

Pulmonary inflammation after lung transplantation

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SCOPE OF THE CLINICAL PROBLEM

THE ANNUAL NUMBER OF LUNG TRANSPLANTS WORLDWIDE has increased 130-fold over the last 20 years. In 2004, the Registry of the International Society for Heart and Lung Transplantation reported that >17,000 lung transplants have been performed worldwide and that >1,600 lung transplants are performed annually. Despite advances in operative management, lung preservation, critical care, and immunosuppression, long-term survival remains limited. In fact, outcomes for lung transplantation are the worst of any solid organ transplant. In most series, 30-day mortality rates approach 15%, and approximately 50% of lung transplant patients die within 3 years.¹

The main obstacles to present day lung transplantation involve (1) lack of donor organs, (2) ischemia/reperfusion (I/R) injury, (3) rejection, and (4) development of bronchiolitis obliterans. Lung I/R injury after transplantation remains the most common cause of respiratory failure and manifests typically during the first 72 hours post-transplantation.¹ I/R injury continues to be a common and substantive cause of morbidity and mortality in the early postoperative period, with reported rates as great as 41%.¹ The 30-day mortality of patients with I/R injury is about 40%, compared with 7% in patients without I/R injury.² I/R injury usually presents with the immediate impairment in lung function after transplantation accompanied by rapid development of pulmonary edema, increased pulmonary vascular resistance, and

decreased airway compliance. Patients with I/R injury require prolonged mechanical ventilation with greater hospital stays and are at an increased risk of multiorgan failure. Lung I/R injury has long-term consequences as well. I/R injury is a risk factor for late graft failure (bronchiolitis obliterans).³ Treatment strategies for lung transplant patients that develop I/R injury consist primarily of maintaining oxygenation and lung function. Use of inhaled nitric oxide has offered only limited benefit. I/R injury occasionally is so severe that extracorporeal membrane oxygenation is required to maintain oxygenation. Clearly, studies into the prevention and treatment of I/R injury are needed in lung transplantation.

I/R-INDUCED INFLAMMATION

Despite advances in donor management and graft preservation, the pathophysiology of lung I/R injury remains incompletely understood. The lungs are particularly susceptible to I/R injury, likely owing to the rich vascularity and relatively large surface area over which blood-borne components interact with the endothelium. Most studies suggest that it is the process of reperfusion of the graft, and not the ischemia per se, that plays a more important role in causing injury. The mechanisms of I/R injury are diverse and include generation of reactive oxygen species (ROS), leukocyte activation/recruitment, complement and platelet activation, abnormalities in pulmonary vascular tone, and increased procoagulant activity. The production of proinflammatory cytokines is increased considerably in the lung after I/R. Several studies, including our own, suggest that lung I/R injury is biphasic, with a distinct, acute injury characterized by macrophage activation followed by a later, neutrophil-dependent injury.⁴ We and others have shown that alveolar macrophages initiate I/R injury via release of cytokines, which contribute subsequently to the activation and infiltration of circulating neutrophils. This acute injury is followed by a

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cascade of events leading to pulmonary inflammation. These studies conclude collectively that I/R injury is largely initiated by activated macrophages, whereas later injury is mediated by activated, infiltrating neutrophils.

MACROPHAGES IN I/R INJURY

Abundant evidence suggests that alveolar macrophages are the primary initiating cell in lung I/R injury. Alveolar macrophages in the donor lung are activated rapidly during reperfusion and release proinflammatory chemokines and cytokines, such as tumor necrosis factor (TNF)- α , interleukin (IL)-1 β , and CCL2 (MCP-1). This early damage is followed by a cascade of events leading to activation of the recipient inflammatory system against the already damaged vascular endothelium and airway epithelium. This model helps explain the acute, neutrophil-independent injury. These data indicate that acute injury is in large part initiated by activated macrophages, whereas delayed injury is mediated by infiltration of activated neutrophils. Recently, our laboratory assessed the impact of alveolar macrophage depletion on lung I/R injury.⁵ Mice were pretreated intratracheally with liposome-clodronate, which induces a >80% depletion of alveolar macrophages. Alveolar macrophage depletion significantly curtailed injury (by decreasing edema and microvascular leak), dysfunction (by decreasing pulmonary arterial pressure and increasing compliance), and proinflammatory cytokine production (TNF- α , CCL2, and CXCL2), indicating that alveolar macrophages are critical to the initiation of lung inflammation, dysfunction, and injury after I/R.

NEUTROPHILS AND I/R INJURY

Neutrophil activation and free radical production is a principal contributor to posttransplant lung I/R injury. Possibly the most critical step mediating injury is the adherence of circulating neutrophils to vascular endothelium after reperfusion, mediated by expression of selectin proteins on cell surfaces, which results in the release of destructive proteases, oxygen radicals, and cytokines that promote tissue injury. Various studies have shown that neutrophil-activating compounds cause lung injury, neutrophil depletion attenuates lung I/R injury, and immunoneutralization of neutrophil adhesion prevents lung I/R injury. Neutrophils are end-effectors of lung injury, and activation and infiltration of neutrophils into damaged lung is orchestrated by the local production of potent chemokines such as CXCL1 (GRO1) and CXCL8

(IL-8) produced by various cell types, including primarily alveolar macrophages, T lymphocytes, and even epithelial cells. Also contributing to neutrophil sequestration is the upregulation of cell surface adhesion molecules after I/R such as leukocyte adhesion molecule CD18 and the endothelial adhesion molecules intracellular adhesion molecule-1 and P-selectin. The resulting damage is then manifested by increased capillary permeability, altered vascular tone, and acute graft failure.

T LYMPHOCYTES AND I/R INJURY

Involvement of lymphocytes in posttransplant lung I/R injury has not been considered until recently. A role for lymphocytes may not seem immediately intuitive, because the requirement for contact with antigen bound to major histocompatibility complex protein to activate T-helper cells seems to exclude the possibility of early activation of lymphocytes. Recent studies, however, demonstrate antigen-independent, T-cell activation by oxygen radicals and cytokines such as TNF- α and CCL5 (RANTES). In the microcirculation, T cells may amplify inflammation by binding simultaneously to endothelial cells, macrophages, platelets, and neutrophils. During initiation of inflammation, professional antigen-presenting cells (dendritic cells, mast cells, and macrophages) respond to various stimuli, including lipopolysaccharide and ROS, and secrete proinflammatory cytokines. The primary inflammatory cytokines, IL-1 β and TNF- α , amplify leukocyte recruitment and their survival in tissue by initiating cross-talk between T cells and antigen-presenting cells.

Several recent studies describe renal and hepatic protection from I/R injury in either null mice (which lack T cells) or T-cell-depleted mice. Our laboratory has tested the hypothesis that CD4⁺ T cells mediate neutrophil-induced lung I/R injury.⁶ In this study, monoclonal antibodies (anti-GK1.5, anti-53-6.7, or anti-GR-1) were used to render the mice deficient in CD4⁺ T cells, CD8⁺ T cells, or neutrophils, respectively. Comparable and significant protection from I/R-induced lung dysfunction and injury occurred after depletion of neutrophils or CD4⁺ T cells but not after depletion of CD8⁺ T cells. In addition, lung I/R injury was proportional to the infiltration of neutrophils but not T cells. Moreover, the production of CXCL1 and TNF- α was significantly decreased only by CD4⁺ T-cell depletion. These data suggest that, although neutrophils mediate I/R injury, CD4⁺ T cells play a critical role in stimulating chemokine production and neutrophil chemotaxis during I/R injury. Thus, both CD4⁺ T lymphocytes and neutrophils

accumulate during reperfusion and contribute sequentially to lung I/R injury.

CYTOKINES AND OXIDATIVE STRESS IN LUNG I/R INJURY

Numerous clinical and experimental studies have shown that I/R of lungs and other solid organs induces a rapid release of both pro- and anti-inflammatory cytokines, such as TNF- α , interferon- γ , CXCL8, IL-1 β , IL-10, and IL-12. In the lung, many of these cytokines are derived largely from resident and/or circulating/infiltrating leukocytes and mediate inflammatory responses, such as activation and chemotaxis of leukocytes. Expression of cytokines in the lung after I/R may not only cause immediate tissue injury, but may predispose the lung allograft to rejection. Because the lung possesses a very important resident population of alveolar macrophages, it is likely that these cells are stimulated rapidly after I/R to initiate an inflammatory cascade. TNF- α is a rapid-response cytokine that likely promotes injury by altering expression of other proinflammatory cytokines and influencing neutrophil recruitment. Neutralizing antibodies to TNF- α have demonstrated the importance of TNF- α in acute I/R injury using an *in vivo* rat lung model.⁷ Our laboratory has shown that TNF- α knockout mice are protected from lung injury and dysfunction after I/R injury.⁸ These studies demonstrate that TNF- α produced from a resident lung cell population, likely alveolar macrophages, is a key initiating factor of lung I/R injury. Not only is TNF- α produced largely by alveolar macrophages, it also has effects on the macrophage respiratory burst, which can lead to oxidative tissue injury.

Oxidative stress and the release of ROS from activated macrophages and other leukocytes also contribute to lung I/R injury and acute graft dysfunction. Ischemia can "prime" the lung tissue, whereby the reintroduction of oxygen during reperfusion induces a rapid production of ROS, such as superoxide anion, hydrogen peroxide, and hydroxyl radicals. Multiple studies have shown that free radical scavengers or selected enzymes that metabolize ROS attenuate I/R injury. Our laboratory has demonstrated an important role of ROS derived from nicotinamide adenosine dinucleotide phosphate (NADPH) oxidase in lung I/R injury by showing that p47^{phox} knockout mice, which lack NADPH oxidase activity, are protected from I/R-induced lung injury and subsequent pulmonary dysfunction.⁹ In this same study, we also demonstrated that NADPH oxidase activity in bone marrow-derived cells contributes importantly to the lung I/R

injury, raising the possibility that NADPH oxidase may represent a novel therapeutic target for the treatment of I/R injury after lung transplantation.

Other contributors to lung I/R injury include lipid peroxidation, platelet-activating factor (PAF), and complement. Lipid peroxidation refers to the oxidative degradation of membrane lipids, whereby free radicals "steal" electrons from lipids in cell membranes resulting in cell damage. ROS production mediates lipid peroxidation in postischemic tissues and promotes the formation of inflammatory agents that recruit and activate neutrophils. In this respect, lung levels of lipid peroxidation have been used as a quantitative measure of lung oxidant injury after I/R. PAF is a proinflammatory phospholipid released by a wide variety of cells, including platelets and leukocytes. PAF seems to play an important role in mediating the adhesive interaction between circulating leukocytes and microvascular endothelium induced by I/R; in addition, PAF promotes leukocyte extravasation associated with I/R. Numerous studies describe activation of the complement system after I/R, which may lead to cellular injury through direct and indirect mechanisms. Inhibition of the complement system also offers a therapeutic target for decreasing tissue injury in various clinical settings of I/R.

PROTECTION FROM INJURY BY ACTIVATION OF THE ADENOSINE A_{2A} RECEPTOR

One anti-inflammatory mechanism used by many cells is mediated through the release of adenosine. Adenosine is a protective agent in I/R injury of the lung, heart, intestine and liver, and mediates its effects through four subtypes of the G protein-coupled, adenosine receptor (AR) family, which includes A₁AR, A_{2A}AR, A_{2B}AR, and A₃AR. Our laboratory focused on the protective effects of A_{2A}AR activation in lung I/R injury. A_{2A}ARs are expressed highly on bone marrow-derived cells, including T lymphocytes, neutrophils, monocytes, macrophages, platelets, and mast cells. Subclassification of the ARs has shown that activation of A_{2A}AR produces anti-inflammatory responses, prevents leukocyte adhesion, and inhibits release of ROS. Our laboratory has shown that specific activation of A_{2A}AR during reperfusion attenuates lung injury and dysfunction in both mouse and rabbit models of lung I/R as well as in a clinically relevant, pig lung transplant model. Specifically, A_{2A}AR activation attenuates pulmonary dysfunction, edema, neutrophil infiltration, proinflammatory cytokine production, and microvascular leak after lung transplantation.¹⁰ Thus, novel, potent,

and specific A_{2A}AR agonists may prove to be an effective therapy for the prevention and treatment of posttransplant pulmonary inflammation.

In conclusion, pulmonary I/R, which leads to lung injury and dysfunction after transplant, is a complex inflammatory process involving many cellular components, as well as intra- and intercellular signaling mechanisms. This process is initiated largely upon reperfusion, with the influx of oxygen and the generation of ROS by several different resident and circulating cell populations. Leukocytes seem to be the most rapidly and extensively activated cell populations after transplantation; however, activation of other cell types, such as alveolar epithelial and vascular endothelial cells, play important roles in this process. It seems that the major players are multiple and include the rapid production of ROS by NADPH oxidase and proinflammatory cytokines such as TNF- α from activated macrophages and CD4⁺ T cells. Ongoing studies focusing on the attenuation and suppression of the activity of these major players as well as their potential interactions may very well lead to novel and specific therapies to prevent or treat I/R injury in lung transplant patients.

REFERENCES

1. Granton J. Update of early respiratory failure in the lung transplant recipient. *Curr Opin Crit Care* 2006;12:19-24.
2. McGregor CG, Daly RC, Peters SG, Midthun DE, Scott JP, Allen MS, et al. Evolving strategies in lung transplantation for emphysema. *Ann Thorac Surg* 1994;57:1513-20.
3. Fiser SM, Tribble CG, Long SM, Kaza AK, Kern JA, Jones DR, et al. Ischemia-reperfusion injury after lung transplantation increases risk of late bronchiolitis obliterans syndrome. *Ann Thorac Surg* 2002;73:1041-7.
4. Fiser SM, Tribble CG, Long SM, Kaza AK, Cope JT, Laubach VE, et al. Lung transplant reperfusion injury involves pulmonary macrophages and circulating leukocytes in a biphasic response. *J Thorac Cardiovasc Surg* 2001;121:1069-75.
5. Zhao M, Fernandez LG, Doctor A, Sharma AK, Zarbock A, Tribble CG, et al. Alveolar macrophage activation is a key initiation signal for acute lung ischemia-reperfusion injury. *Am J Physiol Lung Cell Mol Physiol* 2006;291:L1018-26.
6. Yang Z, Sharma AK, Linden J, Kron IL, Laubach VE. CD4⁺ T lymphocytes mediate acute pulmonary ischemia-reperfusion injury. *J Thorac Cardiovasc Surg* 2009;137:695-702.
7. Eppinger MJ, Deeb GM, Bolling SF, Ward PA. Mediators of ischemia-reperfusion injury of rat lung. *Am J Pathol* 1997;150:1773-84.
8. Maxey TS, Enelow RI, Gaston B, Kron IL, Laubach VE, Doctor A. Tumor necrosis factor-alpha from resident lung cells is a key initiating factor in pulmonary ischemia-reperfusion injury. *J Thorac Cardiovasc Surg* 2004;127:541-7.
9. Yang Z, Sharma AK, Marshall M, Kron IL, Laubach VE. NADPH oxidase in bone marrow-derived cells mediates pulmonary ischemia-reperfusion injury. *Am J Respir Cell Mol Biol* 2009;40:375-81.
10. Reece TB, Ellman PI, Maxey TS, Crosby IK, Warren PS, Chong TW, et al. Adenosine A2A receptor activation reduces inflammation and preserves pulmonary function in an in vivo model of lung transplantation. *J Thorac Cardiovasc Surg* 2005;129:1137-43.