REVIEW ARTICLE

Translational Insights on Lung Transplantation: Learning from Immunology

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ABSTRACT

The introduction of ex vivo lung perfusion (EVLP) in the practice of lung transplantation has allowed the reconditioning of the marginal grafts and their conversion into transplantable grafts. In addition, EVLP can provide a platform for the application of various preventive measures to decrease the incidence of post-transplant complications. While the Toronto team targets the attenuation of the cytokine production within the graft through gene therapy to up-regulate IL-10, other measures could be applied to achieve significant attenuation of the cytokine load of the graft. This manuscript provides a short overview on the importance of the attenuation of the cytokine production within the transplanted lung grafts and some possible strategies to achieve this goal.

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INTRODUCTION

Lung transplantation is the main therapeutic strategy for end stage pulmonary failure that results from various chronic lung diseases. The major problems facing lung transplantation could be summarized in the following points; the wide unmet gap between graft demands and availability. The incidence of primary graft dysfunction (PGD) following transplantation and the development of chronic lung allograft dysfunction (CLAD) (1,2). Research progresses rapidly and strongly to overcome those three major obstacles, considering the improvement of the activity and the outcome of lung transplantation, as well as the increased patient survival and the improved quality of life as the common targets of work (1). In this manuscript, light will be shed on some basic immunological data that could be translated into the clinical lung transplantation practice.

The Wide Unmet Gap between Graft Demands and Availability

The majority of lung grafts would be excluded and not considered for transplantation, based on the failure to meet the standard selection criteria. This is usually related to the presence of edema, minor contusions or other minor conditions within the graft, which potentiate the already present decline in the donor availability (1,3). To increase the organ availability through the reconditioning of the marginal brain death donor grafts and the recruitment of circulatory death donor grafts, *ex-vivo* lung perfusion (EVLP) technique was introduced (4).
Further improvement of EVLP was achieved by the Toronto team, who reported for the first time the ability to keep and reassess the graft under *ex-vivo* perfusion for 12 hours, where the *XVIVO* system is used (5,6) (Figure 1). In addition, other EVLP protocols are followed in the surgical practice of lung transplantation (7), with the results confirming the ability of the technique to improve and recruit the previously rejected grafts, where the post-transplant graft functions and the patient's survival are comparable to those of the standard transplantation (1-7). Nevertheless, EVLP could be used as a platform for the application of measures that aim at the prevention of post-transplant complications (1).

PGD and CLAD Following Lung Transplantation

The incidence of PGD following lung transplantation could significantly worsen the prognosis in the immediate post-transplant period. In addition, survivors of PGD would have longer ventilation periods, longer ICU and hospital stays, higher 30 days mortality and increased incidence of CLAD (8,9). Moreover, the long term survival rates after lung transplantation are less than those after the other solid organ transplants, with appreximately 55% 5-year survival rate (2).
Within the multiple risk and causative factors participating in the development of post-transplant complications, inflammatory cytokines have an important contribution to its pathogenesis (10). This could be clearly seen by investigating the effect of the ischemic-reperfusion injury (IRI) on the cytokine production within the lung. Exposing the lung graft to ischemia followed by reperfusion during EVLP resulted in the increased production of IL-1β, IL-6, TNF-α and IL-8. However, the patterns of the increase of these cytokines were not the same, where TNF-α starts to increase with the onset of

reperfusion, and reaches its peak after 2 hours, then its level starts to decline gradually. Meanwhile, the level of IL-8 production increases after 2 hours of perfusion and lasts for 6 -7 hours (11,12).

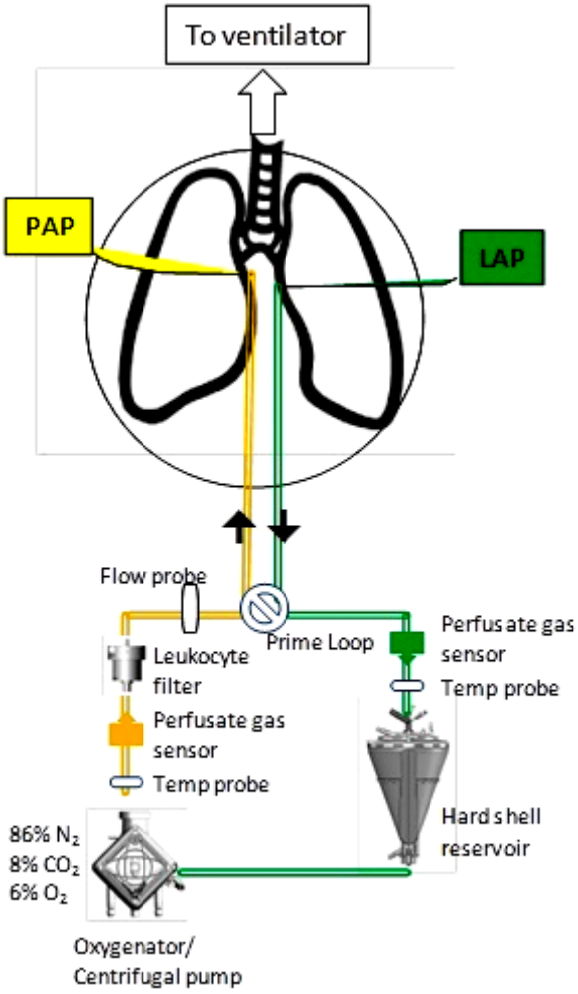

Figure 1. Diagrammatic representation of the XVIVO system describing the principle of the EVLP technique. The graft is placed in a hard shield and connected to a ventilator and a circuit, which is subjected to perfusate gas sensors, temperature probes, a perfusate reservoir, a centrifugal pump, an oxygenator, a flow probe and a leukocyte filter. Downloaded from http://www.xvivoperfusion.com (05.11.2014)

Basis of the Increased Cytokine Production in Response to IRI

In response to various tissue injuries, pathogen associated and damage associated molecular patterns are released, which activate intracellular macromolecular complexes called inflammasomes. Activation of inflammasome occurs on two levels; the level of a first signal (priming) and the level of a second signal (13). Toll-like receptors (TLRs) (which are activated during infection and by ischemic-reperfusion injury) are able to induce the first signal of inflammasomes activation, where the priming of NLRP3 inflammasome requires the activation of extra-cellular signal regulated kinase 1, and is reactive oxygen species (ROS) dependent (13). The second signal can be induced by many effectors, which all share a common end result of K^+ efflux. Once the cytosolic

K^+ level drops, the primed inflammasomes become functional and activates caspase 1. Caspase 1 proteolytically activate pro-IL-1β and pro-IL-18 (14,15).
As the graft would be exposed to a period of ischemia followed by reperfusion (which occurs in the recipient in case of standard transplantation or during EVLP), the IRI results in the activation of TLRs. Activation of TLR4 on parenchymal lung cells leads to pulmonary edema through MAPKs, NF-κB and MyD88 independent pathway. Activation of TLR4 on pulmonary fibroblasts results in increased extracellular versican (16,17). In addition, TLRs activation induces the priming of NLRP3, where MyD88 is required for the immediate early phase priming, while ‘’Toll/IL-1R domain-containing adapter inducing IFN-β‘‘ is required for the subsequent intermediate phase (14).
The absence of shearing stress (due to no flow) is translated into cell membrane depolarization and increased activity of NADPH oxidase (NOX2), resulting in increased ROS production (18). ROS, augment priming of NLRP3 inflammasome (13,15). Cold ischemia is also associated with inhibition of Na^+/ K^+ ATPase, leading to increased K^+ efflux and drop of intracellular K^+ level, which is the second signal for inflammasome activation, leading to activation and release of IL-1β and IL-18 (13,15). Both cytokines are able to induce IL-6.
With the onset of reperfusion, the production of TNF-α increases. Together with IL-1β, they up-regulate TLR2 on pulmonary endothelial cells. Versican (which was induced by fibroblasts TLR4) binds TLR2 resulting in a significant increase in IL-8 production (19,20,21). Now within the graft, there is an enhanced activation of IL-1β and IL-18, leading to the induction of IL-6 (22), in addition to the enhanced production of TNF-α and IL-8. The origin of these cytokines is not only the graft macrophages, but also other cell types within the graft. These cytokines are pro-inflammatory cytokines that induce other cytokines, inflammatory cell infiltration and inflammatory reactions. The continued production of cytokines post-transplant recruits the inflammatory cells of the recipient and plays a major role in the development of PGD and various forms of CLAD, where IL-6 correlates with the 30 days mortality post-transplant. IL-8 correlates with the incidence of PGD, and is important for the neutrophilic infiltration, which is a major step in the development of CLAD (8). In addition, versican correlates with the incidence of CLAD (9) (Figure 2).

How to Interfere with This Cascade

Based on the above mentioned scenario, a very important strategy to prevent post-transplant complications would be the inhibition of the cytokine production within the transplanted graft, which could be applied on different levels. For example, the inclusion of cytokine filters within the EVLP circuits would reduce the amount of cytokines within the transplanted graft through removal. However, intervening with the cytokine production would be of more value.
While many studies have confirmed the EVLP transplantation to be comparable to the standard one regarding the incidence of PGD (23,24), a recent study reported for the first time the observation that the incidence of CLAD was similar between both groups when the transplanted grafts belonged to circulatory death donors. Meanwhile the incidence of CLAD was significantly reduced in the EVLP group when the transplanted grafts belonged to brain death donors, which might mark out the ability of EVLP to attenuate the pro-inflammatory stimuli associated with brain death (25). Although the grafts subjected to EVLP were initially marginal, which would be expected to result in

less CLAD-free survival in comparison to the grafts meeting the standard criteria, the exposure to EVLP conditioning contributed to a decrease in the risk of CLAD through manifesting the IRI outside the recipient, and through the application of the various measures of immune modulation. Further confirmation of that ability might potentiate the suggestion of the routine application of EVLP to all grafts, even when meeting the standard criteria.

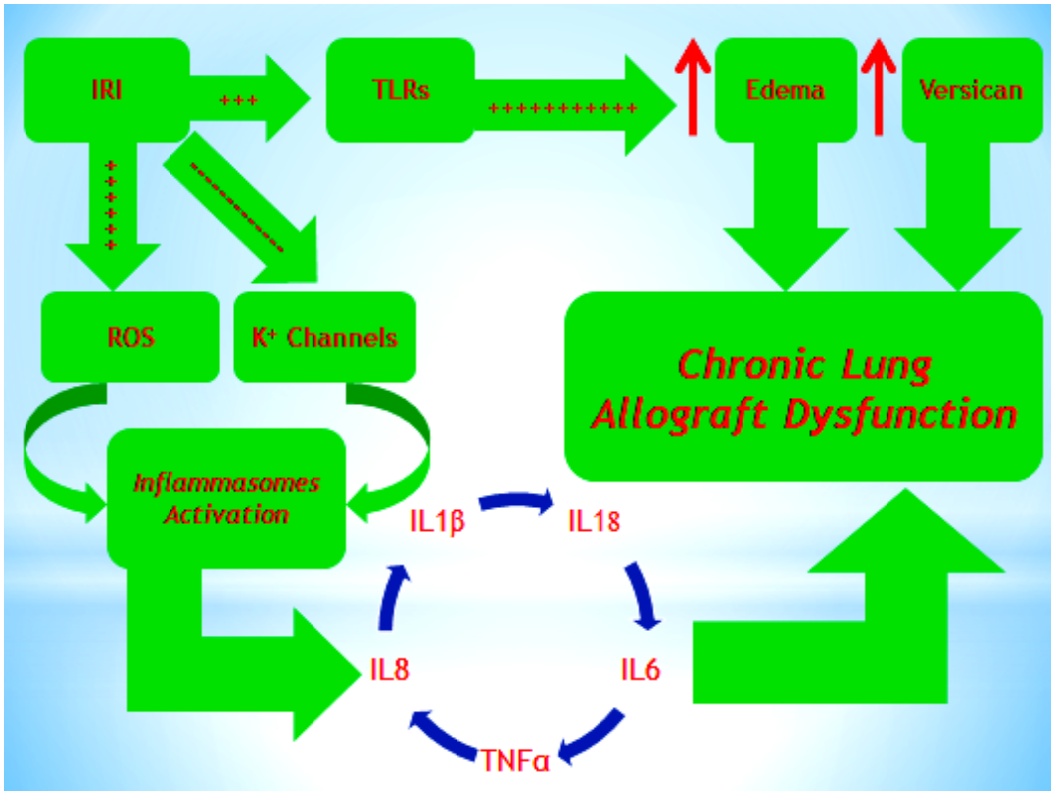

Figure 2. Diagrammatic representation of the effects of the IRI during lung transplantation. IRI stimulates TLRs, which lead to lung edema and increased versican production. IRI is also associated with increased ROS production and inhibited activity of K^+ channels, which result in inflammasomes activation, ending up at a vicious circle of increased inflammatory cytokine production. All that contributes to the development of CLAD.

Strategies for the Attenuation of the Graft Cytokine Production

To intervene with inflammasomes priming, inhibition of ROS production would be a very important step. The inclusion of antioxidants and/or NOX2 inhibitor during graft cold preservation would attenuate ROS production. To intervene with inflammasomes recruitment (second signal), prevention of K^+ efflux and drop of intracellular K^+ levels would be essential. This could be achieved through the inclusion of K^+ channel agonists during cold preservation. The inclusion of K^+ channel agonists seems to have many

positive aspects. For example, KATP activity antagonizes cell membrane depolarization, which mediates increased ROS production during pulmonary ischemia. In addition, KATP activation antagonizes the opening of mitochondrial permeability transition pore during reperfusion (18).

In two different studies that applied 2% hydrogen inhalation during EVLP (one used rat and the other used pigs as the animal models), a significant inhibition of cytokine production within the graft was reported, which correlated with improved graft physiological and histological parameters (26,27). This was mediated through the potent antioxidant effect of hydrogen, in addition to heme-oxygenase-1 (HO-1) up-regulation. HO-1 catalyzes carbon monoxide production, which acts as an antioxidant and activates ATP-sensitive K^+ channels and big conductance calcium sensitive K^+ channels (28).

Transient inhibition of TLRs and or versican blocking antibodies could be theoretically another strategy to intervene with the above discussed scenario, however, this might be risky as knockouts of TLR4 and TLR2 in mice were associated with increased pulmonary apoptosis and increased mortality (29).

Preconditioning of the graft prior to cold ischemic preservation (e.g. ischemic or pharmacological preconditioning) might also provide protection for the graft during preservation and subsequent reperfusion, which would correlate with better post-transplant outcome. As adenosine was claimed to be an important mediator of ischemic preconditioning, the use of adenosine A_{2A} agonist potentiates the ability of EVLP to improve lung graft quality and functions, and to decrease the cytokine production within the graft (30).

Nevertheless, the extension of the inflammatory hazards beyond the lung graft has been recently confirmed. $CCR9^+CD4^+$ T cells were found to migrate from the lung to the intestine (31). In addition, the increased levels of circulating cytokines after transplantation can transmit the injury to other organs. TNF-α increases the expression of monocyte chemotactic protein-1 in the liver, which is the major monocytes recruiter across endothelial cells. Moreover, exposing the lung to IRI up-regulates NF-κB in the liver (32). Nevertheless, there is a confirmed increased risk of acute and chronic renal injury after lung transplantation (33). Accordingly, the care should be paid, not only to the transplanted graft, but also to the other organ systems. Therefore, adoption of a preventive strategy should also consider such consequences.

While the Toronto EVLP team has achieved a significant level of attenuation of cytokine production within the graft through IL-10 gene therapy (in Mouse) (34), Sevoflurane inhalation before graft retrieval, as well as during the transplantation operation has been found to attenuate the cytokine production within the lung graft and the liver following lung transplantation (35,36). Again, sevoflurane inhalation was found to up-regulate heme-oxygenase-1 (HO1) (36).

Recently, a new protocol for EVLP was theoretically described (Shehata protocol) (37). This protocol targets the attenuation of the inflammatory consequences within the lung graft due to IRI. The inhibition of inflammasomes priming and activation would inhibit IL-1β and IL-18 production, as a result, IL-6 production would be attenuated. Blockade of TNF-α by Etanercept, together with the inhibition of IL-1β, would attenuate the up-regulation of TLR2 and the production of IL-8. This would be achieved in Shehata protocol through the use of Steen solution™ supplemented with antioxidants, K^+channel agonists and Etanercept, during both cold static graft preservation and EVLP. A recent study reported a unique antioxidant function of Steen solution™ (38), which supports the recommendation of Shehata protocol to use it instead of Perfadex™ for the graft

cold static preservation. In addition, a recent study has documented a significant inhibition of the cytokine production within the graft in response to the inclusion of the bronchial arteries during the *ex-vivo* perfusion procedure (39), the concept that was previously suggested within the Shehata protocol, where the designed Shehata system entitles, in addition, cytokine filtration during EVLP (Figure 3).

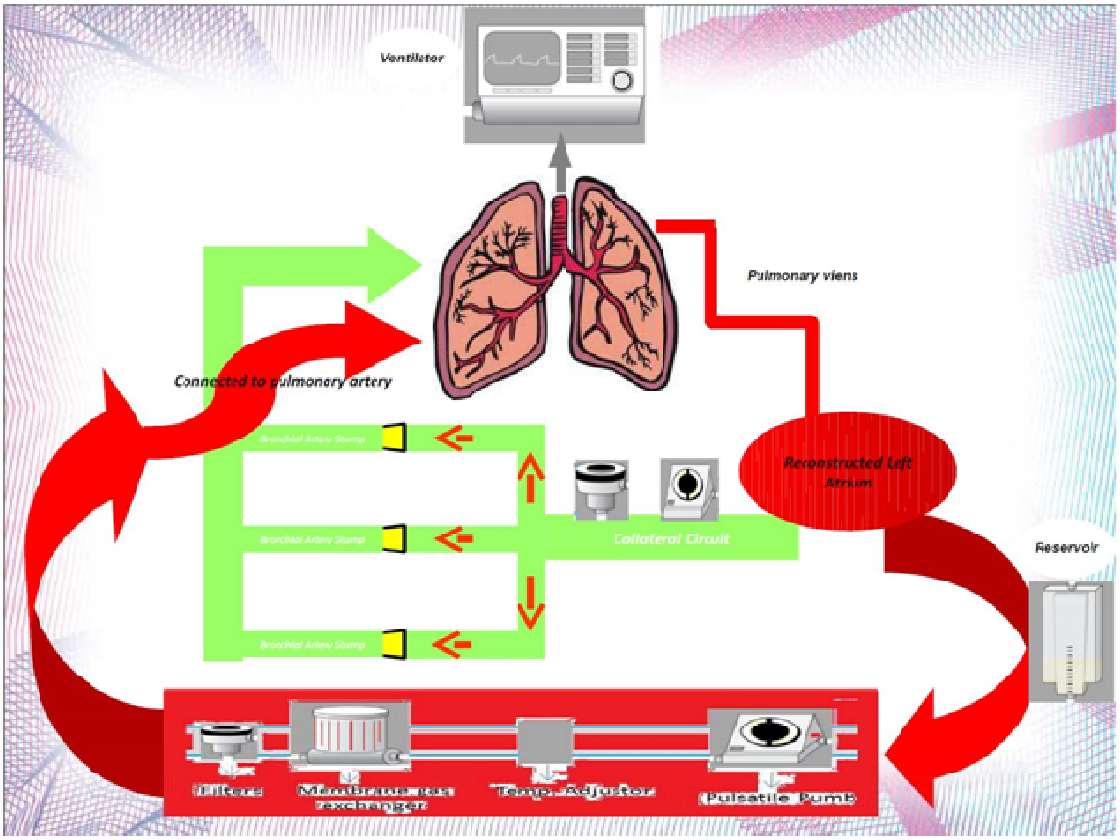

Figure 3. Diagrammatic representation of Shehata EVLP system. The main unique modifications in this model are the inclusion of cytokine filters in addition to the leukocyte filters, and the introduction of a collateral circuit for the inclusion of the bronchial arteries in the ex vivo perfusion procedure.

While sevoflurane inhalation up-regulates HO-1, which catalyzes the production of carbon monoxide (CO), which functions as an antioxidant and stimulates big conductance Ca^{2+} dependent- and ATP sensitive-K^{+} channels, Shehata protocol may be favored in this regards because it is totally applied *ex-vivo* and entitles no systemic side effects (Sevoflurane might raise the intracranial pressure and can cause respiratory depression) (35).

In conclusion, understanding the molecular bases of IRI in the lung, and how it would be linked to the cytokine production and the inflammation, could lead to the improvement of the EVLP protocol and the lung transplantation practice. Accordingly, the assessment of EVLP effects on the lung graft should be confirmed by molecular studies, in parallel to the clinical ones.

The application of the above mentioned measures might have the potential to improve the performance of the lung grafts, recondition the marginal grafts, increase the donor graft availability, decrease the mortality within the waiting list, and decrease the incidence of PGD and CLAD following transplantation, in addition to the protection of the liver and the other organs from experiencing inflammatory manifestations secondary to lung alloimmune reactions. However, these theoretical notions should be confirmed through clinical and molecular studies.

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