Lung ischemia-reperfusion injury: a molecular and clinical view on a complex pathophysiological process

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Lung ischemia-reperfusion injury remains one of the major complications after
cardiac bypass surgery and lung transplantation. Due to its dual blood supply
system and the availability of oxygen from alveolar ventilation, the pathogenetic
mechanisms of ischemia-reperfusion injury in the lungs are more complicated than
in other organs, where loss of blood flow automatically leads to hypoxia. In this
review, an extensive overview is given of the molecular and cellular mechanisms
that are involved in the pathogenesis of lung ischemia-reperfusion injury and the
possible therapeutic strategies to reduce or prevent it. In addition, the roles of
neutrophils, alveolar macrophages, cytokines, and chemokines, as well as the
alterations in the cell-death related pathways, are described in detail.

pulmonary; lung transplantation; ventilated ischemia

IN THE PAST, THE LUNG WAS thought to be relatively resistant to ischemia because of its dual circulation and the availability of oxygen from alveolar ventilation. However, the depletion of lung oxygenation by stopping the ventilation leads to the same degree of lung impairment as a decrease in mechanotransduction by loss of blood flow, as determined by increases in vascular pressure and permeability (11), indicating that any deficiency from oxygenation or mechanotransduction can have significant repercussions (149).

Reperfusion of the ischemic lung is a double-edged sword. Reestablishing the perfusion of the ischemic lung is absolutely required to maintain the viability of the lung, but reperfusion itself can trigger a complex cascade of events, the so-called pulmonary ischemia-reperfusion injury (LIRI) (48), characterized by increased microvascular permeability (Pμvasc), increased pulmonary vascular resistance (PVR), pulmonary edema, impaired oxygenation, and pulmonary hypertension. Ischemia of the lung, without loss of ventilation, results in a complex pathophysiological situation and, therefore, is not comparable with ischemia in other organs. During ventilated ischemia, for example, the adenosine triphosphate (ATP) levels remain normal while reactive oxygen species (ROS) are formed (70), illustrating aspects of complexity of the pathophysiology of ventilated and anoxic lung ischemia. Therefore, a distinction should be made between ventilated ischemia, meaning a loss of blood flow, and anoxia/reoxygenation, indicating the loss of oxygenation and/or perfusion. In this review, the terms anoxia/reoxygenation and ventilated ischemia will be used, if the specific model is known and/or the mechanism is suggested to be model specific. Ischemia alone will be used, if it applies for both mechanisms, if the specific model is not known, or the research involves other organs.

Complete and prolonged lung anoxia for up to several hours is unavoidable during lung transplantation, with dire consequences. Reperfusion of the transplanted lung can lead to nonspecific alveolar damage, pulmonary edema, and hypoxemia within 72 h after lung transplantation. Even with the advancements in lung preservation, immunosuppression, and perioperative management, ~15% of the patients undergoing lung transplantation experience graft complications due to LIRI (48). This can lead to severe primary graft failure (41) in up to 20% of all lung transplantation patients in the first 90 days posttransplantation and remains a significant cause of morbidity and mortality after lung transplantation, because it results in acute lung injury/acute respiratory distress syndrome (41). Other events, such as cardiopulmonary bypass (151), trauma (185), resuscitation for circulatory arrest (185), atherosclerosis (12), and pulmonary embolism (12), are also associated with LIRI and likely contribute to the high morbidity rates and perhaps the mortality in these patients. LIRI is a complex phenomenon involving not only intracellular injury processes, but also injurious inflammatory responses and biochemical changes. In this review, the underlying molecular mechanisms of the several interacting processes during both lung ischemia and reperfusion will be explored in detail, and an overview is given of existing and new therapeutic strategies to limit or prevent LIRI.

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Animal Models of Lung Ischemia-Reperfusion

In the research seeking the mechanisms and possible treatments of LIRI, a wide variety of animal models are used. The different animal models are nicely summarized by Matute-Bello et al. (129), and we will discuss here only the most widely used models. Lung ischemia-reperfusion (IR) models can be divided grossly between studies using ventilated ischemia and anoxic ischemia. The most widely used models of pulmonary ischemia are clamping of the pulmonary artery, which leaves the bronchial circulation and venous return intact, and clamping the hilum, which results in complete ischemia and anoxia. A variation on these models is insuflating or desuflating the lung before or during the clamping and/or during reperfusion. Another variation is transplanting the lung in a warm or cold storage and perfusion with a solution during storage and last, but not least, the type of animal that is used.

As mentioned by Matute-Bello et al. (129), there are five main variables that need to be considered: animal species, desuflation vs. insuflation of the lung, the type of ischemic bed (pulmonary artery, hilum, venous return), experimental preparation (in vivo vs. isolated perfused lungs), and the duration of both ischemia and reperfusion.

IR-induced Physiological Changes

During ischemia, with or without anoxia, hypoxia and lack of mechanotransduction in the arterioles and capillaries (118) induce macrophages, endothelial cells, and other immune cells to generate ROS (70, 211). Calcium/calmodulin-dependent nitric oxide synthases (NOS) (97), nuclear factor-κB (NF-κB) (87), nicotinamide adenine dinucleotide phosphate (NADPH) (6, 211), and proinflammatory cytokines (48) are activated, causing an upregulation of cell-surface adhesion molecules on the endothelial side of the lung. All of these actions may be leading directly or indirectly to several physiological changes in the microvasculature (Fig. 1). These changes translate grossly into increased PVR, increased Pμvasc, and pulmonary

edema that causes gas exchange abnormalities (149) with severe ventilation-perfusion mismatch immediately following lung reperfusion (120). In cases in which the ischemia is unilateral, the physiological changes also appear on the contralateral nonischemic lung immediately after reperfusion (163), suggesting that the injurious signals are humorally secreted as well (163).

After reperfusion, PVR can be increased by up to three times normal levels. The increase in PVR is mainly due to the vasoconstriction of the pulmonary precapillary system after lung IR (120). The increase in PVR, together with an increase of vascular permeability, the latter of lesser importance (11), results in pulmonary edema in both ischemia (23) and reperfusion (93). The increase in total and extravascular lung water content causes poor gas exchange and lung mechanics, which leads to a lower arterial oxygen tension (Pa$_{O_2}$) (93), increased peak airway pressure, and an elevated alveolar-arterial oxygen gradient [(A-a)D$_{O_2}$] (166).

The Pμvasc is measured by the pulmonary capillary filtration coefficient. During reperfusion in lung IR models, this is increased up to 10-fold (106). The changes in Pμvasc have a bimodal pattern that peaks at 30 min after perfusion and again 4 h later (59). It is suggested that the initial phase is more dependent on products from activated pulmonary macrophages, such as IL-8, IL-12, IL-18, TNF-α, and platelet-activating factor (PAF), while the late phase is more dependent on products from activated neutrophils, like ROS, IL-8, PAF, and TNF-α (48, 58, 149). The upregulation of cell-surface adhesion molecules, leukocyte adhesion molecule CD18, endothelial intercellular adhesion molecule-1, and endothelial P-selectin, also seems to have an influence on Pμvasc and leukocyte sequestration (138). In addition, polymorphonuclear cells also have an impact on the Pμvasc of lung tissue by releasing proteolytic enzymes such as matrix metalloproteinases (151). These injuries to the microvasculature can manifest as an increase in dead-space ventilation, which is frequently seen in patients with bilateral lung transplants (91).

Fig. 1. Physiological changes during ischemia-reperfusion (IR) injury in the lung. During ischemia, macrophages and endothelial cells generate reactive oxygen species (ROS) and induce an upregulation of NADPH, nuclear factor-κB (NF-κB), nitric oxide synthases (NOS), and cell surface molecules. When reperfusion occurs, ROS and cytokines activate neutrophils. These, together with platelets and nitric oxide (NO) signaling pathways, result in IR-induced vascular damage, such as increased pulmonary vascular resistance (PVR) and microvascular permeability (Pμvasc). PVR contributes to the development of pulmonary edema far more by comparison (thick line) than to the increase in permeability (thin line), a process that ultimately results in a loss of lung function, illustrated by a wide array of parameters. NADPH, nicotinamide adenine dinucleotide phosphate; Pa$_{O_2}$, arterial oxygen partial pressure; eNOS, endothelial NOS; iNOS, inducible NOS; AWP, airway pressure; (A-a)D$_{O_2}$, alveolar-arterial oxygen gradient.
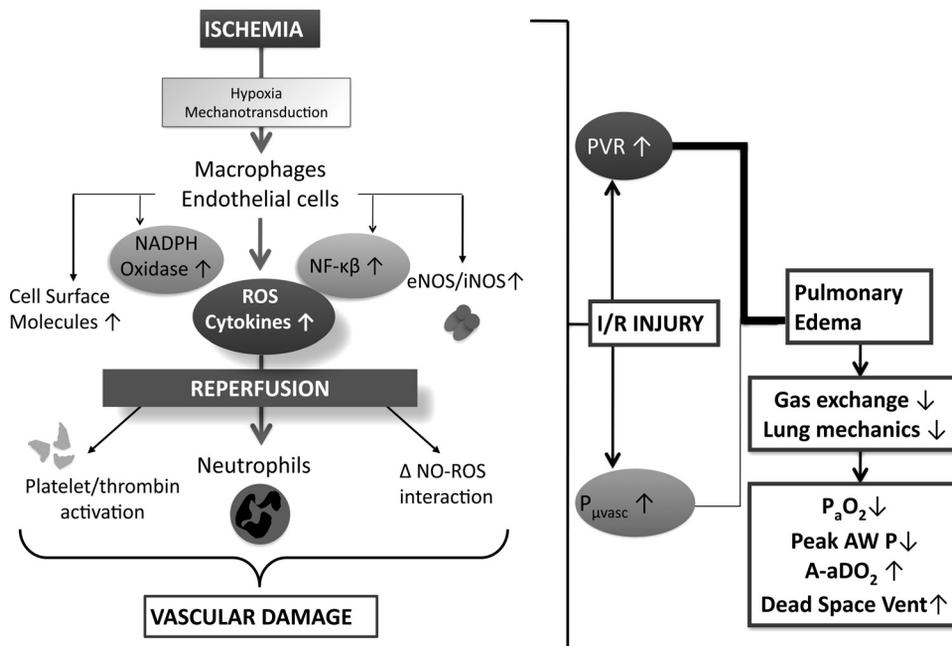
On the epithelial side of the lung, the surfactant inactivation or imbalance of surfactant function plays an important role during lung IR in lung transplant patients (152). The alveolar type II epithelial cells are recognized as important mediators of LIRI because the changes in the composition, function, and metabolism of pulmonary surfactant (152) essentially amount to a loss of function in these cells. The dysfunction in surfactant composition and production causes both static and dynamic lung compliance reduction, and an increase in the $(A-a)D_{O_2}$ (188). This could be caused by the release of phospholipase A_2, a major surfactant degradation enzyme, from apoptotic or necrotic alveolar macrophages (2) (see below). The type 1 epithelial cells, however, are much more resistant to inflammatory injury and response, and their function remains intact, even with high grades of pulmonary damage, which suggests that the function of cells might determine long-term patient outcomes (128).

Morphological Changes During Lung IR

The morphological response of the endothelium to LIRI is initially apoptotic, indicated by cellular rounding and contraction, rather than necrotic (74). Other histological markers include alveolar capillary interstitial edema, hyaline membranization along alveolar ducts, and infiltration from neutrophils, macrophages, and polymorphonuclear cells.

The hypoxia and mechanotransduction induces macrophages, endothelial cells, and other cells to upregulate cell-surface adhesion molecules, as well as generate highly injurious ROS (6, 70, 87, 211). As a consequence, the microvascular endothelial cells are activated and become more dysfunctional and more permeable to macromolecules.

After cold ischemia (in lung transplantation), apoptosis is only found during reperfusion (190) and is influenced by the duration of the cold ischemia. A moderate cold ischemia time of 6–12 h before reperfusion resulted in more apoptosis in the lung tissue than necrosis (30 vs. <2%), whereas a longer cold ischemia time of up to 24 h before reperfusion resulted in necrosis-dominated cell death (21–29% necrosis vs. <1% apoptosis) (67). Functional preservation assessed by Pa_{O_2} was associated with apoptotic, rather than necrotic, cell death after lung transplantation (67). The amount of oxygen delivered to the cell after reperfusion of the lung determines its fate. A cell that receives less oxygen will more likely shift toward necrosis, whereas more oxygen will lead the cell to apoptosis (67).

To the best of our knowledge, no classification scheme has been established for the histological changes during LIRI.

"No-Reflow" Phenomenon

The "no-reflow" phenomenon is suggested to be multifactorial (168). After lung transplantation, ventilation may resume without perfusion, and there may not be a discernible reason for the lack of perfusion. During ischemia, the lung parenchymal cells release chemotactic substances (29), resulting in massive adhesion of inflammatory cells, such as macrophages, neutrophils, and T-cells, which plug the arterioles, venules, and alveolar capillaries (113). The "no-reflow" phenomenon in lung tissue is greatly reduced when these cells are depleted (114). Ischemia-induced tissue edema with subsequent swelling of endothelial cells causes further narrowing of the capillaries (109), which can result in the accumulation of red blood cells in those capillaries. The role of red blood cell accumulation in ischemic lung injury is not fully understood.

In the early phase of reperfusion, these changes in the small blood vessels may contribute to cellular swelling and subsequently to the "no-reflow" phenomenon (81).

A recent study in a model of warm lung anoxia/reoxygenation showed a significant increase in red blood cell accumulation in the lung (203). This study also showed an increase in red blood cell accumulation in the contralateral nonischemic lung after 30 min of reperfusion (203), suggesting a role for chemotactic signaling from the ischemic-reperfused lung to the nonischemic lung (58).

Mechanisms of Lung Injury During IR

LIRI is a complex pathogenetic situation, which involves several biochemical, cellular, and molecular alterations.

High-energy phosphates and lung IR. During anoxia of the lung, the aerobic metabolism has come to a halt, leading to a breakdown of ATP and adenosine diphosphate into adenosine monophosphate (AMP), which is further degraded into inosine monophosphate (IMP). IMP will be eventually dephosphorylated to inosine, which can be degraded to hypoxanthine (Fig. 2). Xanthine oxidase, which is known to have an important role in reperfusion-induced superoxide generation (101), can oxidize hypoxanthine first to xanthine and then to uric acid, although the significance of xanthine oxidase in LIRI is debatable. Von Wichert (200) described, in a model of isolated, normothermic anoxic rabbit lung, a 70% decrease of ATP and a sixfold increase of AMP after 30 min of anoxia. Afterwards, it was demonstrated that dephosphorylation of AMP to adenosine is more important than deamination to IMP (46). Very recently, our group described how folic acid can target this high-energy phosphate pool with a subsequent and significant reduction in myocardial IR injury (135). No data are yet available on the applicability of this new therapeutic strategy to the lung.

Temperature appears to be one of the main factors influencing the rate of high-energy phosphate degradation. The group of Shoji et al. (187) have demonstrated, in an ex vivo rat model, that even a slight decrease from normothermic ambient temperatures during anoxia/reoxygenation can help maintain ATP and AMP levels.

Lung IR-induced superoxide generation. A burst of ROS appears immediately after reperfusion within the hypoxic endothelial cells. They overwhelm the lung's antioxidant defense system to the detriment of cellular metabolic function and signaling, most especially in the mitochondria of the endothelium and alveolar macrophages (see below). Ultimately, IR in the lung causes systemic effects by inducing heart and liver injury due to activated neutrophils and the significant amount of ROS that is produced (60).

ROS causes activation of alveolar macrophages with stimulation of proinflammatory cytokine release, and ROS may react with nitric oxide (NO) to produce reactive nitrogen species, such as peroxynitrite, which are highly reactive and destructive radicals. Oxidative stress enhances the transcription of several genes that initiate the translation and prolonged expression of cell-surface adhesion molecules on leukocytes, and a release of cytokines to further promote the recruitment of neutrophils to the lung after IR (28).

Fig. 2. Xanthine oxidase (XO)-dependent superoxide generation in the lung. Xanthine dehydrogenase, which needs nicotinamide adenine dinucleotide (NAD^+) as an electron acceptor, is converted to XO when ischemia occurs in the endothelia and type II alveolar cells. This conversion changes the normal degradation of hypoxanthine to uric acid into a source of oxygen radicals. Hypoxanthine is the final molecule in the breakdown of high-energy nucleotides, such as adenosine triphospate (ATP) and adenosine monophosphate (AMP). The XO-generated radicals themselves damage the endothelial cell. When released by these endothelial cells, XO is a factor in the process of sequestration of lung neutrophils, leading to further IR injury.
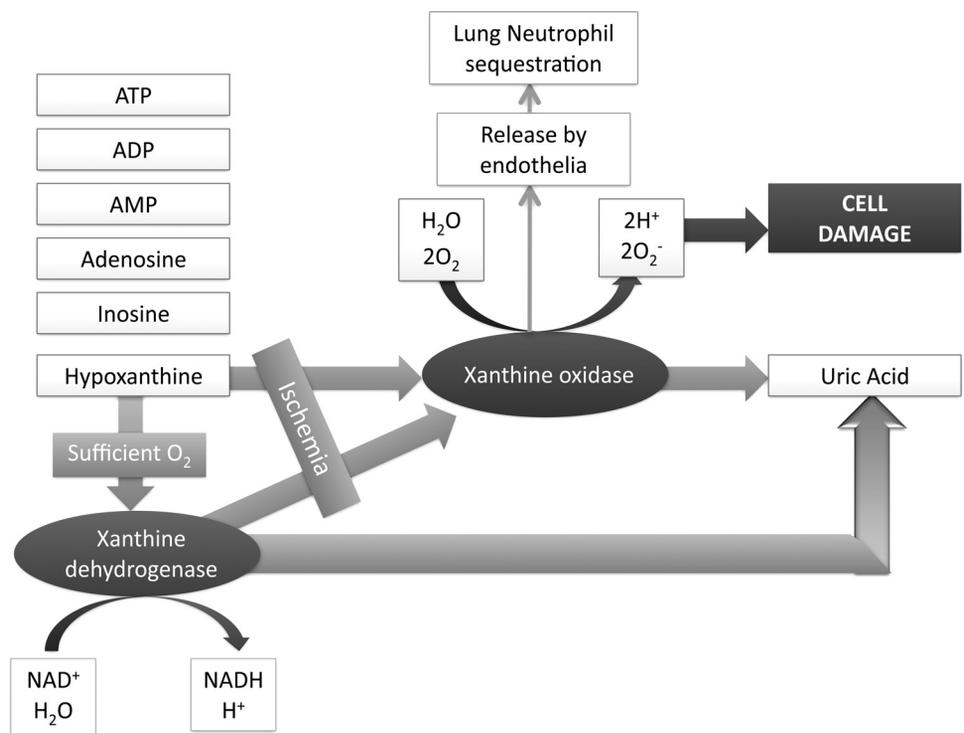
Several groups have shown in animal models that inhibiting the enzymes involved in free radical production in the donor lung can dramatically reduce the proinflammatory profile and the amount of free radical damage upon reperfusion in the recipient animal.

Several generators of ROS are known in the lung, such as mitochondria, activated xanthine oxidase, the NADPH oxidase system, NOS, and neutrophils. Under normal conditions, NOS produces mainly NO and a little ROS, but, under anoxic conditions, NOS produces almost exclusively ROS (see below).

MITOCHONDRIAL-DEPENDENT ROS GENERATION. It is generally accepted that mitochondria are both producers and targets of ROS. Mitochondrial ROS is produced at several sites in the mitochondrial respiratory chain, but mainly by complexes I and III.

The main portion of mitochondrial damage occurs during ischemia and not during reperfusion (37). In most organs, the lack of oxygen means a lack of electron acceptors, ending oxidative phosphorylation and the production of ATP (10). The rapid depletion of ATP causes a decrease in the membrane potential of the mitochondria (79) and allows calcium to escape the mitochondria, triggering apoptotic processes. ROS are generated as a result of the changes in membrane potential (32). In the lung, which has a secondary source of oxygen via ventilation (55), the ATP levels are preserved, so the cellular event triggering the generation of ROS production is the loss of shear stress in the endothelium, which is conveyed through increased NADPH oxidase activity (6) (see below).

ROS create damage through lipid peroxidation, damaging DNA, and inactivating many proteins. This activates a pathway characterized by mitochondrial outer membrane permeabilization (see below), and this is regulated by both pro- and antiapoptotic proteins from the Bcl-2 family (45, 64) (see below). The mitochondrial outer membrane permeabilization releases several mitochondrial molecules, such as cyto-

chrome-c. The loss of cytochrome-c, as the mitochondria release it into the cytosol, is particularly detrimental, as cytochrome-c acts as a free radical scavenger, and it is the final step in the electron transport chain. Mitochondrial cytochrome-c subsequently activates caspase-9 and the apoptotic pathway when it enters the cytosol (26, 45). The membrane potential dysfunction in the mitochondria can also mean that more calcium irreversibly enters the mitochondria by redox-sensitive transient receptor potential channels (1); calcium has been shown to activate mitochondrial NOS (mtNOS) (see below), one of the main sources of mitochondrial ROS (Fig. 3).

XANTHINE OXIDASE AND SUPEROXIDE GENERATION. The role of superoxide generation by xanthine oxidase, located in endothelial and epithelial type II alveolar cells in the lung (141), as a possible mechanism in LIRI remains controversial. It is known that, under anoxic conditions, xanthine dehydrogenase is converted to xanthine oxidase, which uses hypoxanthine as a substrate to break it down into xanthine, H_2O_2, and ROS. Hypoxanthine is derived from the breakdown of high-energy nucleotides, such as ATP and AMP, which cannot be utilized in the absence of oxygen.

Two studies (4, 101) demonstrated, using an in vivo lung IR model in the rabbit, that pretreatment with the xanthine oxidase inhibitor allopurinol protects against anoxia/reoxygenation superoxide generation. In addition, pretreatment with allopurinol attenuated the hypoxia-reoxygenation-induced permeability changes in an isolated dog lung model (11). Furthermore, the xanthine oxidase inhibitor iodoxamide has been shown to attenuate LIRI in an anoxia/reoxygenation rat lung model (123), as measured by decreased PVR and decreased albumin accumulation in the lung parenchyma and in the alveolar space. Also, severe endothelial and epithelial injury during anoxia/reoxygenation was markedly attenuated in the iodoxamide-perfused lungs (123). These investigations suggest a prominent
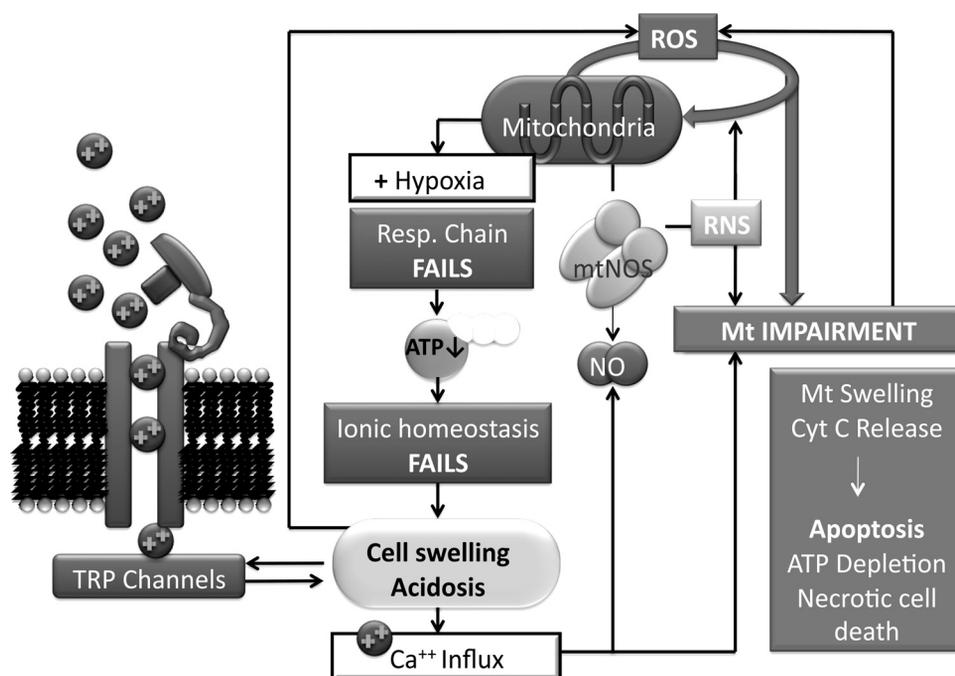
Fig. 3. Mitochondrial-dependent ROS generation in the lung during IR. Mitochondria both produce ROS and fall victim to it (thick arrow). Anoxia depletes ATP by shutting down the ATP-generating system, thereby disturbing the balance of ionic homeostasis. Cell swelling and acidosis cause calcium influx, which further impairs the mitochondria (Mt). This effect is multiplied by the irreversible opening of transient receptor potential (TRP) channels, caused by redox imbalance. Calcium further induces mitochondrial ROS production and NO production by mitochondrial NOS (mtNOS). This NO may form reactive nitrogen species (RNS), which, together with ROS, shuts down several mitochondrial enzymes and increases lipid peroxidation. The mitochondrial impairment ultimately results in the downfall of the cell by activating the pathways toward apoptosis and even necrosis. Cyt C, cytochrome-*c*.

role for xanthine oxidase as one of the producers of ROS during lung IR. However, the results appear to be model dependent because ROS production during ventilated ischemia was not influenced by allopurinol, but ROS production during anoxia/reoxygenation was inhibited by allopurinol, while NADPH oxidase inhibition decreased ROS production during ventilated ischemia and not during anoxia/reoxygenation (see below). This suggests different pathways of ROS generation in these two different models (5, 211).

As alluring as these results are, Kinnula et al. (107) pointed out that xanthine oxidase activity in human lung is very low and that xanthine oxidase is not released into the bloodstream during human heart-lung transplantation, and, therefore, it is unlikely to contribute to postoperative complications in these patients. It is also possible that the measured elevations of hypoxanthine and xanthine levels after lung IR are merely an indication of ATP and AMP degradation, rather than a marker of xanthine oxidase activity.

NADPH-DEPENDENT SUPEROXIDE GENERATION. NADPH oxidase, which is present in endothelial cells, macrophages, and vascular smooth muscle cells, is one of the major sources of ROS in the lung during ventilated ischemia (68). As mentioned above, the absence of mechanical forces such as shear stress in the pulmonary vasculature causes a depolarization of the endothelial cell membranes, followed by an upregulation of NADPH oxidase (6), with subsequent increased production of ROS (6). This may activate NF-κB and activator protein-1 (AP-1), which stimulates the production of proinflammatory cytokines and induces endothelial injury (69). Closure of ATP-sensitive K^+ channels in the endothelial membrane in the absence of shear stress activates NADPH oxidase via membrane depolarization (207). The inactivation of NADPH oxidase by apocynin suggests the importance of this enzyme in LIRI. It has been demonstrated that apocynin can completely prevent pulmonary edema and the increase of Pμvasc in a sheep model of lung IR (52) and can attenuate hypoxemia in a

pig model after cardiopulmonary bypass (53). NADPH oxidase is not as important during anoxia/reoxygenation as it is during ventilated ischemia. ROS production was inhibited during ventilated ischemia with diphenyliodonium, another NADPH oxidase blocker, but had no effect on anoxia/reoxygenation-induced ROS production (5, 211).

NOS-DEPENDENT ROS GENERATION. Four different types of NOS are known in the lung, i.e., endothelial NOS (eNOS) (182), neuronal NOS (184), inducible NOS (iNOS) (184), and mtNOS(31). NO is a powerful anti-inflammatory agent, decreasing leukocyte recruitment (83) and the subsequent release of inflammatory cytokines. Although this is normally a beneficial effect, in high concentrations NO may also react with ROS to form peroxynitrite and other reactive nitrogen species (73, 160). Of these NOS subtypes, the activities of eNOS and iNOS have been shown to be anti-inflammatory in several models (44, 83), although the overinduction of iNOS is implicated in the formation of reactive nitrogen species (160). The mtNOS subtype, on the other hand, seems to play a role in proinflammatory cell processes by generating reactive nitrogen species (199), while the role of neuronal NOS in lung IR remains unclear.

1) mtNOS-dependent superoxide production: mtNOS activity is stimulated by an influx of calcium into the mitochondria and the presence of oxygen radicals, which are both abundant during lung ischemia (49). Together, the superoxide and activated NOS generate peroxynitrite, which, in turn, releases calcium from the inner membrane of the mitochondria (30). It is uncertain how much this particular isoform contributes to ROS damage, since the regulation of mtNOS activity is so complex. Under normal circumstances, it appears that mtNOS is associated with and acts as one of the final electron acceptors of electron-transport chain I (164). Under anoxic conditions, a decrease in the amount of oxygen shuts down the electron-transport chain I, making mtNOS lose most of its NO production capability (164). When L-arginine is depleted due to

ischemia, Ca^+ influx in the mitochondria appears to stimulate ROS production in cardiac myocytes (50). While this suggests that mtNOS, therefore, generates ROS, more research must be done to verify this in the lung. Nevertheless, it is apparent that a decrease in oxygen availability is detrimental to the ability of mtNOS to function properly.

2) iNOS-dependent superoxide production: During IR, iNOS has been activated by endothelial cell membrane depolarization due to the absence of shear stress during ischemia (6, 7) up to 2 h after lung reperfusion (146). The high concentration of NO inhibits cytochrome oxidase, resulting in increased ROS production (94), and reacts with ROS to form peroxynitrite and other reactive nitrogen species (15). Increased production of NO by iNOS may lead to an increase in lung injury (154) and decreased lung compliance (80). Furthermore, the presence of reactive nitrogen species can act as a proinflammatory signal, recruiting macrophages and neutrophils to the site of injury.

However, this is only the case on the vascular side of the lung: several papers have suggested that inhaling NO is beneficial in preventing LIRI (139, 204). However, it should be noted that none of these studies provide, nor prove, a mechanism of action for these effects.

3) eNOS-dependent superoxide generation: eNOS is constitutively expressed under physiological conditions (16, 77, 132) and constantly releases NO for different signaling pathways and plays a pivotal role in protecting the lung from damage due to mechanical stress or IR. eNOS is located in the proximal bronchiolar epithelium (184) and in the endothelial layer of bronchial and large pulmonary blood vessels (63).

During oxidative stress, tetrahydrobiopterin (BH4), an essential cofactor of eNOS, becomes oxidized and subsequently less available to eNOS (136). This leads to "eNOS uncoupling", as the dimeric eNOS will revert to its monomeric form. The depletion of BH4 can also occur if heat shock protein 90 (HSP-90) is downregulated. Both situations result in eNOS uncoupling, less NO being produced, and more ROS causing injuries to the tissue (136, 193).

Neutrophil-induced IR damage. Neutrophils are important mediators of changes in endothelial and epithelial permeability following lung IR (126). The activation of neutrophils may prolong oxidative stress in endothelial cells, resulting in oxidative stress signaling in endothelial cells that further increases a proinflammatory phenotype. The sequestration of neutrophils in the lung occurs during both ischemia and reperfusion (201).

Neutrophils promote their adherence to the endothelium by increasing the expression of CD18 and CD11b surface adhesion molecules that promote adhesion to specific ligands, such as intercellular adhesion molecule-1 and P-selectin from the endothelium (42, 138). Neutrophils further stimulate adherence by producing multiple proinflammatory cytokines and chemotactic agents and cause direct pulmonary injury by releasing elastase and other proteases. Alveolar macrophages can cause a migration of neutrophils to the lung during IR by releasing chemokines (IL-8, epithelial cell-derived neutrophil activating protein-78) (42, 127). The infiltration of neutrophils in the lung is correlated with a poor Pa_{O_2}, and a higher pulmonary arterial pressure, $P\mu vasc$, and PVR (172).

Cell death. Apoptosis is the main mechanism of cell death observed during human and experimental models of lung transplantation-induced LIRI. In contrast to necrosis, apoptosis is only present after reperfusion and increases rapidly with a maximal peak 2 h after reperfusion (65). Apoptosis can be activated by mechanical injury and exposure to certain environmental conditions, leading to the activation of the intrinsic and extrinsic pathways (Fig. 4). Mitochondria are the regulators of both intrinsic and extrinsic pathways, because multiple cell death signals converge on the mitochondria, affecting the integrity of the outer membrane, which leads to multiple death-promoting factors being released in the cytosol (173). These death-promoting factors, as well as other parts of the intrinsic and extrinsic apoptosis pathway, are partially regulated by heat shock proteins like HSP-90 (20).

The intrinsic pathway, also called the mitochondrial pathway, can be triggered by stressors such as ionizing radiation, cytokine deprivation, chemotherapeutic agents, and oxidative stress. This
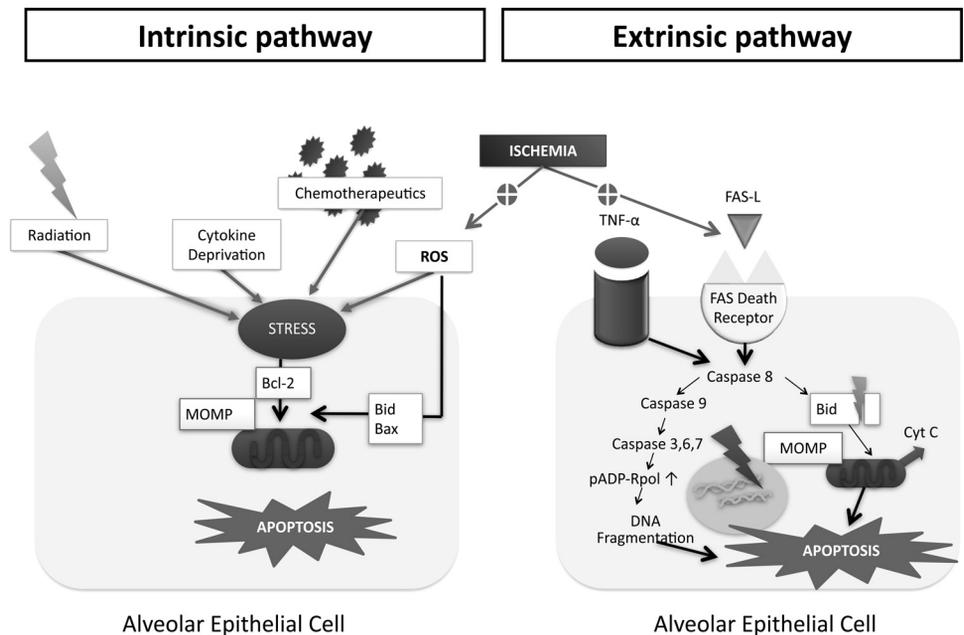

Fig. 4. IR injury-induced apoptosis of the alveolar epithelial cell. Mitochondria regulate both the intrinsic and extrinsic pathway toward apoptosis, of which the extrinsic is the most powerful one. Ischemia of the lung causes the ligands FAS-L and TNF-α to bind on their respective receptors, inducing the caspase cascade. This results in DNA fragmentation in the nucleus and Cyt C release from the mitochondria by mitochondrial outer membrane permeabilization after Bid cleavage. The intrinsic pathway can be activated by numerous factors; a major cause is ROS generated by lung ischemia. The intracellular oxidative stress activates Bcl-2 proteins, which causes mitochondrial outer membrane permeabilization (MOMP), which further enhances BH3 interacting domain death agonist (Bid) and Bcl-2-associated X protein (Bax) induction by ROS, triggering apoptosis.

pathway is characterized by changes in the mitochondrial outer membrane permeability, which is controlled by the Bcl-2 family of proteins. Oxidative stress can induce the proapoptotic proteins, such as Bid and Bax, within alveolar epithelial cells, thus triggering apoptosis through the intrinsic pathway (33).

The extrinsic pathway is characterized by the activation of specific receptor-ligand signaling interactions through Fas/Fas ligand, TNF receptor, or angiotensin II, which activate the apoptotic caspase cascade (209). During anoxia of the heart, there is no activation found of caspase-8 (a caspase activated by the extrinsic pathway), whereas caspase-9 (activated by extrinsic pathway) is already activated during anoxia in the heart (179). Because apoptosis is only found during reperfusion and not during anoxia (65), the initial apoptosis seen immediately upon reperfusion is suggested to be mediated by the completion of the intrinsic pathway alone, whereas the extrinsic pathway can take several hours to fully activate (115).

When lung tissue becomes ischemic, Fas-L ligand binds on the Fas receptor to activate caspase-8 (the initiating caspase for apoptosis). This activates caspase-3, -6, and -7 (the executioner caspases) by proteolytic cleavage (209), leading to a cascade of caspase activation and ultimately to DNA fragmentation by poly-ADP ribose polymerase. Besides this cascade in the ischemic lung, caspase-8 also triggers the intrinsic pathway through Bid cleavage, and this is one of the triggers for mitochondrial cytochrome-*c* release that activates caspase-9 and subsequently caspase-3 (209). After LIRI, the plasma Fas level is prognostic for the clinical outcome of the patient. The higher the level of Fas, the more likely it is that the patient will suffer unstable hemodynamics and multiple organ failure (8).

The peak in apoptotic cell death (2 h after reperfusion) coincidences with a peak in caspase-3 and caspase-8 activity, suggesting a crucial role for the caspase pathway in cell death after LIRI (67, 174).

As mentioned above, the amount of cell death in the lung appears to depend on the ischemic duration. At up to 12 h of cold ischemic preservation, apoptosis is predominant ($<2\%$ necrosis vs. 30% apoptosis), whereas longer cold ischemic periods up to 24 h resulted in predominantly necrosis (21–29% necrosis vs. $<1\%$ apoptosis) in a lung transplant rat model (67). Necrosis is associated with significantly worsened lung function after LIRI, due to the high degree of inflammation (150). Apoptosis, in contrast, is associated with less inflammation, because the apoptotic cells are readily phagocytosed by macrophages, thereby limiting the release of inflammatory intracellular enzymes (125).

An important pathway that is able to put a brake on apoptosis during IR is the phosphatidylinositol 3-kinase (PI3K)/Akt pathway. Carbon monoxide (CO) (210) can be one of the activators of this pathway in the lung, but insulin can also activate this pathway, which has been demonstrated in other tissues (95, 176). PI3K/Akt activates p38 mitogen-activated protein kinase, which activates STAT3, a member of a transcription factor family that decreases Fas expression and caspase-3 activation (210).

Cytokines. During lung IR, there is a rapid release of thrombin, complement (mainly C3 and C5a), PAF, vasoconstrictive and vasodilatory arachidonic acid-derived mediators, transcription factors such as NF-κB and AP-1, and anti- and proinflammatory cytokines, such as TNF-α, INF-γ, IL-1β, IL-2, IL-6, IL-8, IL-9, IL-10, and IL-18 (48, 112, 149).

NF-κB, a transcription factor, has a pivotal role in inducing LIRI during lung transplantation (171). During ischemia, the loss of shear stress induces the generation of ROS by increasing intracellular Ca^{2+} and NADPH oxidase, which activates transcription factors like NF-κB, AP-1, and other pathways (40, 85, 89, 116, 117, 144, 170, 197). NF-κB upregulates the production of inflammatory cytokines, chemokines, cell adhesion molecules (148), and apoptosis signals (18). NF-κB and TNF-α are suggested to be two of the most important mediators for complete development of LIRI (58, 112). Using a TNF-α antibody reduced lung vascular injury by 49% (112), and the use of an inhibitor of NF-κB resulted in almost normal lung histology and almost no pulmonary edema compared with controls during anoxia/reoxygenation (171).

Although extensively studied, the various proinflammatory mediators have a very complex, interrelated role and may be influenced by different circumstances (42). These cytokines and chemokines are released by alveolar macrophages and have an important role in LIRI, causing an upregulation of adhesion molecules (19), endothelial cell activation (61), decreasing the sensitivity of vascular smooth muscle cells for vasoactive signals (both dilatation and constriction) (110), and trafficking leukocytes to sites of inflammation (130). Macrophages are the initial source of TNF-α and the main producers of monocyte chemoattractant protein-1 in the lung (212).

IL-8 is one of the most important proinflammatory cytokines in LIRI. It induces the release of TNF-α and IFN-γ from activated pulmonary neutrophils (48, 49). While concentrations of most other cytokines decrease during reperfusion, IL-8 is one of the few cytokines that increase (49). Two hours after reperfusion, IL-8 concentration in lung tissue is inversely correlated with mean airway pressure, lung function (49), and directly with mortality rate after human lung transplantation (49, 71).

TNF-α and IL-1 expression are also upregulated on reperfusion, as determined by increased mRNA expression levels (35). These cytokine levels are correlated with the degree of early pulmonary neutrophil sequestration (161), pulmonary edema, and Pμvasc, suggesting that these cytokines are important modulators of early LIRI (35, 105).

During IR, there is a rapid remodeling of membrane lipids that causes the generation of several bioactive lipids with intra- and/or extracellular activity. Phospholipases, like the phospholipase A_2 family, are the main source of these mediators. Activation of cytosolic phospholipase A_2 in IR mobilizes arachidonic acid for degradation by two main enzymes, lipoxygenase and cyclooxygenase, into several mediators (see below), and these initiate the production of PAF (48).

Alveolar macrophages (2) are believed to play a major role in surfactant degradation through excreting lysosomal phospholipase A_2 (108) and are suggested to be responsible for decreased lung compliance after alveolar macrophage depletion with clodronate in a mouse model (212). Type II secreted phospholipase A_2 is another phospholipase A_2, present in alveolar macrophages, but also in platelets and other cells (108). A relation has been found between the activation of this type II secreted phospholipase A_2 and subsequent degradation of surfactant (14). Arachidonic acid-derived mediators, such as leukotrienes ($B_4/C_4/D_4/E_4$) are produced by the lipoxygenase pathway. Other arachidonic acid-derived mediators, such as thromboxanes A_2 and B_2 and various prostaglandins, are produced via the cyclooxygenase pathway. These mediators are

both proinflammatory and anti-inflammatory, as well as vaso-constrictive and vasodilative. The balance between these different mediators may be important for LIRI. Increased levels of these mediators are found in blood and bronchoalveolar lavage fluid after lung IR in animal models (186) and are correlated with higher pulmonary arterial pressure (162), pulmonary neutrophil sequestration (162), pulmonary edema, and increase in Pμvasc (186). Thromboxane A$_2$ may cause an increase in the severity of pulmonary edema by increasing PVR and lung polymorphonuclear cell infiltration, which causes reduced cardiac output and lower Pa$_{O_2}$ (196).

PAF can be released by many different cells, such as macrophages, platelets, endothelial cells (48), mast cells, and neutrophils. PAF receptors signal through protein kinase C (155), consequently activating leukocytes, inducing cytokine release, stimulating expression of cell-surface adhesion molecules, and ultimately promoting platelet aggregation (133). Because PAF and endothelin-1 use synergistic pathways in inducing LIRI (189), treatment of rats with endothelin-1 and PAF antagonists together resulted in superior posttransplant graft function after lung transplantation compared with placebo or single treatment (189). Endothelin-1 and its receptors are found abundantly in the lung, where they are located on macrophages, endothelial cells, and smooth muscle cells (27). Endothelin-1 can also stimulate the production of cytokines by monocytes/macrophages (178), increase iNOS expression (183), and, at high levels, it can increase the expression of

vascular endothelial growth factor and increase Pμvasc (3). Endothelin-1 increases pulmonary arterial and vascular resistance (134), decreases Pa$_{O_2}$ (134), and is correlated with increases in pulmonary edema and pulmonary neutrophil sequestration (156) after IR.

IL-10 exerts protective effects against LIRI by inhibition of the inflammatory and T-cell-mediated immune responses in the lung (149). Phospholipase A$_2$ also has some protective effects by mediating the production of PGE$_1$. PGE$_1$ may have some anti-inflammatory properties (165) because adding PGE$_1$ during reperfusion of the lung reduces pulmonary production of inflammatory chemokines such as IFN-γ, IL-12, and TNF-α and increases the IL-10 levels (47).

Therapeutic Possibilities

LIRI is a complex pathology, and hence a large number of possible treatments have been investigated to reduce this. An overview covering the different treatment possibilities for intervening with the different LIRI pathways previously mentioned is given in Fig. 5.

Mitochondrial protection. Mitochondria are a very important link in the IR injury pathways because of their contribution to their own damage with ROS production, apoptosis, Ca^{2+} homeostasis, cell signaling, and other important metabolic pathways in the cell. Several mechanisms to protect the mito-
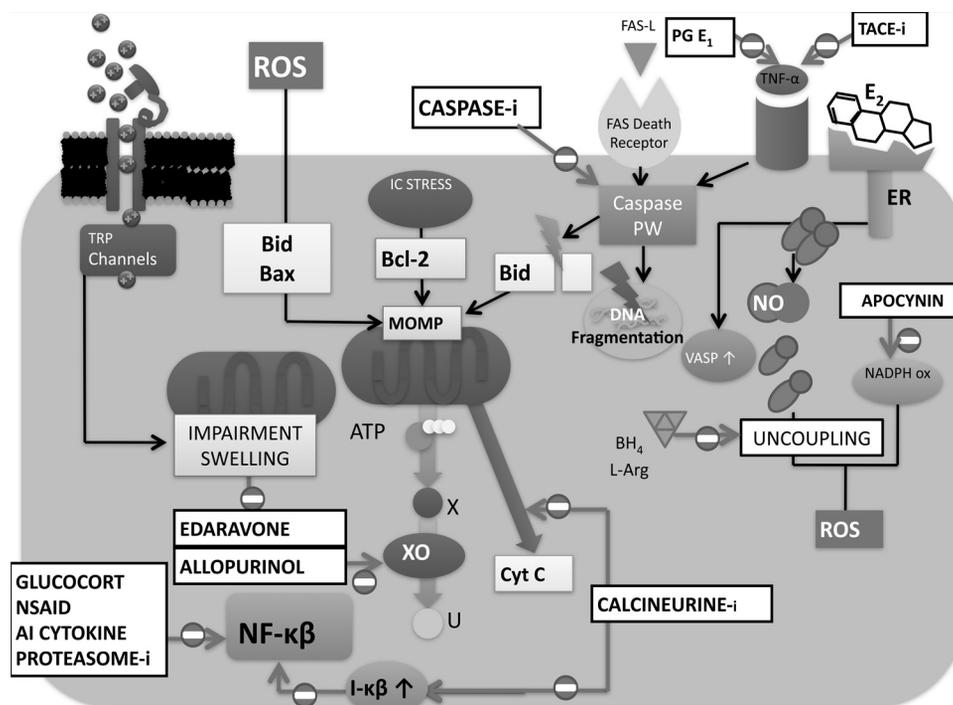

Fig. 5. Overview of different therapeutic approaches in IR injury. Clockwise, starting on the *bottom left*: NF-κB plays an important role in IR injury. Calcineurin inhibitors like sirolimus and cyclosporine A decrease the breakdown of I-κB, which results in an increased inhibition of NF-κB activity. They also prevent Cyt C release from the mitochondria, which is essential for necrosis. NF-κB can also be directly blocked by various agents, such as glucocorticoids and NSAIDs. Allopurinol, a known inhibitor of XO, can act to decrease the amount of ROS from this source upon reperfusion. Edaravone is a free radical scavenger that reduces mitochondrial swelling after IR. Caspase inhibitors block the apoptotic cascade. PGE$_1$ shifts the cytokine production more toward a noninflammatory profile, decreasing production of such proinflammatory cytokines as TNF-α, while increasing the production of IL-10. TNF-α-converting enzyme inhibitors (TACE-i) reduces TNF-α concentrations. Estradiol (E$_2$) directly activates eNOS and leads to an increase in vasodilatator-stimulated phosphoprotein (VASP), providing protection against IR injury. Apocynin is an inhibitor of the cellular NADPH oxidase and works synergistic with TACE-i. Tetrahydrofolate (BH4) and L-arginine (L-Arg) are both substrates for eNOS and prevent its uncoupling, reducing ROS production after IR. PW, pathway; ER, estrogen receptor.

chondria are proposed, although the extrapolation of these new therapeutic strategies to the lung is very limited.

The pretreatment of rabbits with the free radical scavenger edaravone attenuated both mitochondrial swelling and IR-induced decrease of mitochondrial membrane potential after anoxia/reoxygenation of the lung (167). It has been demonstrated that using calcineurin inhibitors, such as cyclosporine A, blocks the mitochondrial transition pore (78), which prevents cytochrome-*c* release and reduces apoptotic cell death.

Complex I of the mitochondrial respiration complex appears to be an important effector of mitochondrial-derived oxidative injury following the release of cytochrome-*c* during apoptosis or ischemia (38). However, when blocking complex I with rotenone under aerobic conditions, indicators of cellular injury increase (119), suggesting that this model is only useful for anoxia and not ventilated ischemia.

These findings suggest that, while directly influencing the mitochondrial pathways can be of therapeutic importance, further investigation is still required to ensure the specificity of action.

Xanthine/xanthine oxidase inhibition. Blocking the xanthine/xanthine oxidase pathway with xanthine oxidase inhibitors, such as allopurinol and oxypurinol, has been investigated and proven in several studies to be effective in LIRI (4, 5, 101, 211). However, the beneficial effect of allopurinol is dependent on the model. During ventilated ischemia, allopurinol had no effect on oxidant generation, but, during anoxia/reoxygenation, it significantly decreased oxidant generation, thereby preventing injury (5, 211). Furthermore, the protection of xanthine oxidase inhibitors seems to be partial, indicating that other sources of ROS may be of greater clinical significance.

NADPH oxidase inhibitors. Apocynin and diphenyliodonium are two unrelated NADPH oxidase inhibitors that have proven to be effective in attenuating LIRI in rodent, sheep, and pig models after ventilated ischemia (6, 53, 211, 213). Apocynin is more effective than diphenyliodonium in preventing LIRI in sheep (52), but this is not the case in all species. When combining apocynin with a TNF-α converting enzyme inhibitor, there is a marked attenuation of the injury to the nonischemic control lung in rats (213). However, both apocynin and diphenyliodonium can cause chronic granulomatous disease by preventing the respiratory burst used for bactericidal phagocytosis made by NADPH oxidase (51, 57). Furthermore, diphenyliodonium is a general flavoprotein blocker and blocks almost all ROS-generating entities, including mitochondria, NOS, and cytochrome *P*-450 monooxygenase (82), making its use in patients very unlikely. Therefore, the use of apocynin needs further investigation before it can be used in humans.

NO administration and NOS modulation. NO can be administrated exogenously by direct inhalation or by infusion of an NO donor, such as FK409, nitroprusside, glyceryl trinitrate, or nitroglycerin. Administration of a NO donor in lung IR has been shown to be beneficial, resulting in lower pulmonary arterial pressure, higher Pa_{O_2}, improved ventilation-perfusion matching, and histologically less edema and polymorphonuclear neutrophil infiltration (205).

Other investigators focused on increasing the NO production of the NOS enzyme by adding one of its cofactors, BH4 (9), in the preservation solution (180), increasing the bioavailability of its substrate L-arginine (142), or transfecting the donor lung just before retrieval with an adenovirus containing eNOS (195). BH4 not only increases NO production in LIRI, but also attenuates ROS formation by decreasing the amount of uncoupled eNOS, as recently demonstrated in other organs, such as the heart (137). No information is available yet about the possibility of the inexpensive and relatively safe molecule folic acid to increase the bioavailiability of BH4 in the lung, although this has been demonstrated in other tissues (135).

Several drugs have been investigated to increase eNOS activity. Statins are able to increase NO synthesis without increasing eNOS expression in a hypoxic rat model (145). Statins attenuate hypoxic pulmonary hypertension and pulmonary vascular remodeling in a hypoxic rat model (75). HSP-90 is able to stimulate eNOS-dependent NO generation and to decrease superoxide generation, suggesting an interesting pathway to prevent ROS production during eNOS uncoupling in a sheep model (193).

It has been described that administration of estradiol can protect against LIRI induced by trauma-hemorrhage, partially by directly activating eNOS after binding the estrogen receptor (98). The NO produced activates a cyclic GMP-dependent protein kinase G pathway and, consequently, the vasodilator-stimulated phosphoprotein (98). It has been demonstrated that estradiol can, therefore, decrease lung injury, neutrophil infiltration, and the expression of cytokines, chemokines, and cell-surface adhesion molecules in the lung tissue (98).

In addition, pretreatment with an iNOS inhibitor (1400W) showed a significant decrease in platelet rolling and vasoconstriction after LIRI in a ventilated rabbit lung IR model (159). Furthermore, iNOS mRNA expression can be reduced by poly ADP-ribose polymerase inhibitors, resulting in less Pμvasc and protein leakage, and less proinflammatory cytokines, NO metabolites, and hydroxyl radicals after LIRI in a ventilated lung IR model in rats (192).

Inhibition of NF-κB. NF-κB activity can be inhibited by several pharmacological agents that block one or multiple steps of its signaling pathway. These agents include glucocorticoids, nonsteriodal anti-inflammatory drugs, anti-inflammatory cytokines, and proteosome inhibitors (39). Ross et al. (171) demonstrated in a lung graft model that pyrrolidine dithiocarbamate, another NF-κB inhibitor, effectively inhibited NF-κB activation and can significantly improve lung function. Beside these agents, anti-NF-κB antibody pretreatment can also attenuate LIRI (39).

The agents cyclosporine A and tacrolimus inhibit calcineurin (111), which prevents the 20S proteosome from degrading I-κB, an inhibitor of NF-κB (111). This ultimately decreases NF-κB activity, which may decrease inflammatory cytokine activation, and results in decreased production of mRNA for proinflammatory cytokines and vasoconstrictive substances (111). These effects may explain the attenuation of LIRI when the patient has been pretreated with tacrolimus or cyclosporine A (111).

Inhibition of AP-1. AP-1, like NF-κB, is responsible for the activation of several proinflammatory cytokines, including TNF-α. Inhibition of c-Jun kinase (JNK) results in decreased expression of AP-1, leading to a marked decrease in protein leakage, lactate dehydrogenase levels, and decreased release of TNF-α in bronchoalveolar fluid, resulting in a decreased lung injury after reperfusion in a rat lung transplantation model (86). Inhibition of the JNK/AP-1 signaling pathway also decreases tissue apoptosis after reperfusion (86). It appears that inhibiting JNK during ischemia and reperfusion is needed to have these beneficial effects (86). Inhibiting JNK, however, has no effect on the activity of NF-κB, but a decrease in TNF-α is still found

during lung IR with JNK inhibition (86). It is suggested that the inhibition of JNK decreases the stability of TNF-α mRNA (22) rather than affect NF-κB modulation of gene transcription (86). These effects of JNK inhibition suggest an effective therapeutic strategy against LIRI during lung transplantation (86). As nicely summarized by Bogoyevitch and Arthur(25), there is substantial difference in results between gene knockout, JNK inhibition, and dominant-negative JNK mutant overexpression studies, and these differences need to be elucidated. The question is, if more specific JNK inhibitors are possible or even desirable because of possible side effects or mechanisms to bypass specific blockades (25).

Thiozolidinediones. Thiazolidinediones, like pioglitazone, troglitazone, and rosiglitazone, are anti-diabetic drugs that stimulate a nuclear transcriptional factor called peroxisome proliferator-activated receptor-γ (88). Peroxisome proliferator-activated receptor-γ prevents the activation of transcription factors, such as NF-κB and AP-1 (92, 169). Preischemic treatment of the lung with pioglitazone significantly reduced LIRI by reducing proinflammatory cytokine production and decreased neutrophil accumulation, resulting in a stable Pμvasc after lung transplantation in a rat model (88).

Alveolar macrophage/neutrophil depletion. Because neutrophils are potentially important mediators for both the changes of endothelial and epithelial permeability following lung IR, and because leukocyte infiltration plays a critical role in pathophysiology of LIRI, both neutrophil and alveolar macrophage depletion are seen as a possible therapeutic strategy to prevent LIRI.

It has been demonstrated that depletion of alveolar macrophages is beneficial in the early phase of LIRI (148, 212). Cyclosporine A pretreatment is a very effective way to reduce reperfusion injury, because it decreases the alveolar macrophage response to hypoxia and reoxygenation (147).

Zhao et al. (212) suggested that clodronate, another agent used for alveolar macrophage depletion, has some negative effects because clodronate induces alveolar macrophage death, and apoptotic or necrotic alveolar macrophages may release lysosomal phospholipase A_2, which destroys surfactant and may decrease pulmonary compliance.

Alveolar macrophages are probably the initial source of TNF-α as a reaction to IR after lung transplantation. Reducing TNF-α levels with a TNF-α-converting enzyme inhibitor prevents rejection and reduces the production of chemokines such as monocyte chemoattractant protein-1 (MCP-1) (76). Reducing TNF-α with an antibody causes marked reduction of leukocyte infiltration in LIRI (105).

Zhao et al. (212) demonstrated that MCP-1 expression is positively correlated with IR-induced lung dysfunction and that alveolar macrophage depletion significantly reduced this expression. MCP-1 is an important monocyte recruiter and activator (130), but it is also important for early accumulation of neutrophils at sites of inflammation (130). These findings suggest that alveolar macrophage depletion could be an important way to minimize the damage to the lung in both early and late phases of LIRI.

While alveolar macrophages (148) are important mediators of the initial phase of LIRI, neutrophils (172) may be responsible for the late-phase injury. Alveolar macrophage depletion may prevent neutrophil sequestration and, therefore, late-phase injury. In a rat model of lung anoxia/reoxygenation, administration of anti-TNF-α and anti-IL-1β antibodies together intra-

venously before reperfusion blocked neutrophil chemotaxis and chemokines production (112). This technique showed a significantly reduced lung permeability, neutrophil content, proinflammatory cytokine production, and morphological injury after lung IR (112). Pentoxifylline is also known to reduce lung injury during cardiac bypass (42). It successfully reduces the amount of cytokine-activated neutrophils, and their degranulation, and prevents endothelial activation (42). Pentoxifylline is able to inhibit TNF-α and IL-1 and increase cAMP levels, and it effectively attenuates LIRI after transplantation (42). Ege et al. (56) compared pentoxifylline, aprotinin (an antifibrinolytic), and placebo for 12 h postoperatively after a cardiopulmonary bypass in patients and demonstrated a similar degree of attenuation of LIRI with aprotinin and pentoxifylline. Aprotinin's effects are especially beneficial in light of a retrospective study of 180 lung transplant patients, which demonstrated a significant decrease of severe posttransplant LIRI, largely through the attenuation of neutrophil activity (24). Thabut et al. (198) found a marked decrease in the incidence of allograft dysfunction (by mortality, pulmonary edema, lung function, and duration of mechanical ventilation) after lung transplantation when administrating pentoxyfilline and inhaled NO before and during perfusion compared with historical controls.

Caspase inhibition. The peak of caspase activity in a pig model matches with the peak of apoptosis during lung IR (174). Caspase inhibitors, such as the broad-spectrum caspase inhibitor zVAD-fmk or small-spectrum caspase inhibitors like IETD-FMK for caspase-8, are commercially available. These molecules might be able to decrease LIRI by blocking the apoptotic cascade during ischemia and so improving the lung graft function.

Therapeutic preconditioning. There are several techniques by which a lung can be preconditioned before undergoing IR, and doing so making the lung more resistant to LIRI. These techniques are as follows: increasing temperature (hyperthermic preconditioning) (90), induction of short periods of ischemia (ischemic preconditioning) (72), and administration of pharmacological agents (chemical preconditioning) (181).

Hyperthermic preconditioning results in an upregulation of the heat shock protein family in the lungs of minipigs, and it is suggested to be protective against LIRI (122).

The induced heat shock protein synthesis after ischemic preconditioning in the lung helps with alveolar fluid clearance and to maintain ion transport, resulting in preserving gas exchange and reducing increases in Pμvasc after lung IR (208). Kaminski et al. (97) found that eNOS plays an important role in the pulmonary protection after ischemic preconditioning because the absence of eNOS completely reversed the protective effects.

Several possible explanations exist to explain the benefits of ischemic preconditioning in the lungs. Featherstone et al. (62) demonstrated in a lung transplantation rat model that blocking perfusion and the ventilation, by collapsing the lung, is more important than blocking the pulmonary blood supply, showing no difference between lung compliance of ventilated ischemia and control. This can be explained because ventilated ischemia does not cause any ATP reduction (70), and lung collapse and reinflation, together with ischemia, may activate stronger signals than the induction of ischemia alone (62). It is of major importance that the preconditioning consists of several brief periods of IR (62). Preconditioning with NO is also beneficial, as it induces pulmonary neutrophil apoptosis, reducing the

ROS production by neutrophils and decreasing the release of inflammatory cytokines from alveolar macrophages (181). Whole body hypoxic preconditioning (WHPC) is another possibility for preconditioning. It appears to enhance the tolerance to lethal hypoxygenation, with longer survival time, less pulmonary edema, and better gas-exchange function of the lung compared with controls (208). Kaminski et al. (96) found that eNOS was transcriptionally upregulated by WHPC in mice, with associated increase of eNOS phosphorylation, and in eNOS-knockout mice the anti-inflammatory effect of WHPC was abolished and the endothelial leakage was further exacerbated. This suggests a new therapeutic approach to improve the outcome of patients who will undergo lung IR by combining an eNOS stabilizing/enhancing drugs and hypoxic preconditioning (96).

Lung preservation solution. The Euro-Collins (EC) preservation solution, an intracellular fluid-type solution, and modifications on this solution have been used widely for lung transplantations (84). However, using EC is correlated with a higher occurrence of LIRI (100), lower posttransplantation lung function recuperation (104), higher cytotoxicity (124), and increased pulmonary edema due to high-potassium concentration (100), compared with an extracellular fluid-type solution named low-potassium dextran solution. Low-potassium dextran solution has been compared with EC in several clinical and experimental studies, and the results suggest that low-potassium dextran solution should be considered the preservation solution of choice, but long-term results of graft function are needed to support this (48).

Temperature has also been shown to be important for preserving lung function (187).

Complement inhibition. A multicenter study demonstrated that complement inhibition before reperfusion, with a soluble complement receptor-1, results in a good outcome for lung transplant recipients, as measured by a higher extubation rate in the first 24 h and shorter intensive care stay compared with placebo (103). This suggests that soluble complement receptor-1 can be a good additional therapy for lung transplant patients. The mechanism by which complement inhibition confers its beneficial effects likely includes the inhibition of signaling to the leukocytes involved in the inflammatory response, as well as limiting the ability of the host to recognize the graft, but further research is needed to see if this is the case. Because of the complexity of lung IR pathophysiology, reducing any inflammatory processes may incur some benefits.

PAF. Blocking PAF is another possible way to decrease LIRI, as PAF is known to activate proinflammatory reactions. A few studies have shown the benefits in administering a PAF antagonist in lung transplantation with better $(A-a)Do_2$ compared with placebo (202) or by blocking the metabolism of PAF with less pulmonary edema, neutrophil extravasation, and reduced Pμvasc relative to control groups (21). A combination treatment of a PAF antagonist with an endothelin-1 antagonist resulted in superior posttransplant graft function after lung transplantation in rats compared with placebo or single treatment (189). However, practical application is limited by their detergent-like effects, which can cause hemolysis and endothelial injury (202). It is probable that PAF antagonism is a viable preventive measure when used in conjunction with other methods, but further research is required to fully determine the mechanism of action to ensure that the risk of blocking one of the key elements of the clotting cascade does not endanger the patient.

Surfactant. The bronchoscopic administration of exogenous surfactant has been proven to be an efficient therapy to prevent LIRI in transplant recipients (102). Surfactant improves oxygenation, prognosis, and radiological clearance of infiltrates in post-lung transplantation, and abolishes atelectasis after lung IR. It has been demonstrated that administering exogenous surfactant both before retrieval of the donor and after perfusion is more effective than single administration, at either time (153). Struber et al. (191) used a nebulized synthetic surfactant for several patients with severe LIRI after lung transplantation, which led to rapid improvement in pulmonary compliance and extubation within 6 days after application. However, bronchoscopic administration appears to deliver the surfactant in the IR lung more accurately (55, 98, 102), and natural surfactants appear to be superior compared with synthetic surfactants used on neonates (98). The surfactant allows reduction in the inspired oxygen levels and ventilator settings, reducing the incidence of pneumothorax and increasing survival (98).

Fluid infusion. The addition of pentastarch, a colloid composed of hydroxyethyl starches, which is usually administered as a plasma volume expander, has recently been shown to be very effective in attenuating LIRI in a rat lung ischemia model, when administered alongside dexamethasone, dibutyryl-cAMP, and β_2-adrenergic agonist via inhalation (121). It is suggested that the pentastarch macromolecules are able to "plug" the injured sites and obstruct capillary leakage due to its extremely hygroscoping nature (140), while the β_2-adrenergic component increases Na^+ transport and edema clearance in both animals and humans (175). A 10% solution of pentastarch administered via intravenous infusion has been demonstrated to have some efficacy in the prevention of endothelial damage; hypothesized mechanisms include the ROS scavenging activity of pentastarch (17), stabilizing cell membranes (140), and decreasing PVR by improving blood flow (131), although it is likely that, if one has had the type of surgery that a lung transplant requires, the effects of increasing the plasma volume should also not be discounted.

Due to the lack of general recommendations for an improved preservative solution to make lung transplantation more successful, but with the rather large amount of new multidimensional information available on the pathogenesis of lung IR, we have taken the opportunity to summarize in Table 1 the components of a solution that should be able to reduce lung IR injury.

Table 1. *Suggested elements for a lung preservation solution during lung transplantation*

Substance	Action
Low potassium-dextran solution (base fluid)	
Folate	Decrease ATP degradation and decrease eNOS uncoupling
Cyclosporine A or tacrolimus	Reduction of mitochondrial permeability transition opening and blocking the activation of NF-κB
Insulin with glucose	Activation of PI3K/Akt pathway
Heparin or soluble complement receptor-1	Inhibition of complement
Free radical scavenger like *N*-acetyl-cysteine	Reduce free radicals

eNOS, endothelial nitric oxide synthase; NF-κB, nuclear factor-κB; PI3K, phosphatidylinositol 3-kinase.

We would like to point out that all of the ingredients of this solution are *1*) commercially available in intravenous format, and *2*) approved for human use. So this strategy to reduce lung IR injury would likely be acceptable to the Food and Drug Administration. We would highly recommend that those involved in the field of lung IR and the relevant scientific institutions start up a large-animal model testing the new solution by using the current consensus preservative fluid and low potassium/dextran solution as two of the controls. Afterwards, the clinical translation of the information acquired should require a fairly quick collaborative trial.

Gene therapy. Gene therapy is a very interesting topic in the prevention of LIRI, although clinical use is limited. Several strategies to transfect the lung have been used experimentally to prevent LIRI. The genes themselves can be delivered with the help of a vector. This vector can be viral, nonviral, or a naked form. These vectors can be administered intravascularly, intramuscularly (194), or transtracheally (66), after which the vector merges with a cell, with subsequent activation and transcription of the transported genes. These vectors with the incorporated genes have been administered to donor lungs before retrieval (34), during cold ischemic time (206), or to the recipient after reperfusion (194).

Transtracheal administration of the gene coding for human IL-10 12–24 h before lung retrieval attenuates LIRI and enhances lung function in a rat single-lung transplant model (66). eNOS delivery just before retrieval with an adenovirus has also been successful in ameliorating LIRI during lung transplantation (195). Further research needs to be done to assess the long-term effects of these procedures on the recipients.

PGE$_1$. Continuous intravenous administration of PGE$_1$ has been shown to be successful in reducing LIRI during the early phase of reperfusion after lung transplantation in a rat (47) and dog model (13). Continuous administration of PGE$_1$ causes a shift in the proinflammatory cytokine profile, including TNF-α, IFN-γ, and IL-12, to an anti-inflammatory cytokine profile with IL-10 (47). Beside these effects, PGE$_1$ has an antiaggregant effect on platelets, which may also explain its potentially beneficial role (157). PGE$_1$ is currently used as an additional substance in preservation solution used in lung transplantation and might be useful in the reducing LIRI by other causes.

CO. CO, like NO, is a messenger gas molecule that regulates vasomotor tone through the production of guanosine $3',5'$-cyclic monophosphate (99). Increasing evidence suggests that CO is a very potent anti-inflammatory and antiapoptotic molecule, as mentioned above. CO is able to mediate its cytoprotective effect through the activation of the PI3K/Akt pathway, which activates p38 mitogen-activated protein kinase and subsequently activates STAT3, independent of the NO/cyclic guanosine $3',5'$-cyclic monophosphate pathway. In cultured cells and small-rodent studies, the inhaled levels of CO ranged between 10 and 500 ppm (36, 43, 143), with reduced inflammatory effects seen from the lowest dose of 10 ppm (54, 177), which is significantly lower than the known toxic level of CO (158).

Using CO to activate the PI3K/Akt pathway and subsequently STAT3 could be a new pretreatment option to decrease LIRI. Due to the fact that there is no universal standard on how much CO a human may be exposed to for a certain amount of time and the fact that this is a potentially lethal gas, further investigations are required to understand the full potential of CO in reducing LIRI and to investigate its clinical usefulness.

Conclusion

LIRI is a complex pathogenetic situation in which multiple molecular and cellular mechanisms are involved. Therefore, it can only be countered if the primary mechanisms leading to it are further explored and better understood. Single treatments do not appear as effective as combination treatments, which tackle the problem at several levels. Furthermore, different strategies must be found for different situations, regarding ventilated ischemia and anoxia/reoxygenation. An important limitation in the archeological build-up of knowledge on lung IR injury is the inappropriate mixing of the terms "ischemia" and "anoxia/hypoxia". Therefore, we would strongly encourage that further publications clearly define this important but complex pathological topic.

It is mandatory that the different treatment options are discussed and a general consensus is made for the treatment of lung transplant patients. Enough pieces are known of the puzzle around LIRI to make a standard treatment for lung transplant patients but also for other causes of LIRI. Further experimental studies, and later multicenter clinical trials, are required to evaluate the effect of this treatment in reducing morbidity and mortality caused by LIRI in different situations. Too many years have gone by without a standard treatment, or at least a general consensus of treatment, for LIRI.

DISCLOSURES

No conflicts of interest, financial or otherwise, are declared by the author(s).

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