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Ischemia-reperfusion Injury in the Transplanted Lung: A Literature Review

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Abstract. Lung ischemia-reperfusion injury (LIRI) and primary graft dysfunction are leading causes of morbidity and mortality among lung transplant recipients. Although extensive research endeavors have been undertaken, few preventative and therapeutic treatments have emerged for clinical use. Novel strategies are still needed to improve outcomes after lung transplantation. In this review, we discuss the underlying mechanisms of transplanted LIRI, potential modifiable targets, current practices, and areas of ongoing investigation to reduce LIRI and primary graft dysfunction in lung transplant recipients.

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Ischemia-reperfusion injury (IRI) is characterized by a complex multisystem response to a prolonged interruption in organ perfusion followed by restoration of that perfusion. This response occurs in a myriad of diverse pathologies to varying degrees; however, it is universal in the setting of organ transplantation. Ischemia during procurement is clinically mitigated by methods of preservation, but does not prevent the injury incurred during implantation and ultimate reperfusion completely. IRI is particularly relevant in the lung transplant population, as transplanted lungs remain the most at-risk clinically of the transplanted solid organs.[1]

IRI in the transplanted lung is characterized by sterile inflammation, microvascular permeability, endothelial cell (EC) dysfunction, and pulmonary edema with increased pulmonary vascular resistance and impaired oxygen exchange.[2,3] It has been described clinically as primary graft dysfunction (PGD) and is associated with significant short- and long-term morbidity and mortality including progression to bronchiolitis obliterans syndrome (BOS).[4-6] Despite modern advances in organ preservation and perioperative support of the lung transplant recipient, PGD continues to occur in up to 30% of patients.[2,5,6] Clinically, PGD is defined by hypoxemia, pulmonary edema, and bilateral pulmonary infiltration on radiography without alternative explanation.[5] The severity of PGD is primarily defined by the degree of hypoxemia utilizing the Pao_2/FiO_2 ratio. Lung IRI (LIRI) and the resulting PGD leads to serious adverse outcomes in patients and increases the overall burden of lung transplantation on the healthcare system. PGD is associated with increased duration of mechanical ventilation, hospital length of stay, and overall mortality.[3,4,7] In the long term, PGD is a major risk factor for the development of BOS and chronic lung allograft dysfunction.[8,9]

This review elaborates on underlying mechanisms for transplanted LIRI, with a particular emphasis on modifiable targets and ongoing investigations to improve outcomes following lung transplantation.

EPIDEMIOLOGY

Donor and Recipient Factors

A number of clinical factors have been identified that are associated with an increased risk for the development of PGD. In the donor, premortem hypoxemia and hypotension, smoke exposure, and demographics including age, race, and sex are all associated with an increased risk of PGD. Of these, donor smoking history is the most significant donor risk factor for PGD.[6] Other donor-acquired risk factors such as trauma, aspiration, and prolonged mechanical ventilation are also risk factors for the development of PGD. Recipient factors including body mass index (BMI), sex, pulmonary hypertension, idiopathic pulmonary fibrosis, and sarcoidosis are also associated with differential risk. Recipient BMI is the most significant modifiable recipient risk factor for PGD, with an increase in absolute risk for PGD by 6% and 11% for a recipient BMI of 25–30 and >30, respectively.[10]

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Procurement practices including prolonged ischemia time (both cold and warm) also contribute to the development of PGD. Transplantation practices, including single versus double lung, use of cardiopulmonary bypass (CPB), transfusion requirements, and high fraction of inhaled oxygen (FiO_2) during reperfusion, have all been implicated in the development of LIRI and PGD. Utilization of FiO_2 >0.4 during lung reperfusion has a high association with the development of PGD.[6] Mechanical ventilation practices in the early postoperative period, particularly the use of lung-protective ventilation strategies with tidal volumes <8 mL/kg have been shown to decrease the risk of PGD.[11,12] Donor–recipient size mismatch can lead to inappropriately large tidal volumes being delivered to undersized lungs. Size mismatch is therefore a major modifiable risk factor for PGD.[12]

Procurement and Preservation

Preservation solutions, flush techniques, approaches to static preservation, and strategies for controlled reperfusion have been studied and refined to reduce the risk of LIRI and development of clinical PGD.

Most institutions utilize a hypothermic low-potassium dextran preservation solution. In the setting of prolonged ischemia time, low-potassium dextran has consistently demonstrated superior graft function in animal models.[13] In general, a flush with high volume and high flow has been shown to decrease the incidence of LIRI and improve posttransplant lung function. A single anterograde pulmonary artery (PA) flush is standard because of its technical ease, targeting a volume of 60 mL/kg and PA pressure between 10 and 15 mm Hg. Prostaglandins and heparin are typically administered during the flush to decrease inflammation, vasoconstriction, and risk for thrombus formation associated with ischemia and hypothermia. Although some centers utilize a second retrograde flush, this has not reliably been shown to decrease the incidence of LIRI. Goal temperature during transport is 4–8 °C with partial inflation targeting 50% of total lung capacity to optimize ongoing surfactant production, pulmonary compliance, and cellular metabolism during the cold static preservation phase.[14,15]

PATHOPHYSIOLOGY AND THERAPEUTIC STRATEGIES

The cellular mechanisms underlying LIRI are multifactorial (Figures 1 and 2). It is thought that the sudden reoxygenation of the previously ischemic tissues results in a unique reperfusion injury via reoxygenation activated cellular pathways.[16] Furthermore, reperfusion results in the activation of local and systemic inflammatory responses that impair local tissue function and can lead to multiorgan failure. It was previously thought that the lung was relatively resistant to ischemic injury because of its dual circulation, the pulmonary and bronchial circulations, and alveolar ventilation that exposes the lungs to oxygen-rich air.[17] However, it has been shown that LIRI plays an active role in the development of PGD in lung transplantation.[2,17,18] Despite extensive research, a thorough understanding of the underlying mechanisms and signal transduction pathways for LIRI is not clear and a specific and effective treatment is not yet available. Here, we summarize the major current evidence for the underlying mechanisms and pathways of LIRI and give an overview of the approaches to target these cellular pathways.

Reactive Oxygen Species

Overproduction of reactive oxygen species (ROS) has been suggested as a key mediator and potential therapeutic target to prevent and treat LIRI.[19] ROS are a family of highly reactive free oxygen radicals, such as superoxide ($O_2^{\bullet-}$) and hydroxyl radicals ($HO^{\bullet}$), and nonradicals, such as hydrogen peroxide (H_2O_2).[20] These radicals were previously thought to be only toxic agents, but recent research has demonstrated their role in various physiologic signaling pathways (Figures 1 and 2).[21,22] First proposed in the 1980s, the concept of ROS-mediated LIRI arose from the idea that reperfusion injury is triggered when oxygen is reintroduced to the ischemic lung.[23] Evidence quickly supported the notion that upon reperfusion of ischemic tissue, an imbalance between the rate of ROS generation and clearance is created.[19] ROS induced by LIRI alter signaling pathways such as activation of apoptosis,[24] calcium overload,[25] and increased innate immune response.[26]

LIRI of the lungs increases ROS production in ECs, alveolar type II cells, vascular smooth muscle cells, and macrophages.[2,27] Several pathways are involved in the production of ROS including: nicotinamide adenine dinucleotide phosphate oxidase (NOX), xanthine oxidase (XO), cyclooxygenase, nitric oxide (NO) synthase (NOS), and mitochondria (Figure 1). Mitochondria and NOX are the main producers of $O_2^{\bullet-}$, which can be spontaneously converted or processed by superoxide dismutase to H_2O_2.[28] H_2O_2 can further be converted to $HO^{\bullet}$, which induces oxidative damage of proteins via carbonylation.[29] $O_2^{\bullet-}$ can also react with NO to produce peroxynitrite, which belongs to the reactive nitrogen species (RNS) and can cause alterations to proteins, lipids, and DNA.[30]

Interestingly, several studies have shown that the ROS process actually begins during the ischemic phase before reperfusion occurs.[25,31,32] In these studies, the residual oxygen present during this ischemic phase is critical in ROS production as ischemia-mediated dysfunction of the mitochondrial electron transport chain occurs, facilitating conversion of residual O_2 into $O_2^{\bullet-}$.[33] A better understanding of ROS-mediated signaling pathways underlying LIRI is critical in preventing and treating LIRI.

Nicotinamide Adenine Dinucleotide Phosphate Oxidase

NOX is the only known enzyme whose primary function is the generation of ROS. NOX is widely expressed in pulmonary cells including vascular (fibroblasts, smooth muscle cells, and ECs), immune (macrophages and neutrophils), and alveolar type II cells.[34,35] The importance of NOX mediated ROS in LIRI has been demonstrated in many studies.[25,36,37] LIRI has been shown to trigger an influx of NOX2-mediated $O_2^{\bullet-}$ into the extracellular environment and its subsequent conversion to H_2O_2 (Figure 1). A reduction in H_2O_2 release after hypoxic events has been markedly reduced by treatment with nonspecific NOX inhibitor in a dose-dependent manner.[38] The NOX subunits, $gp91^{phox}$ and $p47^{phox}$, have been identified as the predominant mediators of free radical damage and oxidative stress caused by NOX during LIRI.[39-41]Phosphorylation of these subunits represents a key activation step for NOX2 isoform of NOX and subsequent development of NOX-dependent oxidative stress.[39-41] NOX2-mediated ROS

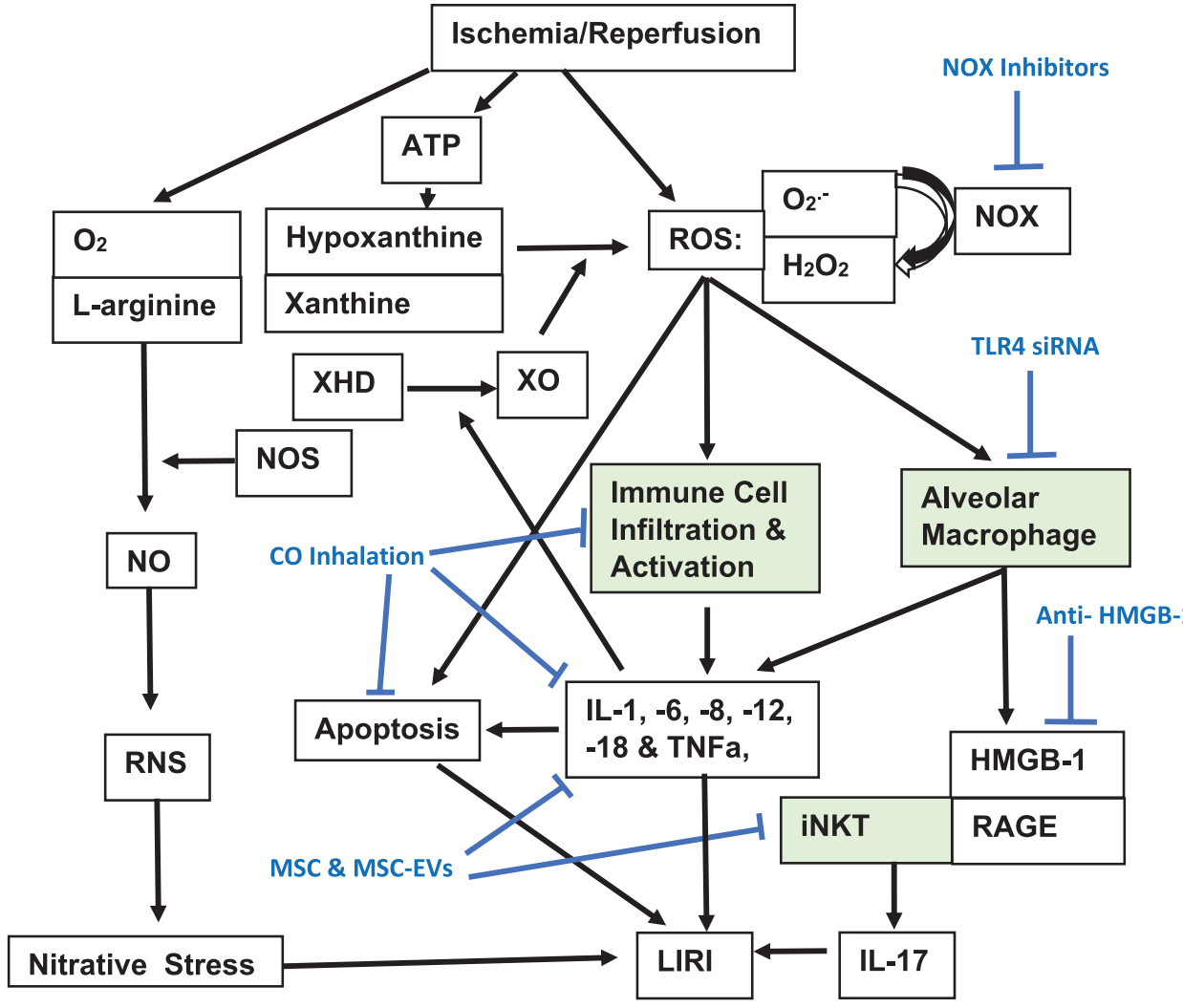

FIGURE 1. Simplified representation of molecular/cellular oxidative stress pathways in lung ischemia-reperfusion injury (LIRI). Boxes filled with light green indicate cells. The blue fonts and lines indicate the potential therapeutic targets. CO, carbon monoxide; EC, endothelial cell; HMGB1, high-mobility group box 1; IL, interleukin; iNKT, invariant natural killer T; MSC-EV, MSC-derived extracellular vesicle; MSC, mesenchymal stem cell; NO, nitric oxide; NOS, nitric oxide synthase; NOX, nicotinamide adenine dinucleotide phosphate oxidase; RAGE, advanced glycation end products; RNS, reactive nitrogen species; ROS, reactive oxygen species; TLR4, toll-like receptor 4; TNF-α, tumor necrosis factor alpha; XHD, xanthine dehydrogenase; XO, xanthine oxidase.

indirectly increases endothelial permeability via an increased calcium influx into ECs (Figure 2).[25,42] Interestingly, this process appears to occur in the early ischemic period of LIRI, suggesting that NOX2-mediated ROS may prime EC to reperfusion injury.[25] Mice models with knockouts of gp91^{phox} and p47^{phox} have been shown to be protective against NOX mediated ROS production.[39-41]

Nitric Oxide Synthase

The role of NO, an endothelium-derived vasodilator molecule, has been extensively studied in both physiologic and pathologic signaling pathways. NO is generated by NOS, which catalyzes the conversion of L-arginine and O_2 into L-citrulline (Figure 1).[43,44] NO activates soluble guanylate cyclase, which converts guanosine-5′-triphosphate into the second messenger cyclic guanosine monophosphate.[45] The most well-known effect of the NO-soluble guanylate cyclase-cyclic guanosine monophosphate pathway is vasorelaxation[46]; however, it also plays a role in other physiological responses.[43,47,48] NO is also a progenitor of RNS, which mediates oxidation of proteins and the irreversible nitration of proteins, fatty acids, and nucleotide guanosine, which causes "nitrative" stress.[27,49-51]

NOS-mediated NO and RNS are important contributors to LIRI.[52-54] Interestingly, treatments targeting NO have shown to be both protective and damaging in LIRI models.[55-57] Previous studies have shown that inhaled NO given at the beginning of reperfusion worsened LIRI, whereas NO given 10 min after the start of reperfusion improved LIRI.[52] A study by Sugimoto et al[58] demonstrated that the addition of nitrite to the preservation solution before lung transplantation (during the ischemic phase) in rats resulted in decreased cytokine and neutrophil infiltration and decreased oxidative damage. These studies suggest that NO and RNS are a double-edged sword in LIRI, and its effect likely depends on which phase of LIRI the alteration in concentration of NO occurs.[52,58]

Xanthine Oxidoreductase

Xanthine oxidoreductase is a flavoprotein that is widely expressed in many organs including the lungs, where it is mainly present in its active dehydrogenase form, xanthine

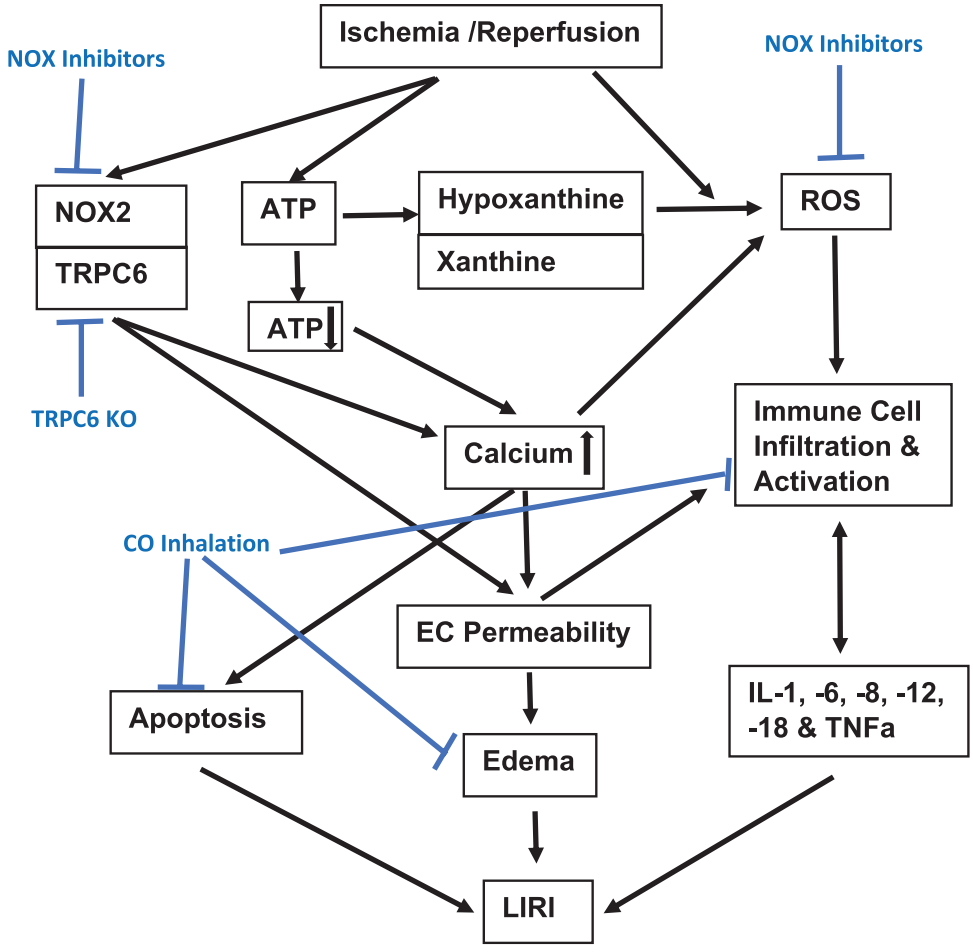

FIGURE 2. The schematic ion channels related molecular pathway with the relevant therapeutic targets in lung ischemia-reperfusion injury (LIRI). The blue fonts and lines indicate the potential therapeutic targets. CO, carbon monoxide; EC, endothelial cell; IL, interleukin; KO, knock out; NOX, nicotinamide adenine dinucleotide phosphate oxidase; RNS, reactive nitrogen species; ROS, reactive oxygen species; TNF-α, tumor necrosis factor alpha; TRPC6, transient receptor potential channel 6.

dehydrogenase (XHD).[59-62] XHD is converted into the oxidase form, xanthine oxidase (XO), under several pathological conditions.[59-61] The primary function of xanthine oxidoreductase is to degrade hypoxanthine to xanthine and xanthine to uric acid[59-61,63]; however, only XO generates ROS via direct transfer of electrons to molecular O_2. XO activation in alveolar type II cells results in DNA strand breakage, decreased mitochondrial integrity, and heightened lipid peroxidation.[64] During reperfusion, accumulated hypoxanthine and xanthine are thought to result in a surge of $O_2^{\cdot-}$ and H_2O_2 production via the following potential pathways: (1) increased XO, which is converted from XHD during ischemia[63]; (2) production of ROS by XHD under acidic conditions[65]; or (3) ischemia or cytokine [interleukin (IL)-1, IL-6, tumor necrosis factor (TNF)-α]-mediated increased activity of XO.[66,67]

Apoptosis

LIRI-induced inflammation mediates increased apoptosis via an increased proinflammatory response (TNF-α, IL-1β, IL-2, and IL-8) that contributes to pulmonary dysfunction (Figure 2).[24,68,69] In addition to the direct cytotoxicity during the ischemic phase, reperfusion contributes significant damage to the pulmonary cells as well. The transition from aerobic to anaerobic metabolism during ischemia increases the accumulation of lactate and intracellular calcium, and decreases cellular pH.[70] Diminished ATP inhibits the function of ATP-dependent cellular ion pumps,[23] which results in the intracellular accumulation of several ions including sodium and calcium.[70] Sodium buildup can disturb water balance across the cellular membrane, leading to increased intracellular calcium and subsequent vasoconstriction and degradation of membrane phospholipids.[27] Apoptosis can be activated via the intrinsic (mitochondrial) or extrinsic (death receptor) pathways. Ion imbalance in the mitochondria can cause an increase in swelling and apoptosis.[23] Specifically, the overload of mitochondrial calcium induces the release of proapoptotic factors via the rupture of the mitochondrial membranes secondary to swelling and increased permeability of mitochondrial transition pores.[71,72] Studies in a canine model of LIRI have shown a direct correlation with severity of edema formation and the presence of apoptotic pneumocytes.[18]

Fas/Fas-ligand

Activation of the extrinsic pathway occurs by the interaction between specific receptors and ligands, such as Fas/Fas-ligand, which leads to subsequent activation of the intracellular apoptotic cascades involving caspases. Fas (CD 95) receptor is a membrane protein of the TNF receptor family, which is expressed in the lungs by endothelial, epithelial, and myeloid cells.[73,74] Fas activation has been associated with the initiation of inflammation in the lungs resulting in altered

barrier integrity, impaired alveolar fluid clearance, and formation of pulmonary edema.[73-76] In fact, ligation of the cell membrane receptor, Fas, by its ligand, Fas-ligand (CD95L), and the activation of effector caspases that follows is one of the most powerful apoptotic pathways in the lung.[77] A study by Herrero et al[74] demonstrated that even a low level of Fas activation in the lung epithelial cells in both in vitro and in vivo models results in a rise in apoptosis and alteration of the permeability of the epithelial barrier.

More recently, Del Sorbo et al[78] demonstrated that silencing of the Fas gene with a specific small interfering RNA (siRNA) significantly reduced apoptosis of the alveolar epithelial cells and severity of LIRI. Although Del Sorbo et al[78] used naked siRNA to downregulate Fas gene expression, transition to clinical use may require an epithelial cell-specific delivery system to avoid undesirable immune responses. Furthermore, the duration of silencing effect after administration of siRNA still remains unknown.

Mitogen-activated Protein Kinase

Mitogen-activated protein kinase (MAPK) signaling pathways have been shown to play an important role in regulating cellular proliferation, differentiation, and apoptosis. p38 MAPK, a specific MAPK module, is involved in membrane-to-nucleus signal transduction for cell cycle regulation.[79,80] Activation of p38 MAPK occurs during metabolic stress, and the level of p38 MAPK activity has been associated with LIRI severity.[81] In a rodent LIRI model, inhibition of p38 MAPK resulted in decreased proinflammatory cytokine levels (IL-1β), pulmonary edema, and alveolar injury.[82] Furthermore, partial pressure of oxygen (Pao_2) was improved after reperfusion.[82,83] Further studies are needed to differentiate the characteristics of various MAPK isoforms during LIRI.

EC Dysfunction and Vascular Permeability

Increased endothelial permeability is a significant contributor to the development of LIRI (Figure 1). LIRI activates an increase in microvascular permeability and an overwhelming immunological response, resulting in pulmonary edema and impaired oxygenation.[19]

Endothelial Gap Formation

LIRI induces migration of leukocytes into the extravascular space, contributing to the increase in microvascular permeability via the release of ROS, proteases, and elastases, and increased gaps between ECs.[84] Neutrophil adherence to the endothelial lining triggers a proinflammatory response mediated by β2 integrin and calcium-dependent cytoskeletal rearrangement resulting in inter-EC gap formation.

Ion Channels

Recent studies have suggested the role of ion channels in the development of endothelial dysfunction in LIRI (Figure 2). Specifically, pannexin, connexin, and transient receptor potential channel (TRPC)6 channels have been implicated in increasing endothelial permeability, resulting in increased pulmonary edema.[25,85,86] Connexins and pannexins are proteins that form transmembranous channels, whereas connexins are also involved in the formation of gap junctions.[86,87]

Pannexin-1 channels only open during pathologic conditions (hypoxia and ischemia).[85,88] In a murine model, treatment with Pannexin-1 inhibitors significantly reduced LIRI, pulmonary edema, and neutrophil infiltration.[85] Furthermore, deletion of Pannexin-1 in vascular endothelium also demonstrated a protective effect against LIRI.[85] Connexin-40 and Connexin-43 are prominent endothelial connexins expressed in the pulmonary vasculature.[89] Prior studies have demonstrated that acute lung injury (ALI) is associated with decreased Connexin-40 expression, whereas decreased expression of Connexin-43 is associated with idiopathic pulmonary fibrosis.[86,90,91] Deletion of these 2 proteins in a murine model resulted in premature deaths secondary to severe spontaneous lung fibrosis and alveolar wall thickening.[89] Another study in a murine model demonstrated that Connexin-43 is involved in abnormal remodeling of the pulmonary vasculature in response to hypoxia.[86]

TRPCs play a predominant role in cation homeostasis and regulation.[92] TRPC6 is highly expressed in the lung, and has been shown to play a role during acute hypoxic vasoconstriction.[93] Recent studies have demonstrated that calcium entry via TRPC6 plays a critical role in increased endothelial permeability in pulmonary vasculature.[92-94] Evidence to support the mechanism of TRPC6 in LIRI has been found to involve an NOX2-mediated increase in endothelial ROS production resulting in diacylglycerol kinase inhibition and subsequent diacylglycerol-mediated TRPC6 activation.[93,94] Global depletion of TRPC6 in mice resulted in protection from LIRI edema, and ECs isolated from TRPC6 knockout mice demonstrated reduced permeability in response to hypoxia.[92]

Innate and Adaptive Immunological Response

LIRI results in the activation of sterile innate and adaptive immune responses[95-97] by various lung cell types, including ECs, alveolar type II cells, vascular smooth muscle cells, and resident macrophages.[1,98] These immune responses are driven mainly by the release of chemokines and cytokines (TNF-α, IL-1β, IL-2, and IL-8) (Figures 1 and 2).

Neutrophil Graft Infiltration

The first immunological response is mounted by the innate immune system and is characterized by neutrophil infiltration of extravascular and alveolar spaces.[95] Infiltration of the graft with circulating host neutrophils is a critical step in the development of LIRI. The process is driven largely by the activation of chemokines [IL-8 and C-X-C Motif Chemokine Ligand 2 (CXCL2)] produced by donor lung epithelium, endothelium, and macrophages.[99]

DAP12, a membrane-associated protein expressed in myeloid cells, can augment or dampen innate inflammatory responses. Spahn et al[100] demonstrated that donor deficiency in DAP12 attenuated LIRI injury in a murine lung transplantation model. Further analysis showed that DAP12 mediates neutrophil chemoattractant production, specifically CXCL2, and also revealed that DAP12 –/– lungs had a transendothelial migration defect that improved with local administration of CXCL2.

Adenosine, an endogenous purine metabolite, is amplified during inflammation or injury.[101] It has been well established that adenosine inhibits neutrophil superoxide production, chemotaxis and adhesion, and production of proinflammatory cytokines.[101,102] Adenosine receptor signaling is cytoprotective during inflammation or stress. Previous studies have shown that selective adenosine 2A receptor activation is protective against LIRI,[103,104] and that adenosine 2B receptor in contrast,

exerts proinflammatory effects in the setting of LIRI.[105] Given the promising results in animal models, adenosine agonists are currently being studied in human clinical trials.[106]

Neutrophil Extracellular Traps

Neutrophil extracellular traps (NETs) are extracellular elaborations of DNA complex with histones and neutrophil granular proteins thought to be an effector function of neutrophils. They are generated by a regulated cell death program called "NETosis", and a study by Laubach and Sharma[1] provided the first evidence of a potential pathologic role in solid organ transplantation. A study by Sayah et al[107] demonstrated that NETs are induced after LIRI in both murine models and human lung transplants. In this study, NET formation was attenuated by DNAase I treatment and was associated with reduced LIRI. NETs were also found to be more abundant in the lungs of patients with PGD.

Platelets

The influx of cytokines during LIRI initiates platelet aggregation and coagulation,[17,23] which leads to microvascular constriction and microthrombus formation. This pathway also activates several vasoactive modulators including serotonin, thromboxane A2, platelet activating factor, and ROS and contributes to the formation of edema.[17,23,108,109]

Complement

The complement system plays an important role in activating the innate immune system, but uncontrolled activation can result in tissue injury in LIRI.[110,111] Complement system activation enhances the inflammatory response by generating anaphylatoxins (ie, C3a and C5a), which results in increased vascular permeability.[112] Membrane attack complex formations (C5b-9) have also been implicated in ALI.[113,114]

In a rat model of LIRI, pretreatment with cobra venom factor, a functional analogue of C3, resulted in complement depletion and subsequently reduced proinflammatory factors as well as neutrophil infiltration.[110]

In post–lung transplant patients, increased plasma C5a levels at 6 and 24 h posttransplantation were associated with severe PGD and increased mortality.[114,115] Furthermore, in a randomized, double-blinded, multicenter trial, prereperfusion treatment with TP10, an inhibitor of C3 and C5 convertases, decreased the duration of mechanical ventilation after lung transplantation compared with the placebo group.[114,116] A more recent multicenter trial found that patients with PGD had increased bronchial alveolar lavage levels of complement activation fragments at 24 h posttransplantation.[114] These findings present complement targeted strategies as a potential treatment strategy for LIRI.

Macrophage Activation and Toll-like Receptors 2 & 4

Alveolar macrophages are activated as part of the initial immunological response in LIRI.[96] ROS mediates the activation of alveolar macrophages triggering a release of proinflammatory cytokines (IL-8, IL-12, IL-18, and TNF-α), which results in neutrophil recruitment and pulmonary dysfunction.[26] These recruited neutrophils and alveolar macrophages further enhance ROS generation, creating a self-perpetuating cycle of ROS release.[117,118]

Receptors that mediate responses to infection, specifically the toll-like receptors (TLRs) have been implicated in LIRI (Figure 1). TLR4, the mammalian lipopolysaccharide receptor, has demonstrated response to endogenous molecules, termed damage-associated molecular patterns, during sterile inflammation[119] and has been implicated as a key modulator of LIRI.[120] Damage-associated molecular patterns include high-mobility group box 1 (HMGB1), fibronectin, and oxidized phospholipids, among others. TLR4 activation results in nuclear factor (NF)-κβ activation, which mediates lung inflammation and injury.[121]

Studies have shown that TLR4 modulates cytokine responses of alveolar macrophages after acute hypoxia-reoxygenation and TLR4 knockout mice attenuate LIRI.[96,122,123] Furthermore, another study revealed that alveolar macrophage secreted TNF-α and IL-1β amplify the response of endothelial and epithelial cells after exposure to hypoxia-reoxygenation.[124]

Activation of Invariant NK T Cells

The activation of Invariant NK T (iNKT) cells and signaling via the receptor for advanced glycation end products (RAGE) have been shown to be independent mediators of LIRI (Figure 1).[125,126] RAGE belongs to an immunoglobulin superfamily that binds to ligands, including HMGB1, under inflammatory conditions.[127] HMGB1 is a nuclear alarmin that is rapidly released from activated macrophages, dendritic cells, and ECs,[128] and can act as a chemokine, NF-κβ activator, and proinflammatory cytokine producer via binding to RAGE and TLR.[129] Previous studies have demonstrated a protective effect of using anti-HMGB1 antibody in attenuating inflammatory cascades in the lung.[129-132] More specifically, HMGB1-mediated RAGE activation results in increased IL-17 production by iNKT cells resulting in neutrophil infiltration and LIRI.[132] Overall, these studies suggest that the HMGB1/RAGE axis on iNKT cells is critical for the initiation of LIRI.

Cell-based Therapies

Mesenchymal Stem Cells

Stem cells have potent anti-inflammatory and immunomodulatory properties. The application of mesenchymal stem cells (MSCs) for LIRI has been investigated in animal models with promising results. Previous studies have demonstrated that LIRI-induced damage to alveolar epithelial type II cells was attenuated by differentiation of human MSCs (hMSCs) into these cells.[133,134] hMSCs are an appealing therapeutic strategy because of their paracrine, immunomodulating, and tissue remodeling properties.[133,135,136] In contrast to embryonic stem cells, bone marrow-derived hMSCs are abundant, easy to access, and without ethical issues.

Studies have shown that MSCs as well as MSC-derived extracellular vesicles (EVs) have the potential to attenuate lung inflammation (HMBG1, TNF-α, and IL-17) in LIRI (Figure 1).[137,138] EVs are small vesicles (100–1000 nm) without nuclei that are released by the cell and can offer a viable cell-free approach to target inflamed or injured tissues.[137]

A critical aspect of EV-mediated protection is that in addition to direct modulation of the immune response it also has the ability to protect the endothelial barrier integrity and resultant edema during ex vivo lung perfusion (EVLP). Despite the favorable safety profile of MSCs, they have been shown to have the ability for spontaneous malignant transformation depending on the preparation of the cells.[139,140] Thus further studies are needed to enhance and isolate the beneficial effects of MSC before clinical application.

Ventilatory Maneuvers

Refractory hypoxemia plays a significant role in postoperative mortality after lung transplantation.[141,142] In most patients with PGD, protective mechanical ventilation and fluid restriction are sufficient supportive measures, but in the event of refractory hypoxemia, further interventions may be needed to improve gas exchange and prevent multiorgan failure. In these cases, some patients have been rescued by nonconventional interventions.[141]

Inhaled Carbon Monoxide

Carbon monoxide (CO) is an endogenously produced byproduct of heme catalysis that has gained recognition as a signal transduction effector molecule in the reduction of LIRI. Inhalation of CO has been shown to improve survival and provide protection against oxidative lung injury in rat and mouse models via upregulation of the p38 MAPK pathway (Figure 1).[143,144] Additional studies in animal models have demonstrated cytoprotective effects of low-dose CO inhalation, with significant improvements in oxygenation and cellular integrity.[145,146]

Taken together, the effects of CO on LIRI include vasodilation, antiaggregation of platelets, anti-inflammatory activity, and inhibition of apoptotic pathways.[147,148]

Previous clinical trials have demonstrated the safety profile of inhaled CO application in humans.[149-151] These promising findings and the feasibility of clinical translation suggest the potential for CO inhalation as a potential therapy for LIRI in lung transplantation.

Inhaled NO

There has been some data to suggest the potential benefit of inhaled NO during the acute period of LIRI after lung transplantation.[152] Inhaled NO studied in 28 patients undergoing lung transplantation demonstrated improved gas exchange and reduced pulmonary arterial pressure in patients who develop LIRI.[153] In a double-blinded, placebo-controlled randomized trial, however, inhaled NO did not significantly decrease the incidence of LIRI, time to extubation, or 30-d mortality, although the study was underpowered.[154] Unfortunately, the majority of studies on NO in reducing LIRI have been nonrandomized or uncontrolled studies, or have a small sample size.[154-156] Further randomized controlled studies are needed to better define the potential benefit of inhaled NO in reducing LIRI after lung transplantation.

Therapeutic Hypercapnia

The potential of hypercapnic acidosis (HA) to produce therapeutic effects, termed therapeutic hypercapnia, has been of interest for some time.[157] A study by Shibata et al[157] demonstrated that HA attenuated LIRI in the isolated rat lung. Subsequent studies demonstrated that HA reduced lung injury after in vivo pulmonary and systemic ischemic-reperfusion injury via reduction of TNF-α, vascular permeability, and apoptosis.[158-160] HA also appears to inhibit the NF-κβ pathway, and that decreased NF-κβ activity is critical to protecting against LIRI.[160] Last, HA has been shown to decrease free radical generation and cellular apoptosis.[157,158,161] Additional studies in relevant preclinical models are needed to further understand the action of HA before it can be considered for clinical testing to treat LIRI.

CURRENT CLINICAL PRACTICE

Protective Mechanical Ventilation

Mechanical ventilation maintains adequate oxygenation while allowing the respiratory muscles to rest, and is life-prolonging in many patients with ALI. However, many studies have shown that mechanical ventilation can worsen preexisting lung injury or produce de novo ALI secondary to mechanical shear stress. Specifically, mechanical ventilation forces block stretch sensitive cation channels resulting in increased endothelial permeability, activation of NF-κB and MAPK pathways, and neutrophil infiltration, among other mechanisms.[162-164]

Protective ventilator strategies minimize the effects of mechanical stress on pulmonary inflammatory responses to improve outcomes. Studies have shown that lung-protective ventilation strategies decrease neutrophil infiltration as well as the proinflammatory cytokine response.[162,165-171] In the landmark ARDSNet trial, protective mechanical ventilation maneuvers, defined as utilizing a lower tidal volume than traditional tidal volumes, showed decreased plasma IL-6 concentrations and significant reduction in mortality in acute respiratory distress syndrome (ARDS) patients.[165] Another randomized controlled trial in beating heart donors also found decreased IL-6 and TNF-α concentrations in the protective ventilation group.[169] However, no standardized approach to ventilatory management exists yet for lung transplantation.

Overall, protective ventilatory therapeutic strategies should be utilized to help reduce pulmonary inflammation and reperfusion injury after lung transplantation.

Extracorporeal Membrane Oxygenation

Extracorporeal membrane oxygenation (ECMO) has been increasingly utilized in patients with LIRI-induced PGD and refractory hypoxemia.[172-176] Although there have been substantial improvements, ECMO is still associated with significant complications, most notably bleeding events, and requires high resource consumption.[177,178] In LIRI, venoarterial (VA) ECMO is more beneficial, as venovenous cannulation cannot reduce pulmonary arterial output and vascular stress.[179,180] Furthermore, severe PGD secondary to LIRI can be accompanied by right ventricular dysfunction that is not supported by venovenous ECMO. Utilization of ECMO in place of CPB for intraoperative mechanical support has been recognized to decrease bleeding and PGD.[181,182] A study by Ius et al[181] found a significant reduction in in-hospital mortality with intraoperative ECMO compared with CPB in patients undergoing lung transplantation, and even identified intraoperative CPB as an independent risk factor for in-hospital mortality by multivariate analysis. Venoarterial ECMO has shown promising results as a treatment for LIRI-induced PGD and even in reducing rates of PGD, and should be considered in place of CPB in lung transplantation.

Prone Positioning

Prone positioning has been shown to be effective in improving oxygenation in patients with ARDS in the ICU setting.[183] Prone positioning is thought to improve oxygenation by improving V/Q matching.[184,185] Major thoracic surgery has traditionally been considered a contraindication for prone positioning after the landmark PROSEVA study considered a sternotomy in the previous 5 d or the presence of chest tubes with air leaks as exclusion criteria.[186] However,

the role of prone positioning as a rescue therapy in the setting of refractory hypoxemia after lung transplantation was evaluated more recently in a prospective study of 131 patients undergoing lung transplantation, and the 22 patients underwent prone positioning had a significant increase in their P/F ratio.[143] Prone positioning is a low-cost, noninvasive treatment for LIRI-induced PGD that has been shown to improve gas exchange and should be considered as a potential intervention in the postoperative lung transplant patient with refractory hypoxemia.[143,189]

FUTURE DIRECTIONS

Ex Vivo Lung Perfusion

EVLP is an increasingly utilized modality in both preclinical and clinical studies for the advancement of lung transplantation. In EVLP, following procurement, the donor lung is placed into a normothermic chamber and ventilated while being perfused with a buffered solution. There are 4 commercially available EVLP systems: Organ Care System, XVIVO Perfusion System and LS, Vivoline LS1, and Lung Assist. Of these models, Organ Care System is a mobile system, which allows the organ to be placed immediately on EVLP after procurement. The potential benefit of the mobile system is reduced cold ischemia time, while providing normothermic perfusion during transport. EVLP has been used to assess and improve the quality of donor lungs, and study novel targets and therapeutic interventions to both expand the donor pool and reduce IRI.[6,187-189] Cypel et al[188] demonstrated that high-risk donor lungs could be used for transplantation following treatment with 4 h of EVLP with similar results to conventional lungs in a small, prospective, nonrandomized clinical trial. Overall, there has been a global increase in lung transplants performed as a result of EVLP.[190]

The proinflammatory state from which donor lungs are removed contributes to LIRI through multiple pathways outlined previously; EVLP allows for removal of proinflammatory substances through flushing with a buffered solution or through the addition of pharmacologic mediators of inflammation. EVLP alone has been found to reduce the severity of the inflammatory response of donor lungs after ischemia-reperfusion. Its effects include decreased allorecognition, infiltration, and priming of recipient T cells.[191] Furthermore, the addition of a leukocyte filter to the EVLP circuit in a murine lung transplant model resulted in trapping of pyroptotic leukocytes and decreased expression of IL-6.[192] EVLP has become an established part of the Toronto Lung Transplant Program and is used in at least 20% of the total volume of lung transplantations at the center.[189]

Following the clinical success of EVLP, recent research efforts have focused on the utilization of EVLP to deliver therapeutics with the ultimate goal of increasing the number of useable donor lungs and further improve function after transplantation.[189] Targeting therapies to the lung ex vivo has several advantages: pharmacologic agents can be used without concern for impact on other physiological systems, the effect of treatment on the lung is monitored in real time, and it facilitates the translation of large animal model results to humans. Similarly, gene therapy-based interventions can be investigated with relative safety utilizing EVLP.[6,189,193]

IL-10 gene therapy has been extensively studied to reduce inflammation in the donor lung.[194-196] It has been previously shown that delivery of IL-10 via an adenoviral vector using EVLP in both large animal model and nontransplant human damaged lungs could be effectively used to improve lung function to within transplantable parameters.[194-196] A more recent large animal survival model further demonstrated the safety and effectiveness of EVLP delivery of adenoviral IL-10 gene therapy in attenuating both LIRI and allograft rejection, thus improving lung function compared to conventional cold preservation and controls (EVLP or vector only) before transplantation.[194] As previously mentioned, MSCs or MSC-derived EV-enhanced EVLP has also been studied in a murine model of donation after circulatory death lungs, and significantly reduced LIRI, demonstrating decreased edema, neutrophil infiltration, IL-17, TNF-α, CXCL1, and HMGB1 levels in BAL fluid.[138] Furthermore, the administration of selective A2AR agonists to perfusate during EVLP in a murine model resulted in increased lung compliance, decreased PA pressures, and decreased levels of proinflammatory markers (CXCL1, CCL2, and TNF-α) and neutrophil concentration when compared to EVLP alone.[197]

Several inhaled agents, such as β-adrenoreceptor agonists and sevoflurane, have also been studied in EVLP with promising results. Inhalation of β-adrenoreceptor agonists during EVLP resulted in increased Pao_2 and compliance,[198,199] whereas sevoflurane has been shown to reduce TNF-α and pulmonary edema.[200]

EVLP as a tool in both the laboratory and the clinical setting has been instrumental in the study and treatment of LIRI, and will continue to be invaluable in the years to come.[189,193,201]

Clinical Trials

A number of clinical trials have been initiated or completed to address the mitigation of LIRI. In 2003, Meade et al[154] published a concealed, randomized, placebo-controlled trial of inhaled NO to prevent LIRI in 84 patients. The treatment group received inhaled NO following reperfusion; this did not demonstrate a significant difference in short- or long-term outcomes related to LIRI or PGD.[154] Keshavjee et al[115] published a randomized, placebo-controlled multicenter trial of complement inhibition to reduce LIRI in 59 patients. This study demonstrated a significant difference in early extubation in the treatment group and a significant difference in overall duration of mechanical ventilation.[115]

A phase 2a safety trial utilizing allogeneic MSCs in the treatment of moderate and severe ARDS was published in 2019 with an acceptable safety profile.[202] These results are generalizable to the lung transplant population as well, and provide a basis for clinical trials to evaluate the efficacy of MSCs in the treatment of LIRI.[203] Several clinical trials utilizing MSCs to treat ARDS, ALI, and LIRI in lung transplant are currently ongoing. Safety of MSCs in BOS have similarly been established in early clinical trials, and their efficacy in this population is being investigated in clinical trials at this time.[204]

Slama et al[205] published a prospective randomized trial of EVLP in standard lung transplantation. In a cohort of 160 patients, EVLP was demonstrated to safely extend total preservation time without significant effect on the short-term clinical outcomes including 30-d survival.[205] The utility afforded by prolonged time for evaluation of lung function on EVLP was also described.[205] Clinical trials to apply EVLP to expand the use of marginal donor lungs are actively enrolling at this time.

Finally, a phase 1 open label study to evaluate adenosine 2A receptor agonist in the lung transplant population is currently ongoing with an estimated enrollment of 21 patients and estimated completion in 2022.[206]

CONCLUSIONS

In recent years, there have been significant research efforts focused on LIRI and PGD in lung transplantation. Mechanistic studies have elucidated targetable pathways with promising early results. Pathways including dysfunction of the endothelial and immunologic response as well as mitigation of the toxic effects of ROS have all prompted further evaluation. Success of EVLP, MSCs, and A2A receptor agonists in animal models has led to further investigation in clinical trials. Ultimately, translation of the myriad of preclinical studies to current clinical practice in lung transplantation to reduce LIRI and PGD complications in lung transplantation gives hope for improved outcomes and extended long-term survival for transplant recipients.

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