State of the Art

Ischemia–Reperfusion–induced Lung Injury

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Ischemia–reperfusion–induced lung injury is characterized by nonspecific alveolar damage, lung edema, and hypoxemia occurring within 72 hours after lung transplantation. The most severe form may lead to primary graft failure and remains a significant cause of morbidity and mortality after lung transplantation. Over the past decade, better understanding of the mechanisms of ischemia–reperfusion injury, improvements in the technique of lung preservation, and the development of a new preservation solution specifically for the lung have been associated with a reduction in the incidence of primary graft failure from approximately 30 to 15% or less. Several strategies have also been introduced into clinical practice for the prevention and treatment of ischemia–reperfusion–induced lung injury with various degrees of success. However, only three randomized, double-blinded, placebo-controlled trials on ischemia–reperfusion–induced lung injury have been reported in the literature. In the future, the development of new agents and their application in prospective clinical trials are to be expected to prevent the occurrence of this potentially devastating complication and to further improve the success of lung transplantation.

Keywords: lung transplantation; primary graft failure; acute lung injury; early graft dysfunction; lung preservation

CONTENTS

Since 1983, lung transplantation has enjoyed increasing success and has become the mainstay of therapy for most end-stage lung diseases. The last decade has been marked by both a significant increase in the number of centers performing lung transplantation and in the number of recipients on the waiting list. The Registry of the International Society for Heart and Lung Transplantation reported in 2002 that almost 15,000 lung transplants have been performed worldwide and that more than 1,500 lung transplants are performed annually (1).

Despite refinements in lung preservation and improvements in surgical techniques and perioperative care, ischemia–reperfusion–induced lung injury remains a significant cause of early morbidity and mortality after lung transplantation. The syndrome typically occurs within the first 72 hours after transplantation and is characterized by nonspecific alveolar damage, lung edema, and hypoxemia. The clinical spectrum can range from mild hypoxemia associated with few infiltrates on chest X-ray to a picture similar to full-blown acute respiratory distress syndrome requiring positive pressure ventilation, pharmacologic therapy, and occasionally extracorporeal membrane oxygenation (2). A number of terms have been used to describe this syndrome, but ischemia–reperfusion injury is most commonly used, with primary graft failure attributed to the most severe form of injury that frequently leads to death or prolonged mechanical ventilation beyond 72 hours (Table 1). In addition to significant morbidity and mortality in the early postoperative period, severe ischemia–reperfusion injury can also be associated with an increased risk of acute rejection that may lead to graft dysfunction in the long term (3).

Primary graft failure is the end-result of a series of hits occurring from the time of brain death to the time of lung reperfusion after transplantation. Ischemia–reperfusion injury has been identified as the main cause of primary graft failure. However, other injuries occurring in the donor before the retrieval procedure can contribute to and amplify the lesions of ischemia and reperfusion (Figure 1). Attention of lung transplant physicians has therefore been focused on selective assessment of donor lungs, effective technique of lung preservation, and careful management of transplanted lungs after reperfusion to reduce the severity of ischemia–reperfusion injury and the incidence of primary graft failure. Donor lung assessment is an attempt to select lungs that will be able to handle a period of several hours of ischemia without significant impairment in their function after reperfusion. Unfortunately, currently only 10 to 30% of donor lungs are judged suitable for transplantation (4).

Lungs that have been selected for transplantation are generally flushed with a preservation solution and hypothermically preserved to decrease their metabolic rate and energy requirement until implantation in the recipient. The period of cold ischemic storage is kept as short as possible and usually ranges between 4 and 8 hours according to the location of the donor. Although hypothermia is essential for organ storage, it is associated with a series of events such as oxidative stress, sodium pump inactivation, intracellular calcium overload, iron release, and induction of cell death that may induce upregulation of

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TABLE 1. TERMS USED TO DESCRIBE ISCHEMIA–REPERFUSION–INDUCED LUNG INJURY

Reimplantation edema
Reimplantation response
Reperfusion injury
Reperfusion edema
Primary graft failure
Early graft dysfunction

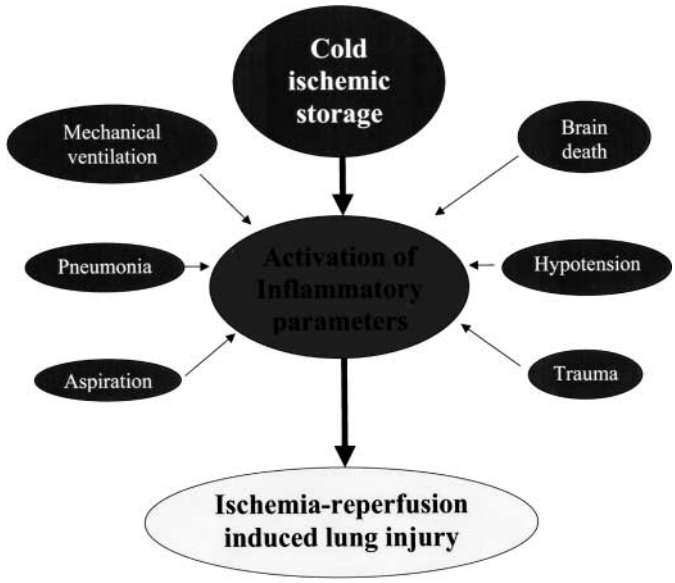

Figure 1. Ischemia–reperfusion–induced lung injury may be aggravated by a number of events occurring in the donor before lung retrieval.

molecules on the cell surface membrane and the release of proinflammatory mediators that will eventually activate passenger (donor) and recipient leukocytes after reperfusion. Prolonged ischemia may also result in a "no-reflow phenomenon" demonstrated by significant microvascular damages leading to persistent blood flow obstruction and subsequent ischemia despite reperfusion.

Over the past decade, numerous studies have been performed to optimize the technique of lung preservation. A new preservation solution, which combines a low potassium concentration and dextran, has also been developed specifically for the lungs (5, 6). Several strategies for the prevention and treatment of ischemia–reperfusion–induced lung injury have been introduced into clinical practice and have translated into a reduction in the incidence of severe ischemia–reperfusion injury from approximately 30 to 15% or less (7, 8).

This review will initially focus on donor lung assessment, then the effect of cold ischemic storage with its consequences after reperfusion will be reviewed, and finally the technique of lung preservation and the current strategies for prevention and treatment of ischemia–reperfusion–induced lung injury will be presented.

DONOR LUNG ASSESSMENT

The success of lung preservation primarily depends on proper organ selection. Currently, the parameters used to assess donor lungs are based on donor history, arterial blood gases, chest X-ray appearance, bronchoscopy findings, and physical examination of the lung at the time of retrieval (9). These parameters attempt to determine function and viability of the lungs, but their accuracy in determining the risk of reperfusion injury is not optimal and several centers have extended their donor selection criteria to the use of nonideal (i.e., extended or marginal) donors without significant effect on early outcome (10–13). The presence of bilateral infiltrates on chest X-rays, persistent pus at bronchoscopy, and signs of bronchoaspiration remain, however, strict contraindications to the use of donor lungs for transplantation (14). Table 2 defines the criteria for ideal and extended donors as well as *some* factors considered to be strict contraindications to the use of donor lungs for transplantation. It is recognized, however, that one may chose to accept increased risk in using lungs for recipients who are desperately ill.

The deleterious effect of brain stem death on organ function has been increasingly recognized over the last few years. Brain death can induce disruption in homeostatic regulation with profound disturbances in endocrine function and an intense inflammatory reaction that may reduce the tolerance of the organs to handle a period of ischemia (15–17). Follette and colleagues have shown that a bolus of steroids (methylprednisolone approximately 15 mg/kg) administered to all donors after brain death declaration can improve Pa_{O_2} and increase lung donor recovery (18). The steroid bolus can potentially reduce the inflammatory

TABLE 2. IDEAL, EXTENDED, AND MARGINAL DONOR SELECTION CRITERIA SUGGESTED BY THE TORONTO LUNG TRANSPLANT GROUP

Selection Criteria	Standard Criteria (*Ideal Donors*)	Extended Criteria (*Extended Donors*)	Contraindications (*Marginal Donors*)
ABO compatibility	Identical	Compatible	Incompatible
Donor history			
Age, yr	< 55	> 55	
Smoking history, pack-years	< 20	> 20	
Chest trauma	No trauma	Localized trauma	Extensive lung trauma
Duration of mechanical ventilation, h	< 48	> 48	
History of asthma	No	Yes	
History of cancer	No (except low-grade skin cancer and carcinoma *in situ*)	Primary central nervous system tumors	History of cancer
Sputum gram stain	Negative	Positive	
Oxygenation, mm Hg*	> 300	< 300	
Chest X-ray	Clear	Localized abnormality	Diffuse infiltrates
Bronchoscopy	Clear	Secretions in main airways	Persistent pus/signs of aspiration

* Last blood gas performed in the operating room with an FI_{O_2} of 100% and positive end-expiratory pressure of 5 cm H_2O.

reaction and compensate for the deficit in hypophyseal hormones observed after brain death.

Comparison of organ donation from living and cadaveric donors presents a unique opportunity to study the effect of brain death on clinical outcome. Some authors have shown that kidney biopsies from cadaveric kidney donors had significantly higher levels of inflammatory cytokines, adhesion molecules, and HLA-DR than biopsies from living donors, and the expression of these markers on tubular cells before transplantation was associated with a higher incidence of primary graft dysfunction and early acute rejection (19–21). In human lung transplantation, the chemokine interleukin (IL)-8 has been shown to be upregulated in bronchoalveolar lavage and lung tissue from brain-dead donors, and the level was found to significantly correlate with the incidence of primary graft failure after reperfusion (22, 23). Hence, there is growing body of evidence suggesting that cadaveric donors are exposed to inflammatory events due to brain death, prolonged intubation, episodes of infection and/or hypotension that may increase organ susceptibility to ischemia–reperfusion injury and alloimmune responses. In the future, methods to rapidly assess the degree of inflammation in the lung, for instance by measuring the levels of proinflammatory cytokines and/or adhesion molecules may be extremely useful to determine the type of lung suitable for transplantation and the potential tolerance to prolonged ischemia. These methods would help to reduce the incidence of primary graft failure and to optimize the use of organs available for transplantation.

EFFECT OF COLD ISCHEMIC STORAGE

Hypothermia decreases metabolic rate. Therefore, biochemical reactions are reduced and the rate of degradation of essential cellular components necessary for organ viability is reduced. Most enzyme systems show a 1.5- to 2.0-fold decrease in activity for every 10°C decrease in temperature (24). However, although hypothermia is essential during organ storage, a number of events can still occur leading to activation of inflammatory mediators that are ultimately deleterious to the preserved organ at the time of reperfusion.

Oxidative Stress

Oxidative stress is characterized by the formation of reactive oxygen species such as superoxide anion, hydrogen peroxide, and hydroxyl radical (25). These molecules, in particular the hydroxyl radical, are highly unstable and react with the first structure they encounter, usually the lipid component of the cell membrane. Cell injury produced by lipid peroxidation can range from increased permeability to cell lysis. The generation of intracellular oxygen species has been found to be present in most lung parenchymal cells, including endothelial cells, Type II alveolar epithelial cells, Clara cells, and ciliated airway epithelial cells as well as in alveolar macrophages (26). Two important mechanisms lead to the production of reactive oxygen species (Figure 2). One results from the accumulation of hypoxanthine and the conversion of the enzyme xanthine dehydrogenase into xanthine oxidase during anoxia, with the degradation of hypoxanthine into superoxide after reoxygenation (27). The other mechanism depends on the NADPH oxidase system, which is present mainly on the membrane surface of neutrophils and monocytes/macrophages and catalyzes the reduction of oxygen into hydrogen peroxide and superoxide anion (27).

Commonly, ischemia–reperfusion corresponds to anoxia–reoxygenation in organ transplantation. However, the lung has to be considered differently because it contains oxygen in the alveoli during ischemic preservation. Alveolar oxygen helps maintains aerobic metabolism and prevents hypoxia (28–30). Hence, in the lung, the oxidative stress resulting from ischemia should be distinguished from the oxidative stress resulting from hypoxia.

Hypoxia and, ultimately, anoxia result in a sharp decrease of adenosine triphosphate (ATP) and a corresponding increase in the ATP degradation product hypoxanthine, which generates superoxide when oxygen is reintroduced with reperfusion and/or ventilation. This phenomenon can occur in the lung when alveolar oxygen tension drops below 7 mm Hg during ischemia (31). It can be blocked by inhibitors of xanthine oxidase such as allopurinol (32, 33).

Ischemia is characterized by the absence of blood flow into the lung, which can cause lipid peroxidation and oxidant injury despite the presence of oxygen (29, 32). The mechanism of oxidative stress is different from that occurring during anoxia–reoxygenation because it is not associated with ATP depletion, and it can occur during the storage period (29, 30, 32). In addition, it cannot be blocked by inhibitors of xanthine oxidase (32, 34).

The endothelium appears to be one of the predominant sources of oxidants during nonhypoxic lung ischemia (34). Endothelial cells are highly sensitive to physical forces resulting from blood flow variation and are able to transform these mechanical forces into electrical and biochemical signals (mechanotransduction) (35). The absence of the mechanical component of flow during lung ischemia stimulates membrane depolarization of endothelial cells with the activation of NADPH oxidase, nuclear factor-κB, and calcium/calmodulin-dependent nitric oxide synthase (NOS) (34, 36). Other cells such as macrophages and/or marginated neutrophils, which are known to have a high NADPH oxidase activity, could also contribute to the lung oxidant burden that takes place during the ischemic storage (37, 38).

Sodium Pump Inactivation

The sodium (Na^+/K^+-ATPase) pump is important to preserve proper intracellular electrolyte concentration (high K^+, low Na^+) and to maintain adequate clearance of alveolar fluid. Hypothermic storage results in the loss of function of the sodium pump, which returns to normal activity with rewarming to 37°C if the epithelial cells are not damaged (39). The loss of function of the sodium pump results in accumulation of sodium in the cell resulting in cell swelling. This is associated with an influx of chloride inside the cell and an efflux of K^+ out of the cell. Preservation solutions contain electrolytes and colloid to create an osmotic pressure gradient in an attempt to prevent hypothermia-induced cell swelling. Preservation of organs at 10°C has been proved to be superior than at 4°C, and this has been attributed to better preservation of function of the Na^+/K^+-ATPase activity (40). The sodium pump activity has also been shown to resume better functional activity at the time of rewarming if the lungs are preserved with extracellular-type preservation solutions that contain low K^+ and high Na^+ concentrations (41).

Intracellular Calcium Overload

Hypothermic storage alters calcium metabolism in cells both by release of calcium from intracellular depots and by pathologic influx through the plasma membrane (24). The alteration of pH and intracellular calcium concentration disrupt many intracellular processes causing cellular damage (24). Elevated cytosolic calcium can also enhance the conversion of xanthine dehydrogenase to xanthine oxidase and potentiate the damaging effect of free radicals on mitochondria (25).

Support of a role for calcium overload in the mechanism of ischemia–reperfusion injury has been demonstrated by the protective effect of verapamil, a calcium channel blocker, on

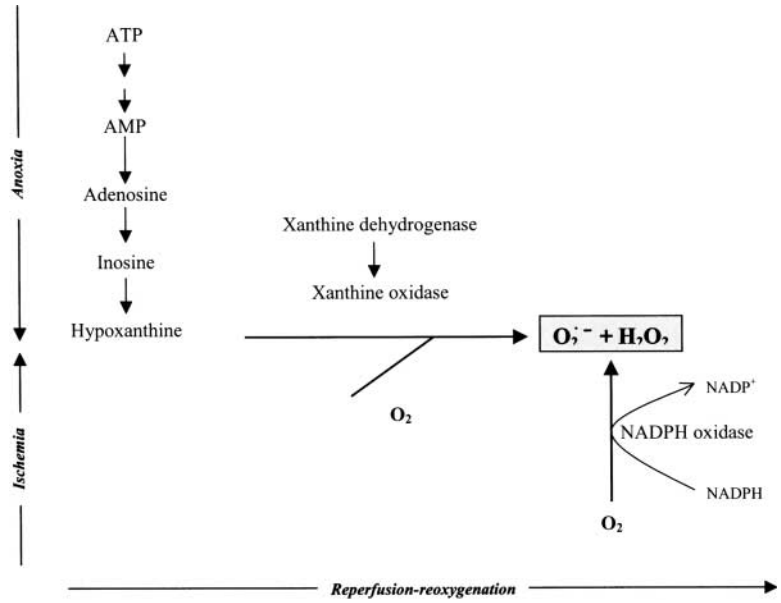

Figure 2. Formation of reactive oxygen species during ischemia–reperfusion and anoxia–reoxygenation of the lung. The lung has to be considered differently than any other organs because it contains oxygen in the alveoli during the ischemic period. Hence, the oxidative stress resulting from ischemia should be distinguished from the oxidative stress resulting from hypoxia.

ischemic injury (42). The effect has been found to be optimal when it is administered to the donor before lung retrieval because it can reduce lipid peroxidation during ischemia and prevent endothelial damage after reperfusion (42, 43). Similar results have been observed with other calcium channel blockers such as nifedipine and diltiazem (44).

Iron Release

Although iron is an essential element for all living cells, it can be highly toxic under pathophysiologic or stress conditions because of its ability to participate in the generation of powerful oxidants. In its free form, iron can cycle between the oxidized (Fe^{3+}) and reduced state (Fe^{2+}), and catalyze the transformation of hydrogen peroxide and superoxide into the highly reactive hydroxyl radical through the Fenton reaction (Figure 3). In addition, free iron facilitates the decomposition of lipid hydroperoxides and accelerates the nonenzymatic oxidation of glutathione. Free iron can be released from ferritin and cytochrome P-450 during ischemia by a number of factors such as acidosis, proteolysis, and superoxide (45–47). In addition to tissue oxidation, iron can be released into the circulation where it can potentially activate platelet aggregation (46, 48).

The importance of iron in promoting ischemia–reperfusion injury has been demonstrated by the increased injury observed in iron-supplemented tissue and by the protection offered by the iron chelator, deferoxamine (45, 49, 50). Recently, a novel iron chelator (desferriexochelin 772SM) has been shown to enhance the effect of a P-selectin antagonist in preventing ischemia–reperfusion injury in a rat liver model (51). Lazaroids, which are aminosteroids inhibiting iron-dependent lipid peroxidation, have also shown good results in protecting the lung from ischemia–reperfusion injury in most studies (52, 53).

$$O_2^{\cdot -} + Fe^{3+} \Rightarrow Fe^{2+} + O_2$$

$$2\,O_2^{\cdot -} + 2\,H^+ \Rightarrow H_2O_2 + O_2$$

$$H_2O_2 + Fe^{2+} \Rightarrow OH^{\cdot -} + OH^{\bullet} + Fe^{3+}$$

$$\text{Net reaction is : } O_2^{\cdot -} + H_2O_2 \Rightarrow O_2 + OH^{\cdot -} + OH^{\bullet}$$

Figure 3. Fenton reaction. Iron can cycle between the oxidized and reduced state, and catalyze the transformation of hydrogen peroxide and superoxide into the highly reactive hydroxyl radical.

Cell Death

Using *in situ* terminal deoxynucleotidyl transferase-mediated deoxyuridine triphosphate nick end-labeling staining as a marker of apoptosis, we have observed in human lung transplantation that lungs with excellent function and good clinical outcome have up to 30% of their cells undergoing apoptosis within 2 hours of reperfusion (54). Similar findings have been observed experimentally after 6 and 12 hours of cold ischemic time in rats, whereas longer ischemic times were associated with a preponderance of necrotic cells in lung tissue (55). In contrast to necrosis, apoptosis is not present during ischemia, its presence peaks rapidly after reperfusion and does not correlate with lung function (54–56).

Apoptosis induction is triggered and modulated by two pathways (Figure 4). The intrinsic pathway involves the mitochondria and is activated by reactive oxygen species, whereas the extrinsic pathway is activated by the ligation of death receptors with their ligands—such as tumor necrosis factor (TNF) with TNF-receptors and Fas with Fas-ligand (57). Although the first pathway is activated in the early phase after reperfusion, the second may take up to several hours to induce apoptosis (58).

Whether apoptotic cells have a deleterious impact on organ function remains controversial. Some authors have demonstrated that ischemia–reperfusion injury of kidneys and hearts is reduced when antiapoptotic agents are injected before reperfusion in mice models of warm ischemia (59, 60). However, other investigators have argued that by blocking the apoptotic molecular cascade after a period of brain ischemia, injured cells may not be able to recover but may instead continue to release proinflammatory agents and subsequently die by necrosis, a mode of cell death more injurious to surrounding tissue (61). We have observed that for a similar amount of dead cells in the transplanted lung, the presence of apoptotic cells was associated with better lung function than if the cells had died by necrosis (62).

CONSEQUENCES OF ISCHEMIA AND REPERFUSION

Upregulation of Molecules on Cell Surface Membrane

Adhesion molecules. Adhesion molecules can be differentiated into three major families, the selectins, the immunoglobulin superfamily, and the integrins. Leukocyte emigration involves the

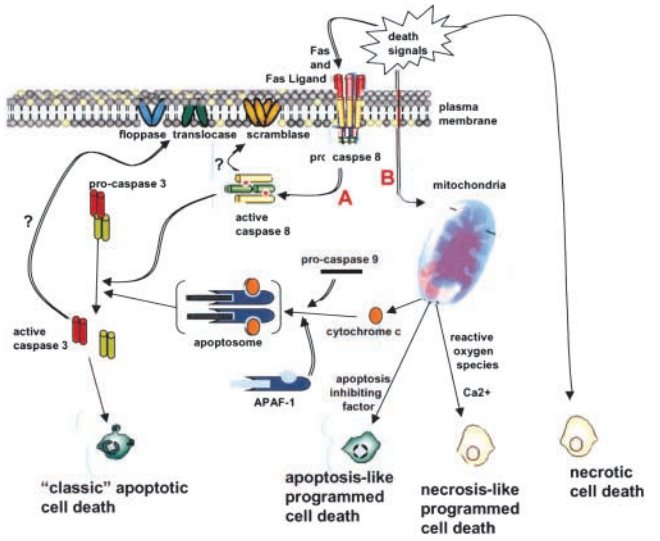

Figure 4. After receiving a death signal, cells can undergo either programmed cell death or necrotic cell death. During the course of the most quiescent form of programmed cell death, "classical" apoptosis, caspase 8 and/or caspase 9 are activated through the external pathway (*A*) or the mitochondrial pathway (*B*), respectively. Both pathways lead to the activation of caspase 3. Cells, which fail to execute the "classical" apoptotic process, may either be salvaged and return to function, or undergo apoptosis-like or necrosis-like programmed cell death.

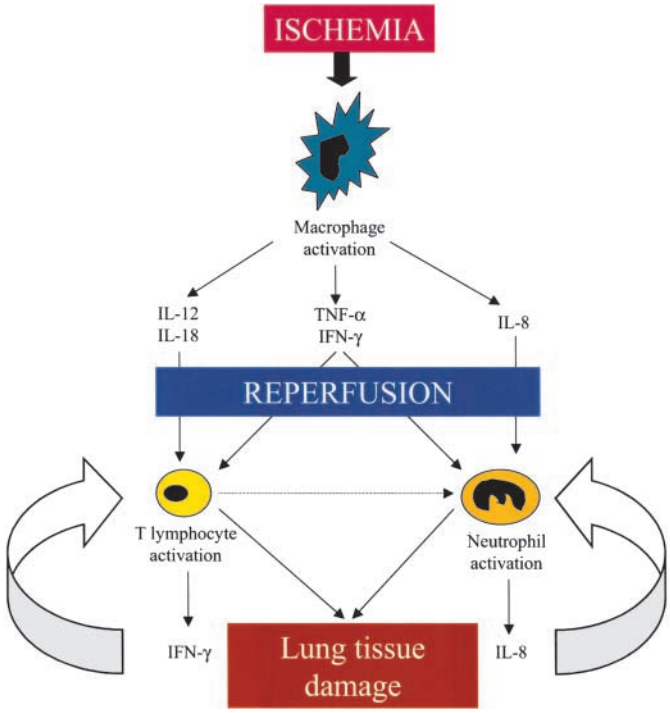

Figure 5. The potential mechanism of interaction between leukocyte activation and cytokine release during ischemia and reperfusion of the lung. Ischemia triggers the activation of passenger macrophages, which release proinflammatory cytokines and mediate reperfusion injury during the early phase of reperfusion. IL-8, IL-12, IL-18, TNF-α, and IFN-γ will then activate recipient neutrophils and T-lymphocytes, which will trigger the delayed phase of reperfusion injury and perpetuate lung tissue damage. T-lymphocytes infiltrate lung tissue more rapidly than neutrophils and may also participate in the activation of recipient neutrophils after reperfusion.

sequential events of rolling, adherence, activation, and extravasation. Leukocyte rolling is dependent on selectin-mediated interaction between endothelial cells (P-selectin and E-selectin) and leukocytes (L-selectin). Firm adherence and activation of leukocytes occur when leukocyte β1-integrin or β2-integrin binds to endothelial cells expressing intercellular adhesion molecule-1 or vascular endothelial adhesion molecule-1, respectively. Finally, leukocyte extravasation into the tissue is dependent on integrin-immunoglobulin interactions, involving intercellular adhesion molecule-1 and platelet endothelial cell adhesion molecule-1.

Adhesion molecules are upregulated on pulmonary endothelial cells during ischemia, and blockade of adhesion molecules such as P-selectin, intercellular adhesion molecule-1, and CD18 (β-chain of the β2-integrin) at the time of reperfusion can reduce lung reperfusion injury (63–67). E-selectin and L-selectin blockade may also be beneficial after several hours of reperfusion when neutrophils have a preponderant role (64, 68, 69). The use of biostable analogs of the oligosaccharides Lewis X and Lewis A, which are potent ligands for selectin adhesion molecules, have also been shown to reduce ischemia–reperfusion injury when given before reperfusion (70–72).

Prothrombotic and antifibrinolytic factors. Hypoxia can induce endothelial cells and macrophages to develop procoagulant properties, which may contribute to the formation of microvascular thrombosis and impede the return of blood flow after reperfusion. *In vitro* studies have shown that endothelial cells subjected to hypoxia can suppress their production of the anticoagulant cofactor thrombomodulin and increase their production of a membrane-associated factor X activator (73). Tissue factor has also been shown to be upregulated on endothelial cells and macrophages by hypoxia and to play a significant role in modulating ischemia–reperfusion injury in a warm ischemia liver model (74). The administration of C1-esterase inhibitor, which inhibits the classic pathway of the complement system as well as the contact phase and the intrinsic pathway of the coagulation system, has been shown to improve early lung function and to reduce ischemia–reperfusion injury in a dog model of single lung transplantation (75). C1-esterase inhibitor has also been used to treat lung graft failure in two patients, but further clinical studies are required to prove its efficiency (76).

Recent experiments have shown that mice placed in a hypoxic environment suppress their fibrinolytic axis by increasing macrophage release of plasminogen activator inhibitor-1 and decreasing macrophage release of tissue-type plasminogen activator and urokinase-type plasminogen activator (77). Additional studies in mice have shown that the beneficial effect of heme oxygenase-1, carbon monoxide, and IL-10 during lung ischemia is partially mediated by their ability to potentiate the fibrinolytic axis (78, 79). The role of prothrombotic and antifibrinolytic agents is a relatively new area of investigation, and further studies are required to determine more precisely the role of fibrinolytic agents in ischemia–reperfusion injury of the lung.

Release of Proinflammatory Mediators

Cytokines. Clinical and experimental studies have shown that ischemia–reperfusion of solid organs such as the kidney (80), liver (81), heart (82), and lung (83) induces a rapid release of proinflammatory cytokines (Table 3). In human lung transplantation, measurable amounts of pro- and antiinflammatory cytokines such as TNF-α, IFN-γ, IL-8, IL-10, IL-12, and IL-18 can be measured in lung tissue during the cold ischemic time and

TABLE 3. SOURCE AND FUNCTION OF CYTOKINES POTENTIALLY INVOLVED IN REPERFUSION INJURY DURING LUNG TRANSPLANTATION

Cytokine	Main Cell Source	Function
TNF-α	Macrophages, lymphocytes	Proinflammatory
IFN-γ	Lymphocytes	Proinflammatory
MCP-1	Immune cells, and lung epithelial cells	Macrophage chemotaxis
IL-1β	Macrophages, fibroblasts	Proinflammatory
IL-2	Lymphocytes	T cell proliferation
IL-6	Macrophages, endothelial cells, and epithelial cells	Proinflammatory
IL-8	Macrophages, epithelial cells, and fibroblasts	Neutrophil chemotaxis
IL-10	Macrophages, lymphocytes	Antiinflammatory
IL-12	Macrophages	T cell activation
IL-18	Macrophages	T cell activation

Definition of abbreviations: IL = interleukin; MCP-1 = macrophage chemoattractant protein-1; TNF = tumor necrosis factor.

after reperfusion (23). Although most cytokine levels decreased after reperfusion, the chemokine IL-8 significantly increased after reperfusion. Donor parameters including oxygen tension, cause of brain death, smoking history, positive sputum cultures, and time on ventilator did not appear to influence the cytokine levels. However, the age of the donor was inversely correlated with the levels of IL-10 after reperfusion. Because IL-10 is an important antiinflammatory cytokine, this may explain why lungs from older donors might be more susceptible to ischemia–reperfusion injury and are associated with higher postoperative mortality rates (84).

A striking relationship between IL-8 levels and graft function can also be observed after human lung transplantation (23). IL-8, which is a potent chemokine-promoting neutrophil migration and activation, rapidly increased after reperfusion. IL-8 levels in lung tissue 2 hours after reperfusion negatively correlated with lung function assessed by the Pa_{O_2}/FI_{O_2} ratio and the mean airway pressure, and positively correlated with the Acute Physiology and Chronic Health Evaluation Score during the first 24 postoperative hours in the intensive care unit (23). In addition, we and others have shown that high levels of IL-8 in donor lung tissue or bronchoalveolar lavage are associated with an increased risk of death from primary graft dysfunction after transplantation (22, 23). The potential importance of IL-8 has also been demonstrated in patients with acute respiratory distress syndrome (85) and in clinical liver transplantation (86). In addition, Sekido and colleagues (87) have shown that the intravenous administration of anti–IL-8 antibody at the beginning of the reperfusion period markedly reduces lung injury and neutrophil infiltration 3 hours after reperfusion in a rabbit model of warm lung ischemia. The potential mechanism of interaction between leukocyte activation and cytokine release in ischemia–reperfusion injury during lung transplantation is shown in Figure 5.

Lipids. Cellular injury is accompanied by a rapid remodeling of membrane lipids with the generation of bioactive lipids that can serve as both intra- and/or extracellular mediators. Phospholipases such as phospholipase A_2, phospholipase C, phospholipase D, and sphingomyelinase play a pivotal role in the generation of these lipid mediators. Among them, phospholipase A_2 has been detected in a wide variety of inflammatory conditions such as ischemia–reperfusion.

The activation of phospholipase A_2 induces the production of platelet-activating factor, an extraordinarily potent mediator of inflammation, and mobilizes arachidonic acid from the membrane lipid pool, which will then be degraded by two major pathways into eicosanoids. The potent vaso- and bronchoconstrictor thromboxane A_2 as well as various prostaglandins (PGs) such as PGD_2, PGE_2, PGF_2, and PGI_2 are produced via the cyclooxygenase pathway. The lipoxygenase pathway, on the other hand, catalyzes leukotrienes such as leukotriene-B_4, C_4, D_4, and E_4, which can increase capillary permeability.

Phospholipase A_2 comprises a constantly growing family of enzymes that have been divided into subgroups based on structural homology and numbered by their order of discovery (88). These enzymes differ in cellular localization and mechanisms of release (88). Recently, Group II secretory phospholipase A_2 has been found to play a major role in acute lung injury. Its level has been found to be elevated in bronchoalveolar lavage fluid from humans with acute respiratory distress syndrome (89), and animal studies have shown that this form of phospholipase A_2 induces acute lung injury after acid aspiration (90), intracheal injection of lipopolysaccharides (91), and after intestinal ischemia–reperfusion injury (92). In addition, Group II secretory phospholipase A_2 has been shown to directly mediate surfactant dysfunction in guinea pigs (91).

To date, only few studies have analyzed the effect of phospholipase A_2 inhibitors in lung ischemia–reperfusion injury (93, 94). However, these inhibitors were not specific for Group II secretory phospholipase A_2, and they may well have blocked the generation of some PGs such as PGE_2 and PGI_2. Specific Group II secretory phospholipase A_2 inhibitors have been developed recently, and further studies should help elucidate this issue in the future (95).

Platelet-activating factor can be released by a wide variety of cells including macrophages, platelets, endothelial cells, mast cells, and neutrophils. It exerts its biological effects by activating the platelet-activating factor receptors, which consequently activate leukocytes, stimulate platelet aggregation, and induce the release of cytokines and the expression of cell adhesion molecules (96). Platelet-activating factor has been difficult to analyze because it is rapidly degraded by tissue and plasma platelet-activating factor acetylhydrolases. Because there are no specific inhibitors for the biosynthesis of platelet-activating factor, most studies have shown the importance of platelet-activating factor by blocking its receptor.

Platelet-activating factor has been shown to play a critical role in initiating lung injury. The most direct evidence was published recently by Nagase and colleagues who demonstrated that platelet-activating factor receptor knockout mice developed less severe acute lung injury after acid aspiration, whereas the overexpression of platelet-activating factor receptor in transgenic mice exaggerated the injury (97). A number of studies have demonstrated that the administration of antagonists of platelet-activating factor during the ischemic storage and after reperfusion reduce ischemia–reperfusion injury and improve lung function (98–100). Similar results have been observed when platelet-activating factor acetylhydrolase was administered to the flush solu-

tion and after reperfusion to increase the degradation rate of the molecule (101).

Arachidonic acid metabolites such as leukotrienes and thromboxanes have been shown to increase in the lung during ischemia–reperfusion injury in a dog model of warm ischemia (102, 103). Thromboxanes may contribute to reperfusion injury and exacerbate lung edema (104). In addition, mast cells, which are known to release large amounts of leukotrienes and histamine, are increased in number after lung ischemia and reperfusion (102). The administration of mast cell membrane-stabilizing agents have also been shown to improve lung function after reperfusion, indirectly demonstrating the importance of leukotrienes (105, 106).

Complement. Studies in ischemia–reperfusion injury of the lung have shown that activation of the complement system after reperfusion may lead to cellular injury through direct and indirect mechanisms (107, 108). Products of complement activation cause smooth muscle contraction and increased vascular permeability and induce degranulation of phagocytic cells, mast cells, and basophils (109). The activated complement fragment C5a is also capable of amplifying the inflammatory response via its chemoattractant properties, its induction of granule secretion in phagocytes, and its ability to induce neutrophil and monocyte/macrophage generation of toxic oxygen metabolites (110). Activation of complement fragments C3 and C5 is also essential for the activation of the complement cascade and the generation of the membrane attack complex, which leads to direct cell lysis (111).

Complement receptor-1 is a natural complement antagonist that has been cloned and the transmembrane portion removed to obtain a soluble form of complement receptor-1. This soluble form suppresses complement activation *in vivo* by inhibiting C3 and C5 convertases, which prevent the activation of both the classic and alternative pathways. In a swine single lung transplant model, we and others have shown that the administration of soluble complement receptor-1 to the recipient before reperfusion reduced lung edema, decreased neutrophil accumulation, and improved oxygenation of the transplanted lung (112, 113). Recently, Stammberger and colleagues have demonstrated in a rat lung transplant model that the administration of a molecule combining soluble complement receptor-1 with sialyl Lewis X, a selectin receptor antagonist, can achieve even better results than the administration of soluble complement receptor-1 alone (72). This study highlights the fact that several pathways may need to be blocked to address the redundancy of the inflammatory system.

Endothelin. Endothelins are powerful vasoconstrictors—10 times more active than angiotensin II or vasopressin (114). Three isoforms have been described in human and other mammals, endothelin-1, endothelin-2, and endothelin-3, of which endothelin-1 has been most extensively studied because it is released by endothelial cells and smooth muscle cells and its expression is predominant in the lung (114). In addition to being a potent vasoconstrictor, endothelin-1 can stimulate the production of cytokines by monocytes/macrophages and promote the retention of neutrophils in the lung (115).

Clinical and experimental studies in lung transplantation have shown that endothelin-1 can accumulate in lung tissue before and during the first few hours after reperfusion (116, 117). High levels of endothelin-1 can then lead to an increased expression of vascular endothelial growth factor and increase vascular permeability (118). The role of endothelin-1 in ischemia–reperfusion injury has been demonstrated by the improvement in lung function when endothelin receptor antagonists are administered before or during reperfusion (100, 119, 120). The administration of endothelin-1 receptor antagonist was associated with a reduction in the expression of inducible NOS and a lower proportion of apoptotic cells in the lung after reperfusion (121).

Leukocyte Activation

Experimental and clinical evidence suggest that ischemia–reperfusion injury occurs in a biphasic pattern. The early phase of reperfusion, which depends primarily on donor characteristics, and the delayed phase of reperfusion, which occurs over the ensuing 24 hours and depends primarily on recipient factors (122). Donor/passenger macrophages are activated during ischemia and mediate the early phase of reperfusion injury, whereas recipient lymphocytes and neutrophils are primarily involved in the delayed phase of reperfusion injury (123–126). The recruitment of lymphocytes and neutrophils into the lung results from the release of cytokines and other mediators before and after reperfusion (23). The potential mechanism of interaction between the cytokine release and the activation of macrophages, lymphocytes, and neutrophils during ischemia–reperfusion injury in lung transplantation is shown in Figure 5.

Macrophages. Alveolar macrophages can produce a large number of cytokines and procoagulant agents *in vitro* in response to oxidative stress (74, 127). In an *in vivo* model of warm ischemia, Eppinger and colleagues demonstrated the importance of TNF-α, IFN-γ, and macrophage chemoattractant protein-1 in the early phase of reperfusion and suggested that alveolar macrophages could have an important role immediately after reperfusion (126). Fiser and colleagues recently confirmed this hypothesis by specifically inhibiting pulmonary passenger macrophages with gadolinium chloride injected into the donor before a period of cold ischemia (125). They showed that lungs, in which passenger macrophages were inhibited, had significantly better function immediately after reperfusion and this was independent of neutrophil inhibition.

Lymphocytes. Evidence suggests that lymphocytes may have an important role in ischemia–reperfusion injury. Richter and colleagues demonstrated that human lung donor parenchyma contains a large number of passenger macrophages and activated lymphocytes, among which T cells and natural killer cells predominate (128). Similar findings have been observed in liver transplantation with a large number of activated CD8+ T cells, $\gamma\delta$ T cells, and natural killer cells being transmitted with the liver graft to the recipient (129–132). Although the role of these passenger lymphocytes has not been extensively explored in the setting of ischemia–reperfusion injury, recent studies have demonstrated that nude mice, CD4+/CD8+ knockout mice, and CD4+ depleted mice have significantly less severe reperfusion injury of the liver and kidney than control mice (123, 133, 134). In addition, Clavien and colleagues have shown in an *ex vivo* model of liver reperfusion that cold preservation induces an increase in lymphocyte adherence within the first 10 minutes of reperfusion and that these infiltrating lymphocytes could be important in mediating graft dysfunction (135). We have recently demonstrated by flow cytometry in a rat lung transplant model that recipient CD4+ T cells rapidly accumulate in lung tissue after reperfusion. CD4+ T cells then upregulate CD25, a potential marker of activation, and participate in ischemia–reperfusion injury in the delayed phase of reperfusion by releasing IFN-γ (136).

Neutrophils. Neutrophils progressively infiltrate the transplanted lung during the initial 24 hours of reperfusion (137). Although they certainly play an important role in perpetuating reperfusion injury, their function in the early phase of reperfusion is less predominant. Using an isolated rat lung perfusion model, Deeb and colleagues demonstrated that the addition of neutrophils to the perfusion system was not necessary for the induction of reperfusion injury after a period of warm ischemia

TABLE 4. COMPOSITION OF PRESERVATION SOLUTIONS

Composition	Euro-Collins	University of Wisconsin	Celsior	LPD-Glucose (Perfadex)
Sodium	10	28	100	138
Potassium	115	125	15	6
Chloride	15	0	41.5	142
Magnesium	0	0	13	0.8
Sulfate	0	4	0	0.8
Phosphate	57.5	25	0	0.8
Calcium	0	0	0.26	0.3
Bicarbonate	10	5	0	1
Dextran 40	0	0	0	50
Glucose	3.5	0	0	0.9
Raffinose	0	30	0	0
Lactobionate	0	100	80	0
Glutathione	0	3	3	0
Adenosine	0	5	0	0
Allopurinol	0	1	0	0
Pentafraction	0	50	0	0
Glutamate	0	0	20	0
Histidine	0	0	30	0
Mannitol	0	0	60	0

Definition of abbreviation: LPD = low-potassium dextran.
All units are in mmol/L except Dextran 40, glucose, and pentafraction, which are in g/L.

(138). Following this line of experimentation, the same group demonstrated that reperfusion injury exhibits a bimodal pattern, consisting of neutrophil-independent events during the first few hours of reperfusion and of neutrophil-mediated events after 4 hours of reperfusion (124). Further studies with specific antibodies against neutrophils have confirmed these findings and show that other leukocytes such as macrophages have a more important role in the early phase of reperfusion (125, 139, 140).

STRATEGIES TO PREVENT LUNG DYSFUNCTION

Method of Lung Preservation and Reperfusion

*Lung preservation solution. C*urrently, the vast majority of centers have adopted a single pulmonary artery flush to preserve the lungs because of its technical simplicity (141). Preservation solutions that have been studied include mainly intracellular-type solutions (high K^+, low Na^+ solutions) such as Euro-Collins and University of Wisconsin solution, and extracellular-type solutions (low K^+, high Na^+ solutions) such as low-potassium dextran (LPD) and Celsior (Table 4). Historically, Euro-Collins was developed for kidney preservation, University of Wisconsin for liver preservation, and Celsior for heart preservation. LPD is the only solution that has been specifically developed for lung preservation. LPD-glucose solution (Perfadex; Vitrolife, Goteborg, Sweden) has been approved for clinical practice, and many centers have switched to the use of LPD-glucose as their clinical lung preservation solution.

The concept of using a modified extracellular fluid solution to preserve the lung was developed in Japan in the mid-1980s. Fujimura and colleagues demonstrated that a modified extracellular solution was superior to the intracellularly based Euro-Collins solution for prolonged lung allograft preservation (5). After these experiments, Keshavjee and colleagues demonstrated that the association of low-potassium (4 mmol/L) and dextran 40 reliably and reproducibly provided significantly better lung function than Euro-Collins after 12 hours of ischemic time in a canine single lung transplantation model (6). The same group further demonstrated that both dextran 40 and the low-potassium concentration were critical components of the LPD solution (142, 143). After these experiments, Date and colleagues observed that the addition of 1% of glucose to the LPD solution provided a substrate for the aerobic metabolism that takes place in the inflated lungs and allowed safe extension of the ischemic time to 24 hours in dogs (28). Steen and colleagues, as well as other groups, repeated these experiments and found safe pulmonary preservation for 12 to 24 hours with LPD-glucose in porcine, canine, and primate models of left single and double lung transplantation (144–147).

Ultrastructural analyses have shown significantly better conservation of lung integrity with extracellular-type preservation solutions than with intracellular-based solutions (148). Better ultrastructural appearance may not translate into better lung function after short ischemic periods, but after prolonged ischemic time, i.e., 8 hours or longer, lungs preserved with LPD solution have always shown better lung function than lungs preserved with intracellular-type preservation solutions (149–151).

Celsior, which is an extracellular-type preservation solution specifically developed for the heart, has also been shown to achieve satisfactory results in lung preservation (152–155). Some authors have suggested that Celsior might even be better than LPD in lung preservation (156, 157). Celsior, in contrast to LPD, contains high amounts of reduced glutathione, histidine, and lactobionate, which may play an important role in the prevention of free radical injury (158). Future studies should determine if the addition of antioxidants and/or radical scavengers could further enhance the quality of preservation with LPD solution.

As previously mentioned, the beneficial effect of preservation with LPD is due to the combination of both a low potassium concentration and the presence of dextran (142). The low potassium concentration may be less detrimental to the functional and structural integrity of endothelial cells, which may thus lead to less production of oxidants (34, 37, 38) and release of less pulmonary vasoconstrictors (143, 159–161). Dextran 40 is a macromolecule with an average molecular weight of 40,000 D exerting an oncotic pressure of 24 mm Hg when diluted at a concentration of 5% (162). Dextran improves erythrocyte deformability, prevents erythrocyte aggregation, and induces disaggregation of already aggregated cells, in addition to an antithrombotic effect induced by coating endothelial surfaces and platelets (142). These effects improve pulmonary microcirculation and preserve the endothelial–epithelial barrier, which may secondarily prevent the no-reflow phenomenon and reduce the degree of water and protein extravasation at the time of reperfusion (163). In addition, *in vitro* studies have demonstrated that LPD solution

TABLE 5. COMPARISONS OF LOW-POTASSIUM DEXTRAN GLUCOSE AND EURO-COLLINS IN CLINICAL LUNG TRANSPLANTATION

	Number of Patients		30-d Mortality (%)			Initial Graft Function		
Reference	LPDG	ED	LPDG	EC	p Value	LPDG	EC	p Value
Fischer and coworkers (174)	46	48	6.5	10.4	0.82	370 ± 133*	310 ± 134*	0.017
Struber and coworkers (175)	57	63	8	14.2	0.35	34 ± 11†	30 ± 10†	0.04
Muller and coworkers (176)	32	48	6	12	0.36	159 ± 145‡	242 ± 265‡	0.028

Definition of abbreviations: EC = Euro-Collins; LPDG = low-potassium dextran glucose.

* Pa_{O_2}/Fi_{O_2} ratio.

† Lung compliance (ml/mm Hg).

‡ Alveolar-arterial oxygen gradient.

can (*1*) exert a suppressive effect on polymorphonuclear chemotaxis (164), (*2*) be less cytotoxic for Type II pneumocytes (165, 166), and (*3*) maintain better activity of alveolar epithelial Na^+/K^+-ATPase function during the cold ischemic period when compared with Euro-Collins or University of Wisconsin solutions (167). These effects may result in less lipid peroxidation, and better surfactant function at the end of the ischemic time and after reperfusion (168, 169).

Raffinose is a trisaccharide sugar with a mean molecular weight of 594 D that prevents pulmonary water diffusion and cellular swelling in a more efficient way than do monosaccharides and dissaccharides (170). Raffinose has been demonstrated to be one of the essential components of the University of Wisconsin solution when compared with Euro-Collins solution in an *ex vivo* rat model of lung graft reperfusion (171). The addition of raffinose (30 mmol/L) to LPD-glucose has been shown to reduce the peak airway pressures and to improve oxygenation of the transplanted lung after 24 hours of ischemic time in a rat single lung transplant model (172). The addition of raffinose to the LPD-glucose solution can result in less tissue damage and better cellular integrity at the end of the ischemic time (173).

Clinical reports from three centers have compared the effect of LPD-glucose (Perfadex; Vitrolife, Uppsala, Sweden) with an historical control group of lungs preserved with Euro-Collins (174–176). All three reports showed significantly better lung function on arrival in the intensive care unit and a trend toward lower 30-day mortality with LPD-glucose (Table 5). An additional report demonstrated that, after adjustment for graft ischemic time, extracellular-type preservation solutions were associated with a decreased incidence of primary graft failure after lung transplantation when compared with intracellular-type preservation solutions (153). Currently, the limitation in extending the ischemic time is more often related to the increasing use of nonideal lung donors rather than to poor lung preservation (14). In our experience, the ischemic time with LPD preservation has been successfully extended up to 12 hours with excellent donors.

In conclusion, clinical and experimental evidence suggests that LPD-glucose is currently the preservation solution of choice for lung transplantation. Continuous refinement is nevertheless still required, and in the future raffinose as well as other components such as reduced glutathione, histidine, and/or lactobionate may be added to the base solution to enhance the quality of preservation.

Volume, pressure, and temperature of flush solution. Several studies have analyzed the effect of pressure, volume, and temperature of the preservation solution during flushing. In 1986, after observing that flush-perfusion at low flow rates (3–5 ml/kg/min) achieved poor results after moderate- to long-term storage, Haverich and colleagues performed a study where they compared a low perfusate volume given at a low flow rate (20 ml/kg given in 6 min) with a low perfusate volume given at a high flow rate (20 ml/kg given in 1.3 min) and a high perfusate volume given at a high flow rate (60 ml/kg given in 4 min) (177). They found that lungs flushed with a high perfusate volume given at a high flow rate resulted in significantly better cooling of the lungs and better lung function after reperfusion. This study has never been repeated with additional groups below and/or above 60 ml/kg. However, Steen and colleagues have suggested the use of 150 to 180 ml/kg of LPD-glucose to obtain a more uniform and clean washout of the anterior part of the lungs, which is usually less uniformly flushed because of the pressure gradient in the supine position (144, 145).

More recently, Sasaki and colleagues systematically analyzed the influence of the pulmonary artery pressure during the flushing period on lung preservation (178). They observed that flushing pressures of 10 to 15 mm Hg were associated with complete flushing of the pulmonary vascular beds and achieved significantly better lung function after reperfusion than flushing pressures of 5, 20, and 25 mm Hg in an *ex vivo* rabbit lung reperfusion model. They also observed that flushing pressures of 20 mm Hg or higher were associated with significantly less endogenous nitric oxide (NO) production, which may have had a detrimental effect on the lungs after reperfusion (179).

The temperature of the flush solution has been the subject of some discussion. Andrade and colleagues have observed in an isolated rat model that hypothermic pulmonary arterial flushing with 60 ml/kg of Euro-Collins solution at a pressure of 15 mm Hg can transiently increase the capillary filtration coefficient and induce persistent lung damage with increased wet to dry weight ratio and biochemical surfactant changes (180). This finding could be explained by two mechanisms, one being the absence of an oncotic component in the Euro-Collins solution to maintain adequate fluid balance between the intravascular and extravascular compartments and the second being the effect of hypothermia on endothelial cells. The use of a cold flushing solution may induce injuries to the alveolocapillary membrane, which could potentially enhance the abnormal relaxation of the vascular endothelium after several hours of ischemia (181, 182).

Wang and colleagues showed that a temperature of 23°C for the flush solution was associated with a lower pulmonary vascular resistance during flushing and more uniform washout of the pulmonary vascular beds than a temperature of 10°C (183). In addition, several authors have observed that lung function was significantly better after reperfusion if the lungs were initially flushed with a temperature of 15 to 20°C instead of 10°C or lower (183–186). However, all these studies were performed in small animals and surface cooling of the inflated lungs was probably more rapid than with larger lungs, thus limiting the period of warm ischemia until core cooling of the lungs was achieved. Steen and colleagues have recommended that if the temperature of the flush solution is kept at room temperature,

then the lungs should be maintained in a collapsed state during cold storage to reduce the core temperature quicker by avoiding the insulating effect of air (145).

Ultrastructural analysis of the lungs at various time points during the preservation period shows that the injuries induced by the flush itself appear to be minimal when compared with the insult induced by ischemia on the endothelial–epithelial barrier (187, 188). Hence, despite some potential injuries induced by cold flushing, it appears that this contribution to the total injury is minimal when compared with the insult induced by ischemia. Flushing the lungs with a hypothermic preservation solution should therefore still be recommended.

Inflation, oxygenation, and storage temperature. Although atelectatic lungs can be preserved at cold temperature for 5 to 6 hours in humans and for up to 24 hours in pigs (189, 190), there have been a large number of experiments since the early 1970s suggesting that preservation of the lung is improved when they are inflated with oxygen (191). Expansion of the lungs with oxygen during the ischemic period protects the lung from injury by three mechanisms: (*1*) it maintains some aerobic metabolism, (*2*) it preserves the integrity of pulmonary surfactant, and (*3*) it preserves epithelial fluid transport.

During ischemia, lungs inflated with air are still able to consume oxygen and to produce energy through the more efficient aerobic metabolic pathway, which prevents the accumulation of cellular metabolites and delay cell death (192, 193). Hence, alveolocapillary membranes are better preserved and the amount of total protein and lactate dehydrogenase in the bronchoalveolar lavage fluid are significantly lower than if the lungs were preserved in a complete atelectatic state or inflated with 100% nitrogen (193, 194). As well, static pulmonary compliance and surfactant secretion remain significantly better if the lungs are preserved in an inflated instead of a deflated state (193–195). In addition, Sakuma and colleagues have recently demonstrated that lung deflation decreases alveolar fluid clearance, whereas fluid clearance was maintained in inflated lungs, independently of the presence of oxygen (196).

Atelectasis is also associated with higher pulmonary vascular resistance and poorer distribution of the lung preservation solution (197, 198). Hence, a recruitment maneuver before flushing the lungs is certainly an effective measure. However, overdistension of the lung by either static inflation, high V_T, or high positive end-expiratory pressure has been shown to be detrimental during mechanical ventilation, and there is evidence suggesting that hyperinflation during storage increases the pulmonary capillary filtration coefficient (199–201). In rat experiments, we and others have observed that lung inflation during storage should be limited to 50% of the total lung capacity or to an airway pressure of 10 to 15 cm H_2O to avoid barotrauma (195, 202). In our clinical practice, we perform a recruitment maneuver to fully re-expand the lung before flushing them, and we ventilate the lungs with a V_T of 10 ml/kg and a positive end-expiratory pressure of 5 cm H_2O during the flushing period. The lungs are then inflated with a sustained peak airway pressure of a maximum of 15 to 20 cm H_2O before tracheal crossclamping in an effort to obtain complete lung expansion but avoid overdistension. It should be noted that overinflated lungs may be exposed to significantly more overdistension if they are transported in airplanes because of the potentially lower atmospheric pressure during the flight.

Oxygen is required during storage to support aerobic metabolism (192, 202, 203). However, an $F_{I_{O_2}}$ greater than 50% may be associated with more lipid peroxidation during lung storage (29, 192, 202, 204). Hence, inflation with an oxygen fraction of 50% or less is usually recommended in clinical practice.

Several experimental studies have shown that lung preservation at 10°C achieved better results than preservation at 4 or 15°C and higher (40, 202, 205, 206). However, these findings were not confirmed by other groups (207, 208). In addition, lungs preserved at 10°C require a greater amount of metabolic substrate, and the risk of lung injury can increase extremely rapidly if the temperature rises above 10°C during preservation (204). Hence, if a 10°C preservation temperature were used, the temperature of the organs would have to be constantly monitored because of the narrow margin of safety. For this reason, we recommend preservation of the lungs at a temperature ranging between 4 and 8°C (Table 6).

TABLE 6. CURRENT RECOMMENDATIONS FOR LUNG PRESERVATION FROM THE TORONTO LUNG TRANSPLANT GROUP

Volume of flush solution, ml/kg	50–60
PA pressure during flush delivery, mm Hg	10–15
Temperature of flush solution, °C	4–8
Lung ventilation	V_T: 10 ml/kg and PEEP: 5 cm H_2O
Oxygenation	$\leqslant$ 50% $F_{I_{O_2}}$
Lung inflation (airway pressure), cm H_2O	15–20
Storage temperature, °C	4–8

Definition of abbreviations: PA = pulmonary artery; PEEP = positive end-expiratory pressure.

Retrograde flush and late reflush. Retrograde flush, which refers to the administration of the flush solution through the left atrial appendage or the pulmonary veins, and drainage through the pulmonary artery, has been described for lung and heart–lung transplantation (209, 210). The technique adds the potential advantages of flushing both the bronchial and pulmonary vessels and of limiting the effect of pulmonary arterial vasoconstriction on the distribution of the flush solution. Experimentally, a retrograde flush has been found to improve lung preservation when compared with an anterograde flush. This effect was attributed to more effective clearance of red blood cells within the capillaries, better distribution of the flush solution along the tracheobronchial tree, and less severe impairment of surfactant function (157, 197, 211, 212). However, despite the retrograde flush, pretreatment with PGE_1 was still helpful in improving pulmonary dynamic compliance after reperfusion (213). After these results, several groups have adopted a combined procedure with an anterograde flush through the pulmonary artery followed by a retrograde flush through each of the pulmonary veins *in situ* while the lungs are still ventilated (214, 215).

Late reflush was initially described in kidney transplantation and refers to the administration of a second flush immediately before implantation of the graft (216). This method has been shown to wash out inflammatory agents and to improve post-transplant graft function by limiting cell damage after reperfusion (116, 216–218). The University of North Carolina has developed a specific extracellular solution for late reflush (Carolina rinse solution) to replenish important substrates and provide antioxidants and vasodilators to the graft before reperfusion to limit cell injury (219). This solution has been shown to be superior to Euro-Collins for late reflush in an *ex vivo* model of lung reperfusion (218). In clinical lung transplantation, Venuta and colleagues have completed a study with 14 patients demonstrating that the addition of a late retrograde reflush with LPD-glucose to an anterograde flush was associated with improved lung function when compared with an anterograde flush only (215). Future studies are required to determine whether the improvement in lung function that they observed was due to the retrograde flush and/or to the late reflush effect.

Low reperfusion pressure and protective ventilation. The pul-

TABLE 7. RANDOMIZED, DOUBLE-BLINDED, PLACEBO-CONTROLLED CLINICAL TRIALS FOR ISCHEMIA–REPERFUSION INJURY IN HUMAN LUNG TRANSPLANTATION

Reference	Mechanism Involved	No. of Patients	Main Findings
Meade and coworkers (264)	Inhaled NO	84	No difference in oxygenation, extubation time, length of ICU stay, or 30-d mortality
Keshavjee and coworkers (273)	Complement inhibitor	59	Greater proportion of patients extubated within 24 h
Wittwer and coworkers (278)	PAF antagonist	24	Trend toward better $P(a-a)O_2$ during the initial 32 h of reperfusion

Definition of abbreviations: ICU = intensive care unit; NO = nitric oxide; PAF = platelet-activating factor; $P(A-a)O_2$ = alveolar-arterial oxygen gradient.

monary artery pressure during the initial 10 minutes of reperfusion is of prime importance. Indeed, the endothelial permeability is transiently elevated during this early phase of reperfusion, and irreversible lung damage, pulmonary edema, and leukocyte sequestration can occur if the lung is rapidly reperfused after a period of ischemia (220–222). Progressive reintroduction of blood flow over a 10-minute period has been shown to reduce lung injury and to improve function of the transplanted lung (220, 222, 223). A specially designed pulmonary artery clamp with a larger number of ratchets can be used to gradually increase blood flow during reperfusion of the lung over a 10-minute period. When a transplant is done on cardiopulmonary bypass, the rate of reperfusion can be controlled with the pump.

Although mechanical ventilation is essential for patients undergoing lung transplantation, a number of animal and clinical studies have shown that mechanical ventilation can worsen preexisting lung injury and produce ventilator-induced lung injury (199). The effect of different modes of ventilation in the early period after lung transplantation has not been explored clinically. However, we have recently demonstrated in a rat single lung transplant model that injurious ventilation with high V_T and low positive end-expiratory pressure significantly worsened lung function after 3 hours of reperfusion when compared with a protective mode of ventilation (224). In our practice, we have incorporated a protective ventilation strategy during the initial period of reperfusion. The newly implanted lung allograft is gently reinflated with a sustained airway pressure of 20 cm H_2O before reperfusion and then ventilated with an FI_{O_2} of 50%, positive end-expiratory pressure of 5 cm H_2O, and pressure-control ventilation limiting the peak airway pressures to 20 to 25 cm H_2O (225, 226).

Clinical Evidence in Prevention and Treatment of Lung Reperfusion Injury

Clinical studies in the prevention and treatment of reperfusion injury during lung transplantation are limited. Only three randomized, double-blinded, placebo-controlled trials have been reported in the literature (Table 7). Most publications are limited to personal experience and case series.

Nitric oxide. NO is a messenger gas molecule with many physiologic effects, including potent vasoregulatory and immunomodulatory properties (227). It is produced by a family of enzymes, i.e., NOSs that catalyzes the conversion of L-arginine to L-citrulline with the help of five cofactors. NO then stimulates soluble guanylyl cyclase, which catalyzes the formation of cyclic 3′-5′-guanosine monophosphate, which in turn regulates protein phosphorylation, ion channel conductivity, and phosphodiesterase activity.

At least two isoforms of NOSs are constitutively expressed. One is restricted to the endothelium (endothelial NOS or NOS-III), whereas the other predominates in neuronal tissue (neuronal NOS or NOS-I). An inducible form of NOSs is found in a wide variety of cell types, such as macrophages, epithelial, and endothelial cells (inducible NOS or NOS-II). Endothelial NOS and neuronal NOS are responsible for many of the beneficial properties of NO such as reduced vascular tone and prevention of neutrophil and/or platelet adhesion, whereas inducible NOS can be induced by a number of stimuli and has been implicated in the killing of exogenous organisms as well as in the pathophysiology of vascular collapse with septic shock, impaired hypoxic vasoconstriction, and tissue injury (228).

Endogenous NO has been found to be decreased after ischemia and reperfusion of the lung in human and animal studies (229–231). This finding may be associated with an increased expression of the enzyme endothelial NOS, which may suggest that endogenously produced NO may be rapidly destroyed by oxygen-free radicals after reperfusion and/or that ischemia–reperfusion may induce the release of endothelial NOS inhibitors in the lung (228, 229).

Multiple strategies have been developed to compensate for the fall in endogenous NO during lung transplantation. These strategies have been applied to the donor and/or to the recipient and have targeted each step of the pathway described previously, including the administration of the upstream precursor molecule L-arginine (232, 233), methods to increase the downstream effector molecule cyclic 3′-5′-guanosine monophosphate (229, 234), and the administration of exogenous NO. Exogenous NO has been given directly by inhalation (inhaled NO) (235–238) or indirectly by infusion of a NO donor, such as FK409 (239, 240), nitroprusside (241–243), glyceryl trinitrate (244), nitroglycerin (245–247), or SIN-1 (248). Other strategies have been directed at increasing the activity of the enzyme NOS by the addition of one of its cofactors (tetrahydrobiopterin) to the preservation solution (249) or by transfecting the donor with an adenovirus containing endothelial NOS before lung retrieval (250).

These strategies have been shown to be effective experimentally and to have a prolonged effect if they are initiated before the onset of reperfusion injury (236–238, 251). However, NO can react with superoxide anion and form peroxynitrous acid, which is a highly reactive oxidant that can induce the release of endothelin-1, damage alveolar Type II cells even after a short period of ischemic time, and cause structural and functional alteration of surfactant (252). Hence, this reaction may explain why some authors have shown that NO administered during ischemia and/or early reperfusion may be ineffective or even harmful, in particular when it is given with a high FI_{O_2} immediately after reperfusion (235, 253–256).

Inhaled NO has been useful clinically to treat ischemia–reperfusion injury of the lung because it can improve ventilation–perfusion mismatch and decrease pulmonary artery pressures without affecting systemic pressures (257–261). However, the role of inhaled NO in *preventing* ischemia–reperfusion injury during clinical lung transplantation remains controversial. Ardehali and colleagues have shown that the application of inhaled NO to 28 consecutive recipients after lung transplantation did not prevent the occurrence of primary graft failure (262). How-

ever, Thabut and colleagues reported that the administration of inhaled NO in combination with pentoxifylline at the time of reperfusion in 23 patients reduced the incidence of ischemia–reperfusion injury when compared with two historical control groups (263). Our group has recently completed a randomized, double-blinded, placebo-controlled trial of inhaled NO administered to lung transplant recipients, starting 10 minutes after reperfusion for a minimum of 6 hours (264). Among a total of 84 recipients, we observed no significant differences in the immediate oxygenation, time to extubation, length of stay in the intensive care unit, or 30-day mortality (Table 7). In conclusion, although inhaled NO therapy can be useful in improving gas exchange in cases of established reperfusion injury, the role for NO in the prevention of ischemia–reperfusion injury has yet to be demonstrated in clinical lung transplantation.

Prostaglandins. PGE_1 has been shown to be beneficial when added to intracellular preservation solutions such as Euro-Collins and University of Wisconsin (184, 207, 265). The beneficial effect of PGE_1 is attributed to its vasodilator properties that may lead to better distribution of the preservation solution and to the stimulation of cyclic-3′,5′adenosine monophosphate–dependent protein kinase during the cold ischemic time, which may reduce endothelial permeability, neutrophil adhesion, and platelet aggregation on reperfusion (265).

The continuous intravenous administration of PGE_1 to the recipient during the early phase of reperfusion has been shown to reduce ischemia–reperfusion injury of the lung in animal models of lung transplantation (266, 267). Although this effect can be partially attributed to the vasodilator property of PGE_1 during the initial 10 minutes of reperfusion (268), after a longer period of reperfusion a continuous PGE_1 infusion achieved significantly better lung function than other vasodilator agents such as prostacyclin and nitroprusside (269). Hence, the continuous infusion of PGE_1 clearly has a beneficial role on ischemia–reperfusion injury, some of which can be attributable to its antiinflammatory effects. Indeed, the continuous administration of PGE_1 during reperfusion is associated with a shift from a proinflammatory cytokine profile including TNF-α, IFN-γ, and IL-12 to an antiinflammatory cytokine profile with increased IL-10 in a rat lung transplant model (266). Other effects of PGE_1, such as its antiaggregant action on platelets, may also potentially explain its beneficial role (270, 271).

On the basis of experimental evidence, some centers routinely use an infusion of PGE_1 during the postoperative period after lung transplantation, whereas others reserve PGE_1 infusion for the treatment of severe reperfusion injury (272). Prospective randomized trials are required to determine whether routine PGE_1 has an overall beneficial effect in the postoperative course during clinical lung transplantation. Such studies may use the newly developed aerosolized form of PGE_1, which has been shown experimentally to reduce ischemia–reperfusion injury of the lung without having the systemic hypotensive side effect of intravenous PGE_1 (243).

Complement inhibition. After the successful experimental application of the complement inhibitor, soluble complement receptor-1 (112), we performed a multicenter randomized, double-blinded, placebo-controlled trial that included 59 lung transplant recipients (273, 274). Among 29 patients receiving a dose of soluble complement receptor-1 before reperfusion, 14 (48%) were extubated within 24 hours, which was significantly better than in the control arm with only 6 patients extubated out of a total of 30 (20%). In addition, the overall duration of mechanical ventilation and length of intensive care unit stay tended to be shorter in the group receiving the therapeutic drug (Table 7). The effect of soluble complement receptor-1 appeared to be stronger in the group of patients who underwent cardiopulmonary bypass, but the results did not reach statistical significance because of the small number of patients (n = 12). This likely reflects the added potential benefit of inhibiting complement activation related to cardiopulmonary bypass. The results of Phase III trials in cardiac surgery should confirm whether complement inhibition with the soluble complement receptor-1 is protective when patients are placed on cardiopulmonary bypass circuits (275).

Cardiopulmonary bypass is known to activate the release of mediators and to stimulate the activation of complement factors. We therefore limit the use of cardiopulmonary bypass to recipients with pulmonary hypertension and to those who cannot tolerate unilateral ventilation or perfusion (276). Some centers, however, routinely perform lung transplantation using cardiopulmonary bypass with good results (277). One potentially beneficial effect of cardiopulmonary bypass is the ability to reperfuse the newly implanted lungs with controlled pulmonary artery pressures over a prolonged period of time.

Antagonist of platelet-activating factor. Wittwer and colleagues have recently reported their clinical experience with an antagonist of platelet-activating factor (BN52021, Ginkolide B) in 24 patients randomly assigned to a high dose of antagonist in the flush solution and after reperfusion (n = 8), a low dose of antagonist in the flush solution and after reperfusion (n = 8), and a control group (n = 8) (278). They observed a trend toward a better alveolar-arterial oxygen gradient within the first 32 hours after reperfusion and better chest X-ray score in the two groups receiving the antagonist (Table 7). In clinical kidney transplantation, a randomized, double-blinded, single center trial with 29 recipients showed a significant reduction in the incidence of primary graft failure after transplantation in the group of patients receiving the antagonist of the platelet-activating factor (279). These promising results from single centers should encourage large multicenter trials.

Surfactant therapy. Pulmonary surfactant consists of approximately 90% lipids, mainly saturated phosphatidylcholine, and approximately 10% proteins, including the surfactant apoproteins-A, B, C, and D. Type II pneumocytes synthesize, store, secrete, and to a large extent recycle pulmonary surfactants (280). The surfactant pool can be separated into the intracellular surfactant, represented by the lamellar bodies of Type II pneumocytes, and the intra-alveolar surfactant, which consists of several subtypes, including freshly secreted lamellar body-like forms, tubular myelin, the alveolar lining layer, and small unilamellar vesicles. Bronchoalveolar lavage studies usually refer to two subfractions of the intra-alveolar surfactant, large aggregates or heavy forms, largely corresponding to tubular myelin,which are highly active in decreasing the alveolar surface tension, and small aggregates or light forms, largely corresponding to degraded and inactive small unilamellar vesicles (281).

Surfactant dysfunction has been shown to occur during ischemia–reperfusion injury of the lung (281, 282). Ultrastructural analyses have shown an increase in the small to large surfactant aggregate ratio, an increase in sphingomyelin, and a decrease in phosphatidylglycerol and phosphatidylcholine, which correlated with decreased pulmonary compliance and lung oxygenation (281, 283, 284). These changes were also associated with a deficit in surfactant adsorption and a decrease in surfactant apoprotein-A (284–286). Alveolar surfactant dysfunction may occur despite the absence of plasma protein leakage or changes in lamellar bodies of Type II pneumocytes (281, 287). The dysfunction is most likely the result of numerous insults occurring during lung storage such as the production of phospholipase A_2, mechanical distorsion, altered phospholipid metabolism, reduced production of surfactant apoprotein-A, and/or accumulation of C-reactive protein (284, 285, 288). Although some alterations in

surfactant can be observed immediately after pulmonary artery flushing, most of the alterations have been shown to progressively increase during ischemic storage and to be significantly less with extracelullar-type preservation solutions (282, 283, 286, 288).

Experimental studies and clinical observations have found that exogenous surfactant therapy can improve pulmonary function after lung transplantation (289–294). The administration of exogenous surfactant is associated with a higher amount of total surfactant phospholipid, a higher percentage of the heavy subtype of surfactant, a normalized percentage of phosphatidylcholine, and a higher amount of endogenous surfactant apoprotein-A, which has been shown to improve oxygenation and compliance of the transplanted lung (291, 292). Exogenous surfactant has also been shown to enhance immediate recovery from transplantation injury and to be persistently beneficial for endogenous surfactant metabolism up to one week after transplantation (295). Exogenous surfactant given to the donor before retrieval has been associated with better and more reliable results than when it was administered just before or immediately after reperfusion (290, 291, 293, 296). Struber and coworkers have successfully used a nebulized synthetic surfactant in several patients with reperfusion injury after lung transplantation (294). They observed rapid improvement in pulmonary compliance and in alveolar-arterial oxygen gradient, leading to extubation within a few days after the application (294). In the future, these promising results need to be confirmed by a prospective, randomized trial.

Future Strategies

Heme oxygenase pathway. Heme oxygenases catalyze the conversion of heme into biliverdin, carbon monoxide, and free iron. The free iron is then sequestred into ferritin, whereas biliverdin is metabolized into bilirubin. Heme oxygenase consists of three isoforms, the inducible heme oxygenase-1, also known as heat shock protein 32, and two constitutive isoforms, heme oxygenase-2, and heme oxygenase-3. Heme oxygenase-1 activity has been shown to provide potent cytoprotective effects against a variety of agents causing oxidative stress in vivo and in vitro (297). This finding is also supported by recent observations from heme oxygenase-1 deficient mice and humans exhibiting increased susceptibility to oxidative stress among other abnormalities (298, 299).

Heme oxygenase-1 is rapidly upregulated on reperfusion, and its overexpression has been shown to confer marked cytoprotection against ischemia–reperfusion injury. Indeed, after a period of myocardial ischemia, transgenic mice overexpressing heme oxygenase-1 have better cardiac function and reduced infarct size when compared with wild-type mice, whereas heterozygous ($HO\text{-}1^{+/-}$) mice with a 40% reduction in the expression of heme oxygenase-1 display reduced ventricular recovery and increased infarct size when compared with wild-type mice (300, 301). Similar findings have been observed in a liver model of ischemia–reperfusion injury when the expression of heme oxygenase-1 was induced by cobalt protoporphyrin or by adenoviral-mediated heme oxygenase-1 gene transfer (302).

The mechanism by which selective overexpression of heme oxygenase-1 confers protection against ischemia–reperfusion injury remains poorly understood and may be mediated by each of the three by-products generated by the enzyme. Free iron activates the production of ferritin, which may mediate cytoprotection against ischemia–reperfusion injury (303). Bilirubin is a known antioxidant that has been shown to protect isolated perfused rat hearts when heme oxygenase-1 was inhibited (304). Finally, carbon monoxide has recently been shown to protect the lung from ischemia–reperfusion injury through suppression of the antifibrinolytic pathway (78).

Carbon monoxide, like NO, is a messenger gas molecule that can regulate vasomotor tone through the production of cyclic 3′-5′-guanosine monophosphate (305). However, increasing evidence suggests that carbon monoxide is an extremely potent antiinflammatory and antiapoptotic molecule that mediates its cytoprotective effect through the activation of the mitogen-activated protein kinase pathway, independent of the NO/cyclic 3′-5′-guanosine monophosphate pathway (306). The administration of low levels of inhaled carbon monoxide has been shown to decrease lung inflammation induced by ischemia, hyperoxia, and aeroallergen (78, 307, 308). The levels of inhaled carbon monoxide in these studies ranged between 50 and 500 parts per million, which appears to be significantly lower than the known toxic level of carbon monoxide (297).

The effect of heme oxygenase-1 and its metabolites derives from a fine balance between cytoprotection and cytotoxicity. Indeed, carbon monoxide and bilirubin are known to be potentially toxic by, respectively, dissociating oxygen from hemoglobin and by causing kernicterus (309). In addition, overexpression of heme oxygenase-1 may be associated with an excessive accumulation of free iron, which may catalyze the formation of hydroxyl radical through the Fenton reaction, and result in increased oxidative stress (310). Future studies are required to determine the role of manipulation of this novel pathway in protecting the lung from ischemia–reperfusion injury.

Preconditioning. Tissues exposed to one insult can develop tolerance to a subsequent injury. This biological adaptation forms the basis of the concept of preconditioning. Various types of preconditioning have been used *in vivo* to protect the lung from ischemia–reperfusion injury. For instance, short periods of ischemia (ischemic preconditioning) (311–314), increased temperature (hyperthermic preconditioning) (315, 316), and administration of pharmacologic agents (chemical preconditioning) (317, 318) have been shown to be successful in reducing lung injury in most cases (319).

The mechanism by which preconditioning confers protection is not well understood. Hyperthermia (approximately 5°C above normal temperature) was initially shown to upregulate the synthesis of a family of proteins, named heat shock proteins that confer protection against a variety of stresses, including ischemia–reperfusion injury (320). Although ischemic preconditioning and some cytoprotective agents have also been shown to upregulate the production of heat shock protein, the mechanism that renders the organ resistant to ischemia–reperfusion injury is certainly more complex and involves other molecules (321). Recent studies have incriminated reactive oxygen species, ATP-sensitive potassium channel openers, protein kinase C, protein tyrosine kinase, and nuclear factor-κB as potential intracellular signal transduction pathways of ischemic preconditioning (322–324). Currently, ischemic preconditioning has been shown to be effective clinically in hepatic resection (325) and in coronary artery bypass graft surgery (326), but its role remains unproven in clinical lung transplantation.

Gene therapy. The use of gene therapy in the transplantation setting is potentiated because immunosuppressive therapy allows effective and repeated transfection with current generation of viral vectors (327, 328). Multiple strategies have been used experimentally to transfect donor lungs. Genes have been administered to the donor before lung retrieval (329), on the back table during the cold ischemic time (330, 331), or to the recipient after reperfusion (332). They have been delivered intravascularly (333), intramuscularly (332), or transtracheally (329) in a naked form (334) or with the help of a vector, either viral (335) or nonviral (336).

We have demonstrated that transfection of the donor lung is possible through the transtracheal route using a second-generation adenoviral vector without contaminating other organs such as the heart, liver, or kidneys (329). Because the transfection rate is significantly decreased at cold temperature (337, 330), this mode of administration is useful in that it allows for efficient transfection before retrieving and cooling the lungs. Transtracheal administration of the gene coding for the antiinflammatory cytokine, human IL-10, to the donor 12 to 24 hours before lung retrieval reduces ischemia–reperfusion injury and improves lung function in a rat single lung transplant model (335, 338). IL-10 is an antiinflammatory cytokine that exerts antiinflammatory and immunosupressive effects on a large variety of cells including macrophages, lymphocytes, and neutrophils, and it has been shown to be beneficial in various models of ischemia–reperfusion injury (339). We are currently performing similar experiments in large animal models with endoscopic delivery of adenoviral-mediated human IL-10 gene to the donor (340). Once optimal gene delivery to large animals can be achieved, human lung protection from ischemia–reperfusion and immunologic injury by gene therapy may soon be possible.

As researches continue to improve our understanding of the mechanisms of injury related to lung preservation and as the genes that control these processes are identified, we will move closer to the ultimate strategy in preservation-related lung injury. Gene-based therapy promises the exciting potential to genetically modify organs to withstand the stresses of the transplant process.

CONCLUSIONS

Better understanding of the mechanisms of ischemia–reperfusion injury, improvement in the technique of lung preservation, and the recent introduction of a new preservation solution specifically developed for the lungs have helped to reduce the incidence of ischemia–reperfusion–induced lung injury and the development of primary graft failure after lung transplantation. In the future, the development of new agents and their application in prospective clinical trials are to be expected to prevent the occurrence of this potentially devastating complication and to further improve the success of lung transplantation.

One of the major upcoming challenges will be to improve the number of donor lungs available for transplantation. Although the number of patients on the waiting list is constantly increasing, only 10 to 30% of the donor lungs are currently used for transplantation. Hence, the development of new strategies to repair and improve the quality of the lungs could have a tremendous impact on the number of transplants performed. In addition, an improved understanding of the mechanisms involved during lung preservation may help to elucidate the potential link between acute lung injury and chronic graft dysfunction. In the future, genetic analysis using novel technologies such as microarray analysis will help to determine which genes play a role in the transplantation process. Hopefully, this will provide new insights into the mechanisms of injury and reveal potential new strategies to improve lung preservation.

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