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OPINION

Could Ex Vivo Lung Perfusion Be a Platform to Decrease the Incidence of Chronic Lung Allograft Dysfunction?

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The number of patients requiring lung transplantation is increasing, with a significant unmet demand for grafts. Ex vivo lung perfusion has been developed to increase graft recruitment. The major complications of lung transplantation include chronic allograft dysfunction (CLAD) whose cumulative incidence ranges from 43–80% within the first 5 years of transplantation. Many risk factors are listed for development of CLAD and almost all of those risk factors would involve activation of Toll-like receptors. This paper represents the author's overview regarding the development of CLAD as a complication of lung transplantation and the possible protective potential of ex vivo lung perfusion in this regard. © 2015 IMSS. Published by Elsevier Inc.

Key Words: Lung transplantation, Ex vivo lung perfusion, Graft rejection, Toll-like receptor, Versican and chronic allograft dysfunction.

Introduction

With the increasing incidence of various lung diseases, end-stage pulmonary failure has been frequently seen. For such a condition, lung transplantation constitutes the main therapeutic and lifesaving strategy. With the relatively limited donor availability, there is an increasing unmet gap between graft demands and availability. In addition, the majority of the available lung grafts would be excluded and not considered for transplantation, based on failure to meet the standard selection criteria for transplantation. This is usually related to the presence of edema, minor contusions or other minor conditions within the graft (1).

Ex vivo lung perfusion (EVLP) is a method to keep the lung normothermic and metabolically active between donation and transplantation, which would allow the chance for graft reconditioning and reassessment. Accordingly, the chance of graft acceptance would increase, leading to increased donor availability through re-recruiting previously not selected grafts (1,2).

A general simple model to explain the technique of EVLP involves the placement of the graft into a hard shell, where it is connected to a ventilator and a perfusion circuit exposed to a pump, temperature adjustor and leukocyte filter (Figure 1). The oncotic pressure of the perfusate helps to resolve pulmonary edema. Meanwhile, keeping the graft under normothermic conditions allows the healing and regenerative activity within the graft to take place (1).

Accordingly, many teams worldwide have considered EVLP in the clinical settings of lung transplantation (1–3). However, lung transplantation might be complicated with major problems; for example, graft failure, graft rejection and the development of bronchiolitis obliterans, which are included under the recent term of chronic allograft dysfunction (CLAD). CLAD describes a sustained lack of the normal function of the transplanted graft with a persistent decline in forced expiratory volume in 1 sec (FEV_1), Bronchiolitis obliterans syndrome (BOS) describes the persistence of FEV_1 ≤80% baseline FEV_1 for ≥3 weeks, which is pathologically confirmed as obstructive obliterans in lung biopsies by the surrounding of bronchioles with cellular infiltrates and inflammatory reactions (4).

In this manuscript, some basic immunological data would be discussed as a trial to understand the effects of ischemia-reperfusion on lung grafts, and how this could constitute the initial stimuli for the development of CLAD.

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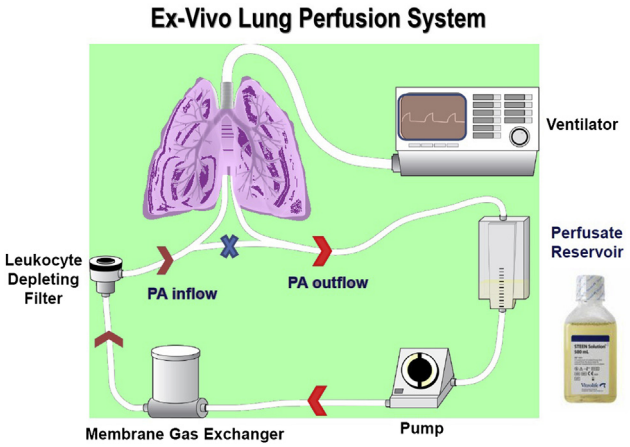

Figure 1. Diagrammatic representation of the EVLP system. The graft is connected to ventilator and circuit, which is subjected to perfusate reservoir, pump, gas exchanger and leukocyte depleting filter. Copied and modified from Andrew Fisher. Reconditioning Donor Lungs for Transplantation. Online EVLP ppt. (A color figure can be found in the online version of this article.)

In addition, the manuscript discusses how EVLP could play a protective role in this regard.

Effects of Ischemic-reperfusion Injury

The role of toll-like receptor 4 (TLR4) in lung ischemic-reperfusion injury (IRI) has been documented. TLR4 is a key mediator of IRI- induced pulmonary edema. This was proved through prevention of IRI-induced pulmonary edema by competitive inhibition of TLR4 (5). Warm ischemia was found to result in actin cytoskeletal rearrangements and formation of gaps in the pulmonary endothelium. That effect was blocked through inhibition of TLR4 (5).

Pulmonary edema in response to IRI was found to develop within 5 minutes after reperfusion and persist longer (at least for 3 h) in TLR4 sufficient mice compared to TLR4 deficient mice, which develop significantly less edema that resolves totally within 1 h after reperfusion (5). Remarkably, TLR4 mediated pulmonary edema is not dependent on activation of MAPKs or NF-κB and is not dependent on MyD88 (5).

Previous experimental data show that TLR4 on parenchymal lung cells, and not on pulmonary macrophages, is essential for IRI-induced pulmonary edema. However, activation of TLRs stimulates pulmonary macrophages to secrete chemokines and cytokines that induce recruitment of neutrophils and monocytes from the blood. In addition, activation of TLR4 on pulmonary fibroblasts results in increased extracellular versican (5,6).

Versican is a major extracellular glycosaminoglycan, which increases in response to many chronic lung diseases. Versican binds TLR2 and induces the production of pro-inflammatory cytokines and inflammatory cell invasion. Meanwhile, neutrophil infiltration was found to induce versican over-expression (7).

Versican was found to be an indicator for pulmonary complication following lung transplantation. Biopsies taken from transplanted lungs at 6 and 12 months after transplantation showed significant increase in versican, in comparison to control individuals. Moreover, the up-regulation of versican was more significant in patients who developed bronchiolitis obliterans. Versican is considered to be an TLR2 agonist (8).

Resting endothelial cells express little or no TLR2; however, the inflammatory cytokines IL1β and TNFα upregulate TLR2 (9,10). Activation of TLR2 (binding to versican) results in a significant increase in IL8 production (11,12).

IL8 is a chemokine produced by macrophages and other cell types such as epithelial cells, airway smooth muscle cells and endothelial cells. Being regulated by NF-κB, activation of TLR4 (e.g., on macrophages), TLR2 and other TLRs would participate in increased IL8 production. IL8 is a potent chemokine and promotes angiogenesis. It is known to play an important role in the development of bronchiolitis (13).

The main target of EVLP is to minimize the graft edema and to recondition the graft to improve the transplantation outcome (14). Investigating the patterns of cytokine production within the lung graft during EVLP revealed a significant increase in TNFα, which started at the beginning of perfusion and reached its peak at 2 h and then decreased gradually (15). Meanwhile, IL8 increases 2 h after onset of perfusion and persists for 6−7 h (15,16).

Taken together, the scenario might be as follows: IRI results in activation of TLR4, leading to graft edema and increased production of versican. IRI results also in increased reactive oxygen species (ROS) production and potassium channel inhibition, which activates inflammasomes and caspase 1, resulting in activation of IL1β and IL18, both cytokines induce IL6, whose production was also found to increase during EVLP (16). In addition, IL1β upregulates TLR2.

With the start of perfusion, TNFα production is enhanced. Both IL1β and TNFα up-regulate TLR2 on pulmonary cells. Vesican binds TLR2 leading to a marked increase in IL8, which starts to increase 2 h after the onset of perfusion and persists for at least 6-7 h. IL8 is a potent chemokine especially for neutrophils and other granulocytes, which are particularly involved in the development of CLAD (Figure 2). With persistence of this vicious circle, cytokine production would increase, inflammatory cell infiltration would increase, versican production would increase, and CLAD would develop.

Although TLR4/TLR2$^{-/-}$ mice were found to have reduced markers of inflammation in broncho-alveolar lavage fluid early after lung injury, the increased pulmonary apoptosis resulted in increased mice mortality (17).