Treatment with placenta-derived mesenchymal stem cells mitigates development of bronchiolitis obliterans in a murine model

Yunge Zhao, MD, PhD,[a] Jacob R. Gillen, MD,[a] David A. Harris, BS,[a] Irving L. Kron, MD,[a] Michael P. Murphy, MD, FACS,[b] and Christine L. Lau, MD, MBA[a]

Objective: Bone marrow–derived mesenchymal stem cells (MSCs) have shown therapeutic potential in acute lung injury. Recently, placenta-derived human mesenchymal stem cells (PMSCs) have shown similarities with bone marrow–derived MSCs in terms of regenerative capabilities and immunogenicity. This study investigates the hypothesis that treatment with PMSCs reduces the development of bronchiolitis obliterans in a murine heterotopic tracheal transplant model.

Methods: A murine heterotopic tracheal transplant model was used to study the continuum from acute to chronic rejection. In the treatment groups, PMSCs or PMSC-conditioned medium (PMSCCM) were injected either locally or intratracheally into the allograft. Phosphate-buffered saline (PBS) or blank medium was injected in the control groups. Tracheal luminal obliteration was assessed on sections stained with hematoxylin and eosin. Infiltration of inflammatory and immune cells and epithelial progenitor cells was assessed using immunohistochemistry and densitometric analysis.

Results: Compared with injection of PBS, local injection of PMSCs significantly reduced luminal obliteration at 28 days after transplantation ($P = .015$). Intratracheal injection of PMSCs showed similar results to local injection of PMSCs compared with injection of PBS and blank medium ($P = .022$). Tracheas treated with PMSC/PMSCCM showed protection against the loss of epithelium on day 14, with an increase in P63+CK14+ epithelial progenitor cells and Foxp3+ regulatory T cells. In addition, injection of PMSCs and PMSCCM significantly reduced the number of neutrophils and CD3+ T cells on day 14.

Conclusions: This study demonstrates that treatment with PMSCs is protective against the development of bronchiolitis obliterans in an heterotopic tracheal transplant model. These results indicate that PMSCs could provide a novel therapeutic option to reduce chronic rejection after lung transplant. (J Thorac Cardiovasc Surg 2014;147:1668-77)

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Lung transplantation is the best therapeutic option for many debilitating pulmonary diseases. Chronic rejection, which manifests histologically as bronchiolitis obliterans (BO), is the single most important cause of late mortality after lung transplantation, affecting up to 50% of patients 5 years after transplantation.[1,2] Despite remarkable progress in improving outcomes through the refinement of surgical technique and the use of more effective immunosuppressive regimens, BO still affects most lung transplant recipients by 5 years; no treatment options have shown beneficial effects for preventing or slowing the progress of this disease.[1,2] Therefore, innovative and effective therapies (such as molecule- and cell-based therapies) to prevent and attenuate the development of BO are urgently needed.

Mesenchymal stem cells (MSCs) originating from the bone marrow have been used as a therapeutic strategy in several in vivo models of acute lung injury, including bleomycin-induced,[3] intraperitoneal and intratracheal *Escherichia coli* endotoxin–induced[4,5] and lipopolysaccharide-induced[6] acute lung injury. Recent research has provided clear evidence that MSCs have great potential as a cell-based therapy for acute lung injury due to several features, including (1) secretion of multiple paracrine factors, including keratinocyte growth factor,[7] interleukin (IL)-1 receptor antagonist (IL-1RA),[8] granulocyte colony-stimulating factor (G-CSF), and granulocyte macrophage colony-stimulating factor

From the Division of Thoracic and Cardiovascular Surgery,[a] Department of Surgery, University of Virginia Health System, Charlottesville, Va; The Vascular and Cardiac Center for Adult Stem Cell Therapy,[b] Indiana University School of Medicine, Indianapolis, Ind.

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Address for reprints: Christine L. Lau, MD, MBA, Department of Surgery, PO Box 800679, Charlottesville, VA 22908-0679 (E-mail: cll2y@virginia.edu).

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Abbreviations and Acronyms

BO = bronchiolitis obliterans
DAPI = 4,6-diamidine-2-phenylindole dihydrochloride
G-CSF = granulocyte colony-stimulating factor
GM-CSF = granulocyte macrophage colony-stimulating factor
H&E = hematoxylin and eosin
HPF = high-power field
HTT = heterotopic tracheal transplant
IL = interleukin
IL-1RA = interleukin 1 receptor antagonist
MSC = mesenchymal stem cells
PBS = phosphate-buffered saline
PMSC = placenta-derived human mesenchymal stem cells
PMSCCM = PMSC-conditioned medium
TNF = tumor necrosis factor

(GM-CSF)[9]; G-CSF and GM-CSF are believed to promote the mobilization of endogenous stem cells into the blood circulation; (2) blocking of inflammatory cytokines, such as interferon-γ, IL-2, IL-1β, IL-4, macrophage inflammatory protein-2, and tumor necrosis factor (TNF)-α,[3,7] all fundamental proinflammatory cytokines involved in lung injury; (3) immunosuppressive effects, by inhibiting the activity of innate and adaptive immune cells[10,11]; (4) alteration of the endothelial and epithelial permeability of the lung[5,12]; (5) reduction of edema by restoring alveolar fluid clearance.[7]

Recently, human placenta-derived MSCs (PMSCs) have been isolated, characterized, and studied by various laboratories. PMSCs not only express common MSC surface markers such as CD11a, CD29, CD44, CD73 CD90, CD105, CD146, CD166, and HLA-ABC but also have the ability to differentiate into adipogenic, chondrogenic, and osteogenic lineages under the proper conditions.[13,14] Compared with bone marrow–derived MSCs, PMSCs are more easily obtained and are available in large numbers. Because they have similar properties and effects as bone marrow–derived MSCs, they are becoming a promising alternative source of MSCs in basic research and clinical applications. In the recent literature, PMSCs have been shown to have immunosuppressive properties by suppressing the activation and proliferation of T lymphocytes.[15,16] In addition, PMSCs show minimal to no immunogenicity.[17]

Because of these unique properties of PMSCs, the present study was designed to explore the use of PMSCs as a novel treatment to prevent the development of BO. We hypothesized that local treatment with PMSCs or PMSC-conditioned medium (PMSCCM) suppresses inflammatory and immunologic cascades and reduces the development of BO in a murine heterotopic tracheal transplant (HTT) model.

MATERIALS AND METHODS

Culture of Human PMSCs

PMSCs[18] were obtained from the laboratory of our collaborator, Dr Michael P. Murphy (Indiana University School of Medicine, Indianapolis, Ind). The PMSCs were grown in Dulbecco's modified Eagle's medium/F12K (1:1) medium (Gibco BRL, Life Technologies, Rockville, Md) supplemented with 10% fetal bovine serum and 1% of penicillin/streptomycin in a humidified incubator containing 5% CO_2 at 37°C. When the cell density reached 90% confluence, the conditioned medium was collected and centrifuged at 1000 rpm to remove the debris. After washing with warm 1× phosphate-buffered saline (PBS), the cells were detached with 0.05% Trypsin-EDTA and centrifuged at 600 rpm. The harvested cells were then washed with 1× PBS twice. The cells were counted using a Cellometer Auto 2000 (Nexcelom Bioscience LLC, Lawrence, Mass). The cells were diluted in 1× PBS (1×10^7) and put on ice until they were injected.

Mice

The experimental mice were purchased from Jackson Laboratory (Bar Harbor, Maine). The Animal Care and Use Committee at the University of Virginia approved all aspects of the experimental protocol before experimentation. All experimental mice received humane care in accordance with *The Principles of Laboratory Animal Care* and *The Guide for the Care and Use of Laboratory Animals*.

Experimental Groups

The mouse HTT model was used as described previously.[19] Male BALB/c mice were used as donors and C57BL/6 mice as recipients (22-27 g). Each recipient mouse received 4 different donor tracheas transplanted subcutaneously. The recipient mice were randomly divided into 6 groups: group 1, PMSC cells (1×10^6 cell in PBS) were injected locally into the subcutaneous area surrounding the transplanted trachea on day 1 after transplantation (4 recipient mice/time point); group 2, the same volume of PBS was injected locally as a control for group 1; group 3, PMSC cells (1×10^5 cell in PBS) were injected directly into the lumen of the transplanted trachea on day 3 after transplantation (4-7 mice/time point); group 4, the same volume of PBS was injected directly into the lumen of the transplanted trachea on day 3 after transplantation as a control of group 3 (3 mice/time point); group 5, PMSC-CM was injected directly into the lumen of the transplanted trachea on day 3 after transplantation (4-6 mice/time point); group 6, the same volume of blank medium was injected directly into the lumen of the transplanted trachea on day 3 after transplantation as a control for group 5 (3 mice/time point). The intratracheal injections were performed as previously described.[20] The recipient mice were killed and the allografts were harvested on day 7, 14, or 28 for histology and immunohistochemical staining.

Histology and Immunohistochemical Staining

The harvested allograft specimens were immediately fixed in 10% formalin. After 24 hours, fixed tracheas were embedded in paraffin and sections were stained with hematoxylin and eosin (H&E) or stained by immunohistochemistry.

Immunohistochemical staining of mouse migratory macrophages and neutrophils. Staining for mouse migratory macrophages and neutrophils was performed as described previously.[19,20] Briefly, rat antimouse neutrophil antibody (AbD Serotec, Raleigh, NC) and rat antimouse Mac-2 antibody (Accurate Chem, Westbury, NY) were used as primary antibodies. Alkaline phosphatase-conjugated donkey anti-rat IgG (Sigma, St Louis, Mo) was used as a secondary antibody. The

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