI in humans is marked by acute inflammation and alveolar epithelial damage that results in airspace flooding and, in many cases, organizing pneumonia consequent to the edema, fibrogenesis, and death (11). The mouse model nicely recapitulates the disease. Interestingly, the Matthay laboratory has treated such mice with MSCs, protecting many of them from premature death by decreasing the edema and reducing the inflammatory response (10). This is an important paper in the field, and it remains unknown if treatment with MSCs would protect the animals against the late stages of alveolar fibrogenesis, and it is not known if treating the mice with stem cells before inducing the injury with LPS will modulate the process. Treatment with MSCs in this model was by the intratracheal route. There is no information on how this form of lung injury will respond to intravascular administration of the cells or how the timing of cell administration by either route will alter the results. This interesting model could be very useful in future studies that focus on acute lung injury.

Among the lung diseases caused by inhaling asbestos fibers is asbestosis (i.e., interstitial fibrosis caused by asbestos) (12). We developed mouse and rat models of this disease decades ago (13, 14), and continue to use the model to answer questions related to distribution and dose response of gene expression determined by laser capture microdissection (15). Recently, we used this model to determine where MSCs would engraft as the asbestos-induced lesions developed (16). Since we had previously determined that the initial lesions of asbestosis develop at the alveolar duct bifurcations in rats and mice (13–15), we could ask if the stem cells were directed to the developing lesions at these anatomic sites, and this proved to be the case (Figure 1). The MSCs from male rats labeled with GFP were transplanted into irradiated females that were exposed to an asbestos aerosol for 3 hours. Within 24 hours after exposure, significant numbers of labeled MSCs had migrated from the bone marrow and were identified in the developing asbestos-induced lesions (Figure 1) (16). Quantitation of stem cell distribution showed that few of the cells were found elsewhere in the lung, indicating that the cells were attracted by an as yet undescribed mechanism. Two and a half weeks later, there still were increased numbers of labeled MSCs in the lesions, and many of them had differentiated to macrophages, alveolar epithelial cells, and interstitial fibroblasts and myofibroblasts (16). There is no information currently available on whether or not the developing asbestos-induced lesions were changed in any way by the presence of the stem cells. Because we know so much about the fundamental details of this model (17, 18), it offers an outstanding opportunity to learn a great deal about what stem cells can do as the lesions develop. In a similar study (19), investigators in Vermont suggested that a bone marrow transplant could ameliorate the fibrogenic effects of asbestos exposure.

MSCs have been introduced into various animal model systems by intravascular injection (4), intratracheal instillation (5), or by bone marrow transplantation (16). The bulk of the evidence suggests that MSCs migrating to lesions produce favorable results, such as in ameliorating fibrogenesis caused by bleomycin (4), radiation and inhaled asbestos (19), and reducing inflammation and mortality in a model of acute lung injury as discussed above (10). In an elegant study from Ortiz's

Figure 1. A and B show asbestos-induced fibroproliferative lesions that have developed at the bronchiolar-alveolar duct junctions in the lungs of rats exposed for 3 hours (see References 12–18 for details). Green fluorescent protein (GFP) labels the mesenchymal stem cells that were used for the bone marrow transplant in these animals and that migrated to the developing lesions. The same lesion is shown in B stained positively with K19, suggesting that several of the GFP-labeled cells in A have differentiated to cells with epithelial characteristics. (Original magnification: $\times 20$.)

laboratory (4), it was shown that MSCs block production of TNF- α and IL-1 that apparently mediate bleomycin-induced lung injury. However, a recent article by Phan's group (6) demonstrated reduced airway remodeling if stem cell factor (SCF) is blocked and bone marrow-derived mesenchymal cells consequently are inhibited from reaching the sites of injury, thus impugning bone marrow-derived progenitor cells. In addition, a new clinical study indicates that circulating fibrocytes signal a poor prognosis for the development of pulmonary fibrosis in patients (20). The mechanisms by which these cells migrate from the vasculature and move among other cell types and along interstitial pathways in the lung or elsewhere remain completely unknown. It is not known where and when these MSCs should be used to provide effective therapeutic approaches.

MSCS PRODUCE TGF- β_1 THAT UP-REGULATES ALPHA-1 PRO-COLLAGEN

We have performed a series of experiments to determine if MSCs produce factors that influence fibroblast growth and matrix production. Here, we report some preliminary data that show the effect of TGF- β_1 produced by the MSCs on collagen synthesis in fibroblasts. Media conditioned by both bone marrow-derived MSCs (BMSC) and human umbilical cord blood (CBMSC) were concentrated, serially diluted and applied to a quiescent fibroblast cell line (16Lu) and normal human lung fibroblasts (NHLF) for 48 hours. Matrix production was determined by analyzing α -1 pro-collagen (COL1A1) levels measured by RT-PCR. When the TGF- β_1 in the conditioned media (CM) from BMSC or CBMSC remained latent, there was no effect on COL1A1 levels. However, acid activation of the CM to remove the latency-associated peptide from TGF- β_1 (21) resulted in a significant dose-dependent increase in COL1A1 levels (Figure 2). Both CBMSC and BMSC CM increased COL1A1 in 16Lu fibroblasts 5- to 7-fold when concentrated 2.5-fold. Primary human fibroblasts were used to confirm the results seen with 16Lu fibroblasts. Both CBMSC and BMSC activated CM increased pro-collagen gene expression in the NHLF to similar levels as in the 16Lu cell line.

ANTIBODY INHIBITION OF TGF- β_1 BLOCKS THE INDUCTION OF COL1A1 IN LUNG FIBROBLASTS

The previous data show that activating TGF- β_1 in the CM increases the COL1A1 gene expression. To demonstrate that it

Figure 2. Activated bone marrow–derived mesenchymal stem cells (BMSC) conditioned media (CM) induces COL1A1 expression in 16Lu fibroblasts. Quiescent BMSC (8×10^5 cells) were cultured in 20 ml serum-free (SF) medium. After 48 hours the mesenchymal stem cell CM was concentrated 5-fold. Quiescent 16Lu fibroblasts were cultured for 48 hours in SF medium, TGF- β_1 (5 ng/ml, positive control) or serially diluted acid-activated or latent CM. COL1A1 up-regulation was determined by quantitative real-time RT-PCR. Data represent the mean $\pm$ SEM from triplicate wells for each treatment group. Significantly different from SF: * $P < 0.05$, ** $P < 0.002$.

was the TGF- β_1 in the CM that induced collagen gene expression, anti-TGF- β was added to the CM before fibroblast treatment. The data indicate that specific blocking of TGF- β_1 abrogates the enhanced collagen expression in fibroblasts treated with MSC CM. Together, these data suggest that the CM is able to induce matrix production in fibroblasts via a TGF- β_1 –dependent pathway.

Recent experiments with these cells show that the CM also contains factors that promote mesenchymal cell proliferation. Studies to identify these factors and the genes that control their synthesis are ongoing in our laboratory. All of these molecules certainly could influence whether or not these stem cells will ameliorate or exacerbate lung injury, and investigators clearly need to know what these factors are and how they function.

CLINICAL APPLICATIONS

The first reported use of MSCs in a therapeutic approach was in 1999 by a team of investigators that performed allogeneic bone marrow transplantation in children suffering from osteogenesis imperfecta (i.e., brittle-bone disease) (reviewed in Reference 1). MSCs engrafted and differentiated into osteoblasts in the recipients, and the cells migrated to sites in the bones where they produced new lamellar bone and improved bone growth.

Regarding interstitial fibrosis, as mentioned above, a clinical analysis showed a poor prognosis for patients who had higher levels of circulating fibrocytes, a class of bone marrow–derived SCs (20). This again raises the unresolved issue of whether or not MSCs should be expected to provide an advantage when used in therapeutic approaches. In another interesting experimental approach, investigators have suggested that MSCs could be engineered to act as vectors for various drugs and gene delivery (22). These investigators stated that MSCs could be “The Perfect Vector” and have proposed very provocative scenarios for treating a variety of lung diseases, from fibrogenesis to cancer (22). In an excellent review (23), Dominici and

coworkers set forth five advantages that are likely to play a role in the successful use of MSCs in clinical applications: (1) Large numbers of cells can be attained; (2) the cells can be expanded many thousand-fold in culture; (3) MSCs differentiate into multiple cell types; (4) gene transfer vectors can readily be used; and (5) the cells can be retroviral producers, thus facilitating gene transfer.

In 2008, an extremely useful review was published in the *Proceedings of the American Thoracic Society* (24). This was the proceedings of the 23rd Transatlantic Airway Conference and was titled “Stem/Progenitor Cells and Lung Repair.” Among the many useful reviews was a chapter entitled “Proof-of-Concept Trials for Stem Cell Therapy” by David Rodman (24). Here, Dr. Rodman raised a number of issues central to whether or not using stem cells in therapeutic approaches will be successful. He pointed out that there are a number of small clinical trials already ongoing, most using bone marrow–derived MSCs as described above. Problems with interpreting the results of these studies include having limited controls, failure of the SCs to engraft, and the generation of inflammatory events by the treatment itself. There clearly are many questions that must be answered before therapeutic approaches will be optimized. Dr. Rodman presented a theoretical timeline for clinical applications where the biology of the MSCs currently is being explored, through perhaps 2010, around which time good laboratory practices are established, toxicity is determined, primate studies are instituted (2011), and through 2013–2014 the first interpretable results are developed in “proof of concept” clinical trials. This very reasonable approach requires the development of acceptable “translational animal models” and “translational biomarkers.” This is where the bulk of the research is proceeding today. Dr. Rodman has proposed the use of lung irradiation as a model that could be used to satisfy many of the proofs of concept. Indeed, in 2004, work from Stephen Rennard’s group suggested that MSCs ameliorate lung injury caused by lethal irradiation (5). Major points raised in Dr. Rodman’s review are that the timing of the application of the stem cells in any therapeutic approach will be critical, and that there are several sources of potential therapeutic cells (e.g., bone marrow, umbilical cord blood, and fetal derivation).

CONCLUSIONS

Mesenchymal stem cells (MSCs) can differentiate into multiple cell types *in vitro* and *in vivo*. The cells can be derived from bone marrow and from umbilical cord blood and engraft in the lung in several models of lung injury. Whether or not MSCs ameliorate or exacerbate various forms of lung injury is becoming an important issue as multiple laboratories move toward the development of clinical trials. The ultimate goal of this research is to develop successful therapeutic approaches for treating such diseases as interstitial fibrosis, emphysema, and lung cancer in which treatments currently are severely limited.

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