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Clinical grade allogeneic human mesenchymal stem cells restore alveolar fluid clearance in human lungs rejected for transplantation


Am J Physiol Lung Cell Mol Physiol 306: L809–L815, 2014. First published February 14, 2014; doi:10.1152/ajplung.00358.2013.—The lack of suitable donors for all solid-organ transplant programs is exacerbated in lung transplantation by the low utilization of potential donor lungs, due primarily to donor lung injury and dysfunction, including pulmonary edema. The current studies were designed to determine if intravenous clinical-grade human mesenchymal stem (stromal) cells (hMSCs) would be effective in restoring alveolar fluid clearance (AFC) in the human ex vivo lung perfusion model, using lungs that had been deemed unsuitable for transplantation and had been subjected to prolonged ischemic time. The human lungs were perfused with 5% albumin in a balanced electrolyte solution and oxygenated with continuous positive airway pressure. Baseline AFC was measured in the control lobe and if AFC was impaired (defined as ≤10%/h), the lungs received either hMSC (5 × 10^6 cells) added to the perfusate or perfusion only as a control. AFC was measured in a different lung lobe at 4 h. Intravenous hMSC restored AFC in the injured lungs to a normal level. In contrast, perfusion only did not increase AFC. This positive effect on AFC was reduced by intrabronchial administration of a neutralizing antibody to keratinocyte growth factor (KGF). Thus, intravenous allogeneic hMSCs are effective in restoring the capacity of the alveolar epithelium to remove alveolar fluid at a normal rate, suggesting that this therapy may be effective in enhancing the resolution of pulmonary edema in human lungs deemed clinically unsuitable for transplantation.

Primary graft dysfunction is the end-result of injury occurring from the time of brain death to the time of lung reperfusion after transplantation, including during cold ischemic storage of the lung allograft. The current clinical practice of organ preservation is cold static preservation. Hypothermia reduces metabolic activity to the point that cell viability can be maintained in the face of ischemia (20). Although hypothermia is essential for organ storage, it can be associated with a series of events (including cell injury and death) that may lead to primary graft dysfunction, which represents a spectrum of injury ranging from mild pulmonary edema to a clinical picture similar to the acute respiratory distress syndrome (ARDS) (6). Longer ischemic times predispose to more injury, and more severe primary graft dysfunction (32).

There is recent interest in reconditioning lungs rejected for transplant using ex vivo lung perfusion (EVLP) (11). With EVLP, lungs are perfused and ventilated ex vivo at body temperature to mimic physiological conditions. EVLP can also facilitate delivery of a pharmacological, gene- or cell-based therapy to repair damaged lungs before transplant (23, 40). We previously reported that intravenous clinical-grade, cryopreserved allogeneic human mesenchymal stem (stromal) cells (hMSC) were effective in restoring alveolar fluid clearance (AFC) in a human EVLP model of acute lung injury (ALI) induced by endotoxin or Escherichia coli bacteria (22, 23). Thus, hMSCs may be a novel strategy to recondition lungs declined for transplantation. Mesenchymal stem cells (MSCs) have the ability to secrete multiple paracrine factors, including growth factors (10) that can potentially treat the major abnormalities that underlie lung injury and pulmonary edema during brain death, including impaired AFC. Specifically, keratinocyte growth factor (KGF) is known to be important in mediating some of the beneficial effects of hMSCs on repair in the lung transplantation; ex vivo lung perfusion; pulmonary edema

LUNG TRANSPLANTATION HAS BECOME the standard of care for selected patients with advanced lung diseases. However, patients are likely to die while awaiting lung transplantation, with waiting list mortality as high as 40% (12), in large part because of the limited availability of donor lungs. There is a worldwide gap between the supply and demand for transplantable organs, and this gap is enlarging (19). However, another major issue is that the use of marginal donor lungs is associated with an increased incidence of primary graft dysfunction, with poor early and long-term outcomes in recipients of such suboptimal organs (3). Hence, transplant teams tend to be conservative in the selection of donor lungs (29).

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ventilator-injured lung (10) and in restoring normal AFC in the human EVLP model of ALI (22).

We hypothesized that hMSCs would be effective in restoring alveolar fluid transport in lungs rejected for transplantation. When the results demonstrated that hMSCs were efficacious for upregulating AFC, we then tested the potential role of KGF as a paracrine mechanism responsible for the therapeutic effect of hMSCs.

**MATERIALS AND METHODS**

Selection criteria for ex vivo perfused human lung preparation. Our laboratory receives one to two human lungs from brain-dead donors from the Northern California Transplant Donor Network weekly. For inclusion in this study, lungs must also have been rejected for transplantation by the Northern California Transplant Donor Network and approved for research use by the family or appropriate surrogate. We previously found that in northern and central California donor lung utilization rate was 14% (102/738) over a two-year period. Lungs from 312 donors therefore did not meet regional lung selection criteria and were rejected. The primary reasons for rejection of these lungs for transplantation were hypoxemia (PaO$_2$/FiO$_2$ < 300), smoking history >20 pack-years, blunt injury, evidence of infection or aspiration, or chest radiographic infiltrates (38). Inclusion of lungs in the study was random and based solely on availability of a qualified surgical team to procure the lungs for the study. After informed consent from the brain-dead subject’s family to use lungs for research if they could not be used for transplantation, the lungs were resected en bloc without preservative flush, and inflated and transported at 4°C.

A previous study (38) found that most human lungs rejected for transplantation maintain alveolar epithelial fluid transport capacity. Furthermore, in a separate study, lungs rejected for transplant with measurable basal and stimulated fluid clearance had mean basal AFC rates of $19 \pm 10\%/h$ and mean stimulated (terbutaline) AFC of $43 \pm 13\%/h$ (17). Lungs were included in this study if the lung demonstrated impaired AFC, with lungs included if baseline AFC was $<10\%/h$ and cold ischemia time <48 h. Based on this criterion, from July 2011 to June 2012, ~50% of rejected lungs were eligible.

MSCs from the University of Minnesota National Heart Lung and Blood Institute Production Assistance for Cellular Therapy program. We established a formal collaboration with the University of Minnesota’s Molecular & Cellular Therapeutics Facility, the current Good Manufacturing Process facility of the University of Minnesota, through the National Heart Lung and Blood Institute Production Assistance for Cellular Therapy mechanism. Clinical-grade allogeneic bone marrow-derived hMSCs, washed as in a clinical trial and resuspended in PlasmaLyteA, were used in these studies.

Allogeneic bone marrow-derived human MSCs were manufactured at Molecular and Cellular Therapeutics of the University of Minnesota (FACT-accredited, CAP no. 18060–01, CLIA no. 24D0688128). Marrow was collected through Tissue Acquisition of Lonza Walkersville (Walkersville, MD). Enrichment of the mononuclear cell fraction of the marrow was accomplished using a manual separation method involving ficoll hypaque density gradient medium, specific gravity 1.077 g/dl (Isolymph; Gallard-Schlesinger Industries, Carle Place, NY). Analysis by flow cytometry was used to characterize the cell types within the final product. Positive markers include CD73, CD90, and CD105; negative markers include CD14, CD19, CD34, CD45, and HLA-DR. MSCs are also tested for their ability to differentiate into bone, fat, and cartilage as per International Society for Cell Therapy guidelines, for viability, using 7-aminocinomycin D DNA staining and flow cytometry, for karyotyping, and lipopolysaccharide (LPS) and mycoplasma contamination.

*Ex vivo perfused human lung and measurement of AFC.* The lung to be used, either right or left, was chosen based primarily on a practical issue, whether or not the pulmonary artery could be cannulated with a catheter. The lung was first separated, and the pulmonary artery was cannulated by passing a Foley catheter 2–3 cm in the surgical stump, securing it in place with a purse-string suture. The Foley catheter was then connected to a peristaltic pump via polyvinyl chloride tubing (Nalgene, Rochester, NY). To measure pulmonary arterial pressure, a pulmonary artery catheter (Cook, Bloomington, IN) was passed through a side port in the tubing and advanced to the end of the Foley catheter. The right or left mainstem bronchus was then intubated with the tip of a size 7.0 endotracheal tube. The lung preparation was weighed and suspended within a sealed acrylic container from a mass transducer (Harvard Apparatus, Holliston, MA). The container was surrounded by a heated (38°C) water bath in which the inner container served as a reservoir for the perfusate solution (DME H-21 with 5% bovine serum albumin, 900 m1) (Fig. 1A). The lung preparation was slowly warmed over 1 h with the peristaltic pump until a flow rate of 0.3–0.4 l/min was achieved, giving an average mean pulmonary arterial pressure of 10–12 mmHg. Venous drainage or left atrial pressure was passive (0 mmHg) (22). When the temperature of the venous drainage reached 36°C, the lung was slowly inflated with continuous positive airway pressure (CPAP) at 10 cmH$_2$O with 95% O$_2$ and 5% CO$_2$. Pulmonary arterial pressure and lung weight were then continuously monitored using a computer-integrated data acquisition system (Biopac, Goleta, CA). Perfusion pH, PO$_2$, and PCO$_2$ tensions were measured hourly with a blood gas machine (Bayer RapidLab 248, Erlangen, Germany). One hour following perfusion and oxygenation (CPAP), a lobe of the lung was
cannulated with a PE catheter (240 tubing; BD, Franklin Lakes, NJ) and advanced until gentle resistance was encountered. Warmed normal saline with 5% albumin (125 ml, alveolar fluid solution) was instilled in the right or left upper lobe. AFC was measured by the change in protein concentration of a 5% albumin instillate in the lung lobe over 30 min and expressed as mean AFC ± SD (%/h) for each condition using the equation, as in our previous experiments (2, 3): AFC (%/h) = 2(1 - Ci/Cf) × 100 (Ci is protein concentration at time 0 and Cf is protein concentration after 30 min). If the AFC of the lung lobe tested was <10%/h (final inclusion criteria), lungs received either intravenous hMSC (5 × 10^6 cells) added to the perfusate or the perfusion only as a control group. After 4 h, AFC in a separate lobe of the control and MSC-treated lungs was measured with the instillation of 125 ml of alveolar fluid solution (Fig. 1B).

Measurement of the receptor for advanced glycation end products in the bronchoalveolar lavage fluid. The alveolar fluid collected during the measurement of AFC in both the control and the MSC-treated lung lobes was used to measure the levels of the receptor for advanced glycation end products (RAGE), a marker of type 1 alveolar epithelial cell injury/activation, using an ELISA-based assay (Antigenix; R&D Systems).

KGF blocking antibody. To test the role of KGF in mediating the effect of MSCs, we used a KGF blocking antibody. In this set of experiments, we used human lungs that had been declined for transplantation with AFC <10%/h and >0%/h in our EVLP model. In the KGF neutralizing antibody experiments, the lungs received MSC (5 × 10^6 cells) added to the perfusate 1 h after administration of 2 μg/ml KGF neutralizing antibody (R&D Systems), or a control IgG antibody, intrabronchially.

Statistical analyses. Results were expressed as means ± SD if the data were normally distributed. Comparisons between two groups were made using the unpaired t-test. Comparisons with a sample over time were made by repeated-measures of ANOVA using the Bonferroni correction for multiple-comparison testing using Statview (SAS Institute).

RESULTS

Baseline data from donor lungs. Baseline demographic data and ischemia time for the 14 donor lungs are listed in Table 1. The right lung was used 35% of the time and the left lung 65% for the experiments. Donor age, evidence of radiographic abnormalities, PaO2-to-FIO2 ratio, and lung compliance (28) did not differ among the groups. The mean time from harvest to EVLP for the perfusion only group was 31 ± 6 h vs. 33 ± 31 h for the hMSC-treated group (P = 0.9).

Pulmonary arterial pressures. Pulmonary arterial pressures remained stable throughout the 4 h of EVLP and were not significantly different among the groups (Fig. 2). Perfusate oxygenation was not different among the groups at any time point, but, as expected, oxygenation deteriorated over time, due in part to the instillation of 125 ml of fluid in a lobe of the lung (to measure AFC) at baseline and in a separate lobe at 4 h (mean PaO2 = 123 ± 62 mmHg at baseline vs. 95 ± 30 mmHg at 4 h for perfusion only; PaO2 = 125 ± 14 mmHg at baseline vs. 71 ± 16 for the MSC group).

Effect of MSCs on AFC. The hMSCs normalized AFC over 4 h, compared with perfusion only (Fig. 3).

Effect of MSCs on air space levels of RAGE. The effect of MSCs on AFC was accompanied by a numerical but nonsignificant decrease in the bronchoalveolar lavage (BAL) concentration of RAGE (P = 0.17) (Fig. 4). BAL RAGE levels at baseline were 13,100 ± 1,395 pg/ml in the perfusion only group and 12,875 ± 914 pg/ml in the MSC group. At 4 h, BAL levels had increased in the perfusion only group to 14,100 ± 141 pg/ml and had decreased in the MSC group to 12,700 ± 547 pg/ml.

MSCs improve AFC in part by a KGF-dependent mechanism. The effect of MSCs on AFC was reduced when a neutralizing antibody to KGF was added intrabronchially with no effect from a control IgG antibody (Fig. 5).

DISCUSSION

The primary findings of this experimental study can be summarized as follows. In lungs that were not used for trans-
plantation and had been subject to prolonged cold storage, ~50% of the lungs demonstrated impaired AFC, a decreased capacity to reabsorb alveolar edema fluid, as defined as <10%/h. Treatment with allogeneic MSCs restored more normal AFC. This effect was mediated in part by KGF secretion. These results may have clinical implications because restoring more normal alveolar fluid transport in lungs before transplantation may reduce their susceptibility to developing reperfusion pulmonary edema or primary graft dysfunction and may make marginal lungs suitable to be used for lung transplantation.

Primary graft dysfunction after lung transplantation manifests itself initially as noncardiogenic pulmonary edema (6), and is a significant cause of morbidity and mortality (7). Experiments in a canine model of lung transplantation (31) and clinical observations in humans (35) suggest that impaired AFC contributes to the development of pulmonary edema in the posttransplant period. An intact alveolar epithelial barrier with preserved AFC has been associated with better clinical outcomes in patients with ARDS (37) and in patients with posttransplant reperfusion pulmonary edema (35).

Pulmonary edema is also a major factor contributing to poor donor oxygen indexes, resulting in refusal of procurement (14). In lungs from 29 donors that were rejected for transplantation, lung wet-to-dry weight ratio, a measure of pulmonary edema, was normal in only seven (24%) (38). In a recent trial of nebulized albuterol in brain-dead organ donors, mean lung weights were elevated by 250 grams compared with normal lung weight, suggestive of pulmonary edema, and chest radiographs confirmed these findings, indicating that pulmonary edema is common in organ donors (34). Strategies to enhance the clearance of pulmonary edema could lead to higher rates of donor lung utilization and a reduced incidence of primary graft dysfunction in recipients. Several stimuli can upregulate AFC (which is driven in part by active vectorial transport of sodium across alveolar epithelial sodium channels), including catecholamines and glucocorticoids (25). The compelling preclinical data highlighting the importance of intact AFC to the resolution of pulmonary edema has led to clinical trials of β-agonists in ARDS (18, 24) and in brain-dead organ donors (36). The failure to demonstrate benefit for systemic or aerosolized β-agonists in these populations suggests the need for a more innovative and fundamental therapeutic strategy. The present study highlights MSCs as a potential therapeutic agent to enhance pulmonary edema clearance in the marginal or injured donor lung before transplant.

MSCs have advantages over a single pharmacological therapy, such as the ability to secrete a variety of immune-modulating and tissue reparative factors, the ability to transfer mitochondria to injured cells, and the ability to home to sites of injury (27). Recent insights into stem cell biology have generated interest in the potential of MSCs to regenerate or repair damaged organs, including the lung (10). MSCs may directly attenuate bacterial pneumonia in donor lungs, a common cause of early morbidity and mortality after lung transplantation, via a number of mechanisms, including enhancement of phagocy-
tosis and increased bacterial clearance (23), and antimicrobial peptide secretion (21). Both the distal lung epithelium and the pulmonary endothelium are selectively accessible to stem cell therapies during EVLP, via the intrabronchial route or the pulmonary vasculature.

Bone marrow-derived MSCs are known to produce several epithelial-specific growth factors, including angiopoietin-1, hepatocyte growth factor, and KGF. In the ex vivo perfused human lung, we previously reported that the intrabronchial instillation of hMSCs 1 h following endotoxin-induced lung injury restored AFC, an effect that was mediated in part by the secretion of KGF (22). More recently, we also reported that clinical-grade hMSCs, given intrabronchially or via the perfusate, were effective in restoring normal AFC following E. coli pneumonia-induced ALI (23). This effect was associated with increased bacterial phagocytosis and killing by monocytes and macrophages, was more effective than antibiotic therapy alone during prolonged pneumonia, and was abolished by the administration of anti-KGF antibody. In the current studies, the beneficial effects of MSCs were mediated in part by KGF in restoring AFC in donor lungs with impaired fluid clearance. The beneficial effect of exogenous KGF has been reported in several ALI models (39), and is currently being tested as a potential therapy for ALI (8). KGF has a wide variety of effects on lung epithelial cells that may mediate its protective effect in lung injury. In particular, KGF stimulates AFC (33). Although MSCs have been shown to be an important source of KGF, and KGF has been shown to mediate enhanced AFC in other models of ARDS (10, 22, 23), we cannot exclude the possibility that the KGF antibody used in these experiments acted on endogenous KGF produced in the donor lung to reduce AFC. In this scenario, the role of MSCs would be to enhance endogenous KGF production. This is unlikely, given that previous experiments have shown that endogenous KGF production occurs late after lung injury (9), and MSCs have been shown to produce larger quantities of KGF than other stromal cells (10, 22). We previously demonstrated that, in an E. coli endotoxin-induced lung injury in the ex vivo perfused lung, KGF small-interfering RNA pretreatment of MSCs eliminated the therapeutic effect of MSC-conditioned medium in restoring the AFC rate (22). In addition, in the endotoxin-injured lobe without treatment, there was no restoration of the AFC rate, although there was endogenous KGF expressed in the injured alveolus, suggesting that MSC KGF secretion was important for restoring AFC.

In addition to AFC, we also measured alveolar fluid concentrations of RAGE as a marker of type 1 alveolar epithelial cell injury/activation. Elevated plasma levels of RAGE are a marker of poor outcome after lung transplantation (5). Elevated airspace levels of RAGE were significantly correlated with impaired AFC in lungs declined for transplantation (4). Our data indicate a trend toward a reduction in the airspace levels of RAGE in lungs treated with MSCs.

There are some limitations to these experiments that will have to be addressed before clinical translation. First, the experimental protocol was designed to assess AFC only, and not alterations in gas exchange or overall lung fluid balance. The procedure to determine AFC requires the instillation of 125 ml of warmed normal saline with 5% albumin in the right or left upper lobe at baseline and after 4 h, which precludes accurate assessment of gas exchange; moreover, only CPAP was used in place of positive pressure mechanical ventilation. Furthermore, the perfusate used, DME H-21 with 5% albumin, is not optimized to maintain vascular integrity over time, which results in increased edema formation (32).

The method used to assess AFC may have some limitations. We have made the assumption that AFC measured in one lobe reflects what is happening in other lobes of the lung, which it might not. However, in earlier experiments with the ex vivo human lung where the AFC rate was intact in the control lobe, we consistently found that both the upper and lower lobes had AFC >10%/h (22). Furthermore, the use of a change of protein concentration in a test solution instilled in the injured lung as a measurement of AFC can be confounded in some situations. Because the concentration of protein in the instilled solution in the lung is equal to the protein concentration present in the circulation, it is unlikely that an increase in protein concentration in edema fluid can be due to an increased movement of protein from the interstitial space into the airspaces. However, movement of liquid from the interstitial space to the alveoli could potentially dilute the protein in the instilled solution. In this circumstance, it can be difficult to determine whether the decrease in clearance is due to a decreased reabsorption of liquid or an enhanced movement of liquid from the interstitial space. This is why a decrease in alveolar protein concentration cannot be used to calculate net fluid accumulation in the air spaces. A rise in alveolar protein concentration, however, as noted in this study, especially above the level of plasma protein concentration, means there must have been net AFC. This method has been validated first in normal lung (2) and has also been validated in multiple models of lung injury (26).

Despite these limitations, we believe that AFC is an important functional measure in the assessment of lungs for transplant. Current selection criteria are poor discriminators of pulmonary injury and infection and lead to the exclusion of potentially usable donor lungs (38). We (38) and others (16) have reported that physiological parameters in the donor, combined with AFC, could increase the rate of successful organ usage.

Although we administered MSCs directly in the perfusate, the primary site of action demonstrated in the study (improved AFC) was the epithelium. We have administered MSCs both intrabronchially in isolated human lungs injured by LPS (22) and intrabronchially and systemically (as in this current study in the perfusate) in isolated human lungs injured by E. coli bacteria (23). We observed similar efficacy with both routes of administration (23). This may be related to the paracrine mode of action of MSCs, as well as to the ability of MSCs to traffic to inflammatory sites. Systemically administered MSCs are also known to immediately accumulate in pulmonary capillary beds (15). Because intravenous administration had equal efficacy to intrabronchial delivery in our recent preclinical studies, and would be a more straightforward method of administration in patients, we delivered MSCs directly in the perfusate for these experiments. We believe, however, that the intrabronchial approach still has research and potential clinical value.

Finally, donor gender differences could have contributed to differences among the groups in regard to AFC. Specifically, the MSC with KGF antibody group was 66% female vs. 0% female in the MSC with IgG antibody control group. However, an increase in female donors in this group is more
likely to have resulted in lungs with enhanced, rather than possible for this is secretion of KGF by MSCs. Organ availability continues to be a major impediment to more widespread use of lung transplantation. Only 15–25% of lungs from donors are transplanted, the lowest graft acceptance rate of any solid organ (30). EVLP has been suggested as a novel method to deliver therapeutic agents to marginal lungs. The current study provides support for the hypothesis that functionally impaired donor lungs could be improved during EVLP before transplantation with human bone marrow-derived allogeneic MSCs. Additional studies will be required to assess the effects of MSCs on oxygenation and lung compliance in clinical-grade EVLP systems using appropriate perfusion solutions and ventilation strategies before this therapy can proceed to clinical trials.

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DISCLOSURES

The authors of this manuscript have no competing interests to disclose.

AUTHOR CONTRIBUTIONS


REFERENCES


