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Primary graft dysfunction: pathophysiology to guide new preventive therapies

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Abstract

Introduction—Primary graft dysfunction (PGD) is a common complication of lung transplantation characterized by acute pulmonary edema associated with bilateral pulmonary infiltrates and hypoxemia in the first 3 post-operative days. Development of PGD is a predictor of poor short- and long-term outcomes after lung transplantation, but there are currently limited tools to prevent its occurrence.

Areas covered—Several potentially modifiable donor, recipient, and operative risk factors for PGD have been identified. In addition, basic and translational studies in animals and ex vivo lung perfusion systems have identified several biomarkers and mechanisms of injury in PGD. In this review, we outline the clinical and genetic risk factors for PGD and summarize experimental data exploring PGD mechanisms, with a focus on strategies to reduce PGD risk and on potential novel molecular targets for PGD prevention.

Expert commentary—Because of the clinical importance of PGD, development of new therapies for prevention and treatment is critically important. Improved understanding of the pathophysiology of clinical PGD provides a framework to explore novel agents to prevent or reverse PGD. Ex vivo lung perfusion provides a new opportunity for rapid development of therapeutics that target this devastating complication of lung transplantation.

Keywords

Primary graft dysfunction; lung transplantation; prevention; ischemia-reperfusion; treatment; acute lung injury; biomarkers

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1. Introduction

Primary graft dysfunction (PGD) occurs in up to 30% of lung transplant recipients within the first 72 hours after organ reperfusion [1]. PGD is characterized by the development of bilateral pulmonary infiltrates consistent with pulmonary edema. The severity of PGD is assessed by the degree of hypoxemia based on the PaO_2/FiO_2 ratio [2]. It is clear from observational studies that PGD is associated with poor clinical outcomes. For example, PGD grade 2 (PaO_2/FiO_2 200–300) or grade 3 (PaO_2/FiO_2 < 200) is associated with increased short and long-term mortality [3, 4]. In addition, PGD is a major risk factor for development of bronchiolitis obliterans syndrome (BOS) or chronic lung allograft rejection (CLAD)[4, 5]. Over the past two decades, there have been numerous studies aiming to understand the clinical risk factors for PGD and to identify the underlying cellular and molecular mechanisms with the ultimate goal to identify targets for prevention of this early and significant complication of lung transplantation. In this review, we will summarize the key findings of these studies with a focus on how such mechanistic information can be harnessed to develop better therapeutic strategies for PGD prevention.

2. Modifiable Clinical Risk Factors

The first step in preventing PGD will be to eliminate or reduce the contributions of potentially modifiable risk factors for this condition. In order to accomplish this, a detailed understanding of both donor and recipient risk factors for PGD is required. In the most comprehensive study to date, Diamond *et al.* reported the findings of a prospective cohort study of clinical risk factors for PGD based on 1,255 lung transplant recipients enrolled in the Lung Transplant Outcomes Group (LTOG) cohort [3]. As shown in Table 1, a number of independent risk factors for PGD were identified, including donor, operative, and recipient factors.

The most prominent modifiable donor risk factor for PGD was donor smoking. Current or previous donor tobacco use increased the risk of PGD from 14% to 21%. Tobacco use has also been associated with increased risk of acute respiratory distress syndrome in critically ill patients with non-pulmonary sepsis (OR 2.3) [6]. In patients with blunt trauma, active tobacco use or moderate to heavy passive tobacco exposure, as measured by urine cotinine levels, was associated with a 2–3-fold risk of acute lung injury [7]. Avoidance of donor lungs with any previous tobacco use is unreasonable, given the prevalence of tobacco use in the organ donor population and in light of reports suggesting that elimination of donor lungs from smokers would increase waitlist mortality [8]. However, it is possible that the detrimental effects of donor smoking could be mitigated by transplantation of these lungs into lower risk recipients. Further work could potentially refine allocation algorithms to maximize donor utilization and reduce the risk of PGD.

Several operative factors were associated with PGD. The fraction of inspired oxygen (FiO_2) used at the time of intraoperative lung reperfusion affected risk of PGD, with FiO_2 of > 0.4 being most strongly associated with occurrence of PGD. Higher reperfusion FiO_2 was associated with a 6% absolute increase in PGD (12% with FiO_2 < 0.4 vs. 18% with FiO2 > 0.4) with incremental increases seen with higher FiO_2. Patients who required

cardiopulmonary bypass support during the transplant operation also had higher risk of PGD. Of these intraoperative risk factors, FiO_2 at reperfusion would be the most straightforward to modify. Limiting reperfusion FiO_2 is consistent with the growing body of literature supporting the concept that hyperoxia is detrimental to the lung in healthy volunteers [9] and in those with acute lung injury [10, 11]. In one study, 74% of mechanically ventilated patients were treated with $FiO_2 > 0.5$ despite having oxygen saturation levels >92% [10]. Use of higher FiO_2 during acute lung injury was associated with increased mortality even in patients with adequate oxygen saturations [12]. In a survey of mechanical ventilation practices after lung transplantation [13], 31% of responding clinicians reported a preference to limit positive end expiratory pressure (PEEP) before reducing FiO_2, suggesting that there is significant room for improvement in recognition of potential detrimental effects of hyperoxia after lung transplantation. Only 36% of responding programs had formal protocols for mechanical ventilation after lung transplantation; of the 5 that shared their protocols, only one used an initial FiO_2 below 0.4. Future studies should test whether incorporation of a protocol for reducing the fraction of inspired oxygen at the time of reperfusion reduces PGD risk. While most lung transplant centers report using low tidal volume ventilation strategies during and after lung transplant surgery, extension of studies of mechanical ventilator management into the early post-operative period may also impact development of PGD. One retrospective single center study in the United Kingdom reported that only approximately half of lung transplant recipients received tidal volumes < 8mL/kg in the first 6 hours after transplant surgery [14]. Use of a specific ventilator management protocol may improve the frequency of low tidal volume ventilation in this peri-operative population. Furthermore, a study of size mismatch between lung donors and recipients reported that undersized lungs received relatively higher tidal volumes because of discrepancy between the predicted weight of donors and recipients [15]. Use of undersized lungs was also associated with an increased risk of PGD [16], a finding that may be even more relevant in lobar transplantation [17]. Together, these data suggest that close attention to size matching between donor and recipient may minimize PGD risk. Donor characteristics only impacted tidal volume considerations in 35% of cases, in part because the post-transplant team was unaware of specific donor information [13]. Further studies of ventilator management during and after transplantation considering both donor and recipient criteria may build on evidence showing that application of a ventilator strategy with lower tidal volume and higher PEEP in organ donors improved rates of lung procurement [18].

There are several recipient-based risk factors for PGD that are also potentially modifiable. The LTOG study by Diamond *et al.* showed that pulmonary arterial hypertension was associated with greater PGD risk, particularly in patients with the highest mean pulmonary artery pressures, independent of use of cardiopulmonary bypass [3]. However, it is uncertain whether the increased risk of PGD associated with pulmonary hypertension impacts subsequent mortality. For example, studies of patients with idiopathic pulmonary fibrosis or cystic fibrosis in the United Network of Organ Sharing (UNOS) registry data have shown no influence of pulmonary hypertension on post-transplant outcomes in these patients[19, 20]. There are no published studies assessing whether specific pharmacologic management of pulmonary hypertension in transplant candidates can reduce risk of PGD or affect mortality, although this is an area that requires further study.

Another important recipient factor that affects PGD risk is recipient body mass index (BMI). Compared to normal weight controls with BMI 18.5–25, recipients who are either overweight or underweight are at greater risk of PGD[3]. Being overweight (BMI 25–30) increased the absolute risk of PGD by 6% whereas obesity (BMI>30) increased PGD by 11%. This finding is consistent with other studies supporting an association between obesity and risk of PGD [21, 22]. In addition to PGD, obesity also affects other short and long term outcomes after lung transplantation[23, 24]. Weight management is a challenge for many potential lung transplant recipients. Patients with CF can struggle with weight gain and maintenance and can require chronic tube feeding and protein supplements to achieve goal weight. On the contrary, overweight patients with end stage lung disease often have difficulty achieving weight loss, given the poor physical endurance and reduced exercise capacity that are characteristic of end stage lung disease. Most transplant centers set strict weight goals for potential lung recipients due to the poor outcomes associated with deviation of the BMI from the normal range. One small retrospective cohort study showed that pre-transplantation weight loss reduced mortality after lung transplantation in patients who were overweight; for each decrease in BMI of 1 unit, there was an associated 11% decrease in mortality [25]. In addition, there is increasing recognition that other measures of body composition may be better measures of nutritional status in lung transplant patients. For example, body fat and skeletal muscle mass measurement by whole-body dual X-ray absorptiometry may more accurately assess for functional obesity [26]. Furthermore, assessment of biomarkers including leptin may help identify patients who have increased risk for poor outcomes despite having a more normal BMI [26].

3. Genetic Risk Factors for PGD

Recent studies have identified polymorphisms in several genes that are associated with increased risk of PGD in the lung recipient (Table 2). A candidate gene study showed that polymorphisms in pentraxin-3 (PTX3), an inflammatory mediator produced by antigen presenting cells, were associated with increased risk for PGD[27]. This is consistent with a prior study that demonstrated that higher levels of PTX3 in plasma were associated with PGD risk[28]. Additional studies have used non-targeted genetic approaches to assess genetic contribution to PGD pathogenesis. For example, a study of genetic variation of lung transplant recipients in the LTOG cohort identified that variation in the prostaglandin E2 (PGE_2) synthase gene was associated with increased risk of PGD whereas other variations in PGE_2 pathway genes were associated with reduced risk of PGD [29]. Another study from this population showed that genetic variation in Toll interacting protein (TOLLIP) is also associated with increased PGD risk and further suggested that this occurs at least in part through association with elevated levels of plasminogen activator inhibitor-1 (PAI-1), a biomarker associated with PGD[30]. Polymorphisms in the IL-17 receptor increase risk of PGD as well[31].

At this time, only one study has investigated whether donor genetic variation also affects PGD pathogenesis. Cantu *et al.* performed genetic analysis of both donor and recipients in the LTOG cohort. In this study, they reported that variation in donor NADPH oxidase 3 (NOX3) and recipient NFE2L2 (Nrf2) are associated with increased PGD risk[32]. This

study suggests that the specific interactions of genetic risk between donor and recipient may be important in modulating risk of PGD.

There will undoubtedly be more studies of genetic contributors to PGD. As the number of common and rare genetic variants that are associated with PGD increases, it will be critical to determine how this information can be translated into better clinical outcomes. One key goal for future studies is to understand the cellular and molecular consequences of genetic variation in the donor and recipient. Detailed understanding of the pathophysiologic implications of a given genetic variant could lead to development of new targeted therapies for PGD. Perhaps even more appealing, availability of genetic polymorphisms for individual patients and prospective donors could allow clinical trials to focus therapies only on the patients most likely to benefit from modulation of specific pathways, bringing personalized medicine to the field of lung transplantation. Finally, development of a genetic risk score for both the donor and the recipient could be incorporated into PGD risk determination models that could be used to more closely match donors to recipients to minimize the overall risk of PGD across the lung transplant recipient population.

4. Ex Vivo Lung Perfusion and Lung Reconditioning

The advent of ex vivo lung perfusion (EVLP) systems has the potential to modify multiple contributors to PGD. In EVLP, lungs are removed from the donor and placed into a chamber where the lungs are maintained at normothermia, ventilated with supplemental oxygen and positive pressure, and perfused with a circulating buffered solution. In a prospective non-randomized trial by the Toronto Lung Transplant Program, 15% of recipients of lungs maintained with EVLP developed PGD as compared to 30% in the control non-EVLP group ($p=0.11$) [33]. Another study by an Italian group showed a similar trend towards a reduction in PGD in lungs reconditioned by EVLP [34]. Since lungs can be maintained on EVLP for at least 12–24 hours, EVLP can extend the time available for assessment of donor lungs to determine suitability for transplantation [35]. In addition, because lungs can be transported and maintained at body temperature. EVLP can limit the time of injurious cold ischemia, another known operative risk factor for PGD [3, 36, 37]. In early studies, despite an extended total ischemic time, EVLP has not increased the incidence of PGD [34, 38–40].

EVLP is increasingly being used as a human experimental model system to test therapies aimed at reducing extravascular lung water (pulmonary edema), resolving inflammation, treating infection, and improving oxygenation prior to transplantation[35]. It is possible that EVLP will allow for reversal or removal of substances implicated in the pathogenesis of PGD, for example, by removing the donor lung from the pro-inflammatory environment of brain death. Alternately, EVLP may allow for use of higher doses of pharmacologic agents that would otherwise be limited by systemic toxicity. Additional studies are investigating whether delivery of genetic therapy to lungs during EVLP can modify the risk of short- and long-term allograft dysfunction. Hirayama *et al.* have demonstrated that lentiviral delivery of IL-10 to the lung before murine transplantation reduces rejection and airway obliteration [41, 42]; clinical trials to translate these results into human EVLP are currently being designed. Further optimization of EVLP strategies is occurring rapidly and will likely impact outcomes after lung transplantation [35]. In the coming years, EVLP-based studies

both in the clinic and the laboratory will be a major driver of progress in the molecular and cellular understanding of PGD and will facilitate rapid translation of new therapies into clinical trials.

5. Experimental evidence for new targets for prevention of PGD

As discussed above, there are limited interventions available to manipulate donor or recipient risk factors for development of PGD. Despite the extensive work on understanding the clinical aspects of PGD, there are no proven pharmacologic therapies for prevention or treatment of PGD per se and additional research is needed to identify the cellular and molecular mechanisms of acute lung injury and pulmonary edema formation after lung transplantation. Based on pre-clinical efficacy, some therapeutic agents such as nitric oxide [43–45] or surfactant [46] have been tested in clinical trials without success. Thus, better understanding of the complex cascade of events that begin in the donor lung and culminate in acute lung injury in the allograft is needed in order to identify new targets for preventative intervention.

The most frequent experimental model systems used to study molecular mechanisms of PGD are ischemia-reperfusion injury (IRI) and animal lung transplant models. These experiments can be performed in both small (mice, rats) and large animal (pigs, dogs) model systems. The advantage of murine or rodent models is the availability of research tools including genetically modified animals and antibodies to help delineate the molecular mechanisms leading to tissue injury. However, lung transplantation in the mouse is technically difficult, a challenge that has limited the number of research groups who are using this model. Nevertheless, with the combination of small and large animal models of PGD, as well as EVLP in human lungs, the lung transplant community has all the essential tools to move quickly towards testing of novel therapies. A number of dysregulated pathways have been identified in experimental systems and several have targeted therapeutics that are already available and in clinical use for other indications (Table 3). For this discussion, we will focus on potential PGD therapies that have not yet been tested in human clinical systems.

5.1. Reactive oxygen species production

Ischemia followed by reperfusion is associated with increased generation of reactive oxygen species (ROS). ROS can self-propagate, leading to activation of inflammatory cells and damage of both epithelial and endothelial cells in the lung [47]. Because ROS are a common element of numerous critical cellular pathways, agents aimed at limiting ROS could have benefit. In a porcine model of lung transplant, IV treatment with N-acetylcysteine (NAC) to both the donor and recipient animals beginning 1 hour prior to organ procurement and continuing for 8 hours after implantation resulted in improved oxygenation, airway pressure, and lung compliance [48]. NAC-treated animals developed less pulmonary edema as measured by extravascular lung water index and had less protein and inflammatory cytokines in the bronchoalveolar lavage (BAL). As expected based on the mechanism of NAC, tissue and red blood cell glutathione were elevated with NAC therapy.

Other studies using a murine hilar clamping model of IRI and murine EVLP showed that IV administration of an adenosine A2A receptor (A2AR) agonist limited IRI-mediated lung injury, resulting in less neutrophil inflammation, cytokine production, and pulmonary edema formation [49]. Further work by this group showed that A2AR agonists function through modulation of NAPDH oxidase 2 (NOX2) to limit ROS-dependent cytokine production by invariant natural killer T cells [50]. Similar studies by this group using A3AR agonist show that other similar pathways may also contribute to IRI through inhibition of neutrophil chemotaxis [51]. Studies such as these may help focus development of new therapies towards specific cell types, rather than global systemic therapy, potentially resulting in more efficacious treatments. Whether these therapies would be confer greater benefit in the setting of polymorphisms in NAPDH oxidase genes has not yet been tested. Furthermore, while these results are promising, but have not yet been evaluated in human systems. The clinical availability of an A2AR agonist (regadenoson) that is currently used in myocardial perfusion imaging should allow clinical testing of targeting the A2AR in prevention of PGD in the near future.

5.2. Apoptosis and permeability

Another cellular mechanism of injury during IRI and PGD is apoptosis and it is possible that inhibition of apoptosis may reduce formation of pulmonary edema. In a rat syngeneic model of lung transplantation, administration of diannexin (a recombinant homodimer of annexin V) given in the pulmonary flush solution of the graft during cold preservation and IV to the recipient at the time of implantation improved oxygenation, reduced peak airway pressures, limited extravascular lung water, attenuated pro-inflammatory cytokine production, and suppressed apoptosis in epithelial and endothelial cells in the lung [52]. PAI-1, a biomarker associated with increased PGD risk, was also reduced in the graft. The authors suggest that prevention of apoptosis was a key element of the mechanism of benefit and identify that annexin was primarily exposed on the vascular endothelium. Diannexin has been studied in renal and hepatic transplant models[53], but has not yet been tested in large animal lung transplant models or in human lung systems. Another study tested whether alpha-1-antitrypsin (A1AT), a serine protease inhibitor, could improve pulmonary IRI by reducing apoptosis[54]. In a rat IRI model using hilar clamping, IV supplementation with A1AT 30 minutes before ischemia reduced pulmonary edema formation and lung injury scores and improved PaO_2/FiO_2 ratios and lung compliance. A1AT-treated animals also showed less apoptosis in the lung by TUNEL staining and had reduced neutrophil influx. A more recent study using a pig model of lung transplantation after EVLP showed that A1AT similarly reduced pulmonary edema formation, reduced pro-inflammatory cytokine production, and improved pulmonary gas exchange [55]. The potential appeal of using A1AT as an agent for PGD prevention is that it is already approved for human use in patients with A1AT deficiency with favorable safety and known pharmacokinetic profiles.

The pathogenesis of PGD involves endothelial cells, lung epithelial cells, and inflammatory cells and each of these cell types has been identified as a potential target for preventive strategies for PGD. Endothelial cell activation and injury plays a major role in pulmonary edema formation. Cingulin is a component of endothelial tight junctions and serves to prevent formation of stress-induced membrane alterations. Overexpression of cingulin in

cultured endothelial cells induced tight junction formation and improves endothelial barrier function[56]. A study in a rat lung transplantation model showed that intravascular administration of a cingulin-derived peptide during donor lung procurement, followed by intraperitoneal administration of the same peptide at the time of reperfusion reduced pulmonary edema and improved oxygenation up to 1 month after surgery [57]. These findings were associated with a reduction in IL-6 expression.

5.3. Lipid mediators of lung injury

Additional evidence suggests that lipids may be key regulators of PGD development and that lipid alterations in endothelial cells may impact development of PGD. Diamond *et al.* reported that patients with PGD had higher circulating levels of lipid peroxidation productions as compared to those without PGD [58]. In addition, the relationship between lipid peroxidation and PGD was present primarily in allografts from donors with a smoking history and in recipients who had a reperfusion FiO2 of > 0.4 [58]. This study suggests that lipid peroxidation may be a common pathway that could link clinical observations of increased PGD risk with donor smoking and reperfusion FiO2 to a molecular pathway that could be manipulated as a potential PGD therapy. One potential contributor to lipid peroxidation during PGD is plasma cell-free hemoglobin. Cell-free hemoglobin accumulates during times of physiologic stress associated with increased red blood cell fragility; as hemoglobin is released from red blood cells, the natural scavenging systems including haptoglobin can become overwhelmed, leading to accumulation of cell-free hemoglobin[59]. Extracellular hemoglobin is a potent oxidant. In critically ill patients with sepsis, circulating plasma cell-free hemoglobin is associated with increased lipid peroxidation, end organ damage, and death[59, 60]. A small single center clinical trial demonstrated that acetaminophen, a specific hemoprotein reductant, given to critically ill patients with sepsis and elevated levels of circulating cell-free hemoglobin reduced formation of lipid peroxidation products and attenuated renal injury[61]. In a subset of the LTOG cohort, elevated pre-operative cell-free hemoglobin was associated with an increased risk of PGD (Ware *et al.*, *JHLT* 32(4):S42–S43, 2013). Whether acetaminophen could modulate the impact of cell-free hemoglobin and lipid peroxidation during PGD has not yet been studied.

Another study tested whether sphingolipids were important mediators of PGD development. Sphingolipids act as potent signaling agents through interactions with G-protein coupled receptors. In a rat model of lung transplantation, S1P treatment before reperfusion improved oxygenation, reduced pulmonary edema, reduced endothelial apoptosis, attenuated cytokine production, and limited neutrophil influx[62]. A subsequent study demonstrated that these effects were modulated through the S1PR1 receptor[63]. Further work is necessary to understand the downstream pathways and cell-specificity of these pathways in IRI and these agents need to be tested in transplant model systems before translation of these agents into human studies.

5.4. Epithelial cell injury

Epithelial cell injury also plays a key role in PGD pathogenesis. The receptor for advanced glycation end products (RAGE) is highly expressed on the alveolar epithelium and plasma levels are elevated during acute lung injury and serve as a biomarker of lung epithelial cell

injury [64, 65]. Several studies have implicated a role for RAGE in the mechanisms of PGD. First, elevated RAGE in BAL of lung donors was associated with increased PGD risk (odds ratio 1.77 per 0.25 mg/mL increase in RAGE) and the level of RAGE correlated with the severity of PGD[66]. Similarly, plasma RAGE in lung transplant recipients within 6 hours of reperfusion was associated with increased PGD (odds ratio 1.28 per 10mg/mL increase)[67]. The initial interpretation of these studies was that RAGE is a biomarker of increasing lung injury; however, more recent work has suggested that RAGE may have a more mechanistic role in the pathogenesis of acute lung injury and PGD. For example, RAGE has been shown to be a critical mediator of acute lung injury related to both traumatic brain injury and LPS instillation [68, 69]. In regards to PGD models, neutralization of RAGE with a soluble RAGE ligand (sRAGE) or elimination of RAGE using knockout mice demonstrated a causative role for RAGE in a mouse model of IRI using hilar clamping[70]. Mice lacking RAGE signaling developed less pulmonary edema and had less histologic evidence of lung injury. Together, these data suggest that activation of injury pathways prior to organ procurement persists after transplant and contributes to PGD. In addition, since RAGE is a common receptor for various injurious stimuli, targeting this pathway as a potential treatment for PGD may have pleiotropic effects.

5.5 Innate immune targets

Whether therapy aimed at specific inflammatory cell types can impact PGD is not well understood. Recent studies have identified neutrophil extracellular traps (NETs) as important mediators of lung injury, particularly in the presence of bacterial infection [71]. NETs are extracellular deposits of chromatin which are extruded by activated neutrophils and serve to sequester microbes and other inflammatory mediators. Recent work has demonstrated that NETs are present in BAL fluid of lung transplant recipients and are present in greater concentration in patients with PGD compared to those without PGD [72]. NETs were detected in the lung and plasma of mice with IRI after hilar clamping and reperfusion as well as in mice after lung transplantation. Platelets were important for NET formation in this model system. Interestingly, aspirin reduced NET formation, limited lung injury, improved lung permeability, and improved oxygenation in a platelet-dependent manner. Furthermore, disruption of NETs using intrabronchial DNaseI treatment improved oxygenation, reduced lung vascular leak, and limited lung injury within 8 hours of reperfusion. NETs are appealing potential targets for new PGD therapies in part because potentially therapeutics such as aspirin and inhaled DNaseI are already approved for human use.

As in many other fields, there is considerable interest in use of mesenchymal stem cells as a therapy for PGD, particularly in light of new clinical trial data showing the safety of intravenous mesenchymal stem (stromal) cell therapy in ARDS[73]. A pilot study in donor lungs not utilized for transplant showed that multipotent adult progenitor cells given intra-bronchially reduced lung inflammation and injury after cold ischemia[74]. Two other studies showed intravenous mesenchymal stem (stromal) cells limited IRI in murine and rat lung transplant models[75, 76]. Intravenous administration of mesenchymal stem (stromal) cells engineered to deliver IL-10 showed improved oxygenation, reduced pulmonary edema, reduced apoptosis, and reduced T cell infiltration after hilar clamping[77]. Additional

studies are necessary to determine how stem cell-based approaches could most effectively impact PGD pathogenesis.

Additional evidence has identified a role for extracellular ATP (eATP) in lung injury after ischemia-reperfusion. Excess levels of eATP lead to expression of pro-inflammatory mediators in part through activation of the NLRP3 inflammasome[78]. Ibrahim *et al.* showed that eATP is elevated up to 4-fold in patients with PGD compared to those without PGD and also that eATP levels increased with severity of PGD[79]. In a dog model of lung transplantation, administration of apyrase, an enzyme that reduces eATP levels, into the pulmonary artery 7 minutes after reperfusion improved oxygenation and reduced pulmonary edema formation. In addition, apyrase treatment attenuated pro-inflammatory cytokine expression and reduced neutrophil infiltration in the allograft. This study is important because unlike the majority of pre-clinical experimental studies, the therapy was tested in the presence of methylprednisolone so it more accurately reflects the immunosuppressive strategies that are currently in use in human lung transplant recipients.

While lung transplant recipients receive multiple immunosuppressive agents peri-operatively, these medications do not have their full pharmacologic effects for several days, leaving a window of time when donor and recipient interactions may be occurring in the absence of maximal immunosuppression. One hypothesis is that early inflammatory interactions between donor and recipient could be attenuated by initiating anti-inflammatory therapy in both the donor organ and in the pre-operative recipient. Along these lines, addition of prednisolone to the perfusate of rat lungs during lung preservation improved survival after lung transplantation and reduced the degree of pulmonary edema[80]. Further investigation demonstrated that the mechanism of reduced edema was through attenuation of VEGF-A-mediated tissue injury. Prednisolone therapy in this model also reduced pro-inflammatory cytokine production and shifted macrophage polarization towards an M2 anti-inflammatory phenotype. In a porcine model of acid-induced lung injury using EVLP, pre-treatment of the lungs with corticosteroids improved oxygenation parameters without significant impact on inflammatory mediators[81]. Another study using porcine EVLP demonstrated that administration of methylprednisolone into the perfusate during EVLP after cardiac arrest led to reduced pulmonary edema formation[82]. While many brain-dead organ donors are already treated with high dose systemic corticosteroids, it is unknown whether additional treatment during organ preservation in human lungs (with or without EVLP) would reduce PGD.

5.6. Anti-coagulants

Another contributor to IRI is the presence of thrombus in the donor lungs that can persist despite routine flushing of vasculature with heparin during organ procurement. Treatment of marginal donor lungs with plasmin in the perfusate during EVLP improved pulmonary vascular resistance, lung compliance, and reduced pulmonary edema formation [83]. To test whether plasmin would also confer benefit during reperfusion in the presence of leukocytes, the same group administered plasmin into the perfusate during rat EVLP followed a period of cold storage with subsequent reperfusion. As seen in marginal lungs, plasmin treatment during rat EVLP also improved oxygenation and lung compliance, reduced pulmonary

edema, and reduced apoptosis[84]. Interestingly, the benefit of plasmin in this study persisted after cessation of EVLP and through reperfusion, suggesting that treatments applied during EVLP may have lasting impact for at least several hours afterwards.

6. Conclusions

In summary, in the past decade, our understanding of primary graft dysfunction mechanisms has greatly improved. Greater understanding of clinical biomarkers and genetic factors that increase the risk of PGD will likely allow development of new algorithms to facilitate organ allocation with specific consideration of donor and recipient matching to reduce the risk of PGD. Based on clinical observations and extensive studies in animal model systems, we now have a long list of potential pathways involved in the mechanisms of PGD that can be tested. Some agents, such as DNaseI, aspirin, or A1AT are already clinically available for other indications and experimental model systems like EVLP present an opportunity for rapid testing of new therapies directed solely at the lung. These novel preventive interventions coupled with improved risk prediction and mitigation based on donor and recipient genetic and clinical characteristics have great potential to reduce the incidence and consequences of PGD by moving novel therapies through targeted clinical trials into routine clinical use for the benefit of lung transplant recipients.

7. Expert commentary

Development of PGD is a critical event for lung transplant recipients. Early events, even within the first few hours of transplant, dictate the major complications of transplant. More specifically, PGD is a major risk factor for development of chronic rejection (CLAD, BOS) and for mortality [3–5]. Because of the major impact of PGD for the transplant recipient, development of new therapeutic options for this condition is paramount. While animal models of lung transplantation do not recapitulate the pathology of chronic rejection, models of ischemia-reperfusion are excellent in mimicking the features of human PGD. As such, these model systems have identified a number of different pathways and cells involved in PGD that form a framework for ongoing research and development of new therapeutics. The increasing use of ex vivo lung perfusion will be invaluable to this process because it will allow testing of agents directly on human lungs with less risk to other transplantable organs or the organ recipient. One critical advantage in therapeutic interventions for prevention of PGD is that unlike other forms of acute lung injury, the timing of the major insult is known so potential therapies can be instituted prior to ischemia-reperfusion, which may increase the likelihood of meaningful clinical responses. Some therapies may be most beneficial when started prior to organ procurement. EVLP may also allow for reversal of lung injury that developed in the organ donor, potentially reducing PGD in this manner in addition to expanding the pool of suitable donor lungs. This is a time of incredible opportunity for the field of lung transplantation, now poised with the tools and mechanistic insight to test a series of new therapies to prevent PGD, thereby improving both short and long term clinical outcomes for lung transplant recipients.

8. Five year view

Primary graft dysfunction remains a common complication after lung transplantation. Agreement on a standard definition and grading system in 2005 has allowed focused studies of this complex clinical syndrome [85]. The advances in our understanding of clinical risk factors for PGD in conjunction with advances in mechanistic understanding of pathways involved in PGD pathogenesis has laid the groundwork for the next phase of translational studies and clinical trials.

Over the next five years, we anticipate that ex vivo lung perfusion technology will be a major driver of advances in prevention of PGD. The potential for benefit from EVLP is great. First, EVLP may facilitate improved utilization of donor organs. This is because of increased ability to assess the lungs over time to ensure adequate physiologic function. Second, EVLP provides a platform with which potential PGD treatments can be studied in human tissues. This type of organ specific human research will be invaluable for moving rodent or large animal based information towards clinical use more rapidly. The novelty of transplanting organs that have been treated ex vivo as “study subjects” with experimental therapies will require balancing of risks and benefits for the potential recipient and the process of informed consent for these studies will need to be carefully considered. Third, EVLP will allow for novel therapies to be applied only to the lung allograft prior to transplantation. Restricting treatment to only the lungs, in the absence of the pro-inflammatory milieu of brain death, may allow for safe usage of medications that would potentially be suboptimal for other organs. Intravenous therapies administered in the perfusate could also be flushed from the allograft prior to implantation, reducing exposure of the potential recipient.

Another major area in which we expect substantial progress in the next five years is in personalized medicine for PGD prevention. The feasibility of large scale genetic and biomarker studies will likely yield a number of new potential targets for PGD. As streamlined systems are developed to determine the mechanistic contribution of these pathways to PGD, we will increasingly be able to identify the patients who are most likely to benefit from a particular treatment strategy. Tailoring of therapy to the patients most likely to benefit, and then restricting treatments to those with biomarker evidence of positive responses, will allow for more efficient and targeted clinical trials. These studies will also shed light on how the donor, the recipient, and the interaction between the two drive PGD. This will guide whether therapies should be given to the donor, the recipient, or both. In addition, greater mechanistic understanding of which pathways are disrupted in which cell types will allow novel treatments to be targeted to the primary cell type affected, by delivering therapeutics systemically (or in perfusate solutions) to target the vascular endothelium or intra-bronchially to target the lung epithelium.

In summary, 5 years from now, the numbers of lung transplants will have continued to increase and the median survival after transplant will hopefully exceed today’s 6 years. Based on the current breadth of research into mechanisms of PGD, we anticipate that we will have more therapeutic tools moving through clinical trials and approaching clinical utilization. This is an exciting time for lung transplant research and clinical practice. Careful

clinical observation and diligent mechanistic research will merge for the benefit of the patients to reduce the incidence of PGD.

9. Key issues

- Primary graft dysfunction is a serious and common complication of lung transplantation with serious short- and long-term adverse effects on lung recipients
- Use of FiO2 <0.4 at the time of organ reperfusion has been associated with a reduced risk of PGD.
- Recipients who can achieve a body mass index in the normal range (>18.5 and <25) have a reduced risk of PGD.
- Genetic variation in both lung transplant recipients and in donor lung allografts modulates pathways critical to the pathogenesis of PGD and may allow for personalized medicine approaches to PGD prevention and treatment as well as improved allocation and preoperative risk assessment.
- Ex vivo lung perfusion systems are critical for translational and clinical research. EVLP will likely allow reconditioning of lungs damaged by critical illness and the pro-inflammatory milieu of brain death in the donor. EVLP also provides a novel platform for testing and implementation of new therapies for PGD prevention.
- Experimental models of ischemia-reperfusion and non-human orthotopic lung transplantation have yielded great mechanistic insight into mechanisms of PGD. The lung endothelium, lung epithelium and inflammatory cells each have key roles in the pathogenesis of PGD.
- Potential therapies that warrant testing in human lung transplantation include anti-oxidants (NAC, adenosine receptor agonists), anti-apoptotic agents (diannexin, A1AT), endothelial targeted agents (sphingosine analogs, cingulin), epithelial targeted agents (RAGE pathways), neutrophil targeted agents (DNaseI, aspirin), stem cell therapies, eATP suppression (apyrase), immunosuppressants, and anti-coagulants (plasmin).
- EVLP may allow for rapid translation of these mechanistic targets into human model systems and then into lung transplant recipients

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Table 1

Potentially modifiable independent clinical risk factors for primary graft dysfunction.

Risk factor	Odds ratio	95% confidence interval
Any donor smoking [a]	1.8	1.2–2.6
Reperfusion FiO_2 (per 10% increase) [a]	1.1	1.0–1.2
Pulmonary arterial hypertension [a]	3.5	1.6–7.7
mPAP (per 10mmHg) [a]	1.3	1.1–1.5
Body mass index <18.5 [a]	1.3	0.6–2.8
Body mass index 25–30 [a]	1.8	1.2–2.7
Body mass index >30 [a]	2.3	1.3–3.9
Packed red blood cell transfusion >1L [a]	1.9	1.1–3.2

Adapted from data reported in Diamond *et al.* and limited here to only modifiable risk factors [3].

[a]Adjusted for transplant type (single or bilateral), cardiopulmonary bypass use, recipient gender, ischemic time, and diagnosis.

Table 2

Single nucleotide polymorphisms associated with risk of primary graft dysfunction.

Gene	Protein or pathway	SNP	Odds ratio	95% confidence interval	Reference
RECIPIENT GENETICS					
Ptx3	Pentraxin-3	rs2120243	1.5	1.1–1.9	[27]
		rs2305619	1.4	1.1–1.9	[27]
Ptges2	Prostaglandin E2 synthase	rs13283456	2.0	1.4–2.9	[29]
Ptger4	Prostaglandin E receptor	rs4434423	0.5	0.4–0.8	[29]
		rs4133101	0.6	0.5–0.8	[29]
		rs11957406	1.7	1.3–2.3	[29]
Tbc1d1	Cell growth and obesity	rs2996044	0.5	0.4–0.7	[29]
		rs2925956	0.6	0.4–0.8	[29]
		rs13132184	0.5	0.3–0.7	[29]
Pmch	Neuronal signaling	rs7973796	0.6	0.5–0.8	[29]
F13a1	Coagulation factor XIII	rs3024388	1.8	1.3–2.5	[29]
Gaa	Glycogen degradation	rs12452616	1.8	1.3–2.5	[29]
Cav3	Caveolin 3	rs237865	1.7	1.3–2.2	[29]
Col4a1	Type IV collagen	rs17588591	1.7	1.3–2.3	[29]
Casp8	Caspase 8	rs16836965	3.2	1.7–6.0	[29]
Irx4	Cardiac development	rs260400	2.1	1.4–3.1	[29]
Itgb5	Integrin beta 5	rs3772843	1.9	1.3–2.6	[29]
Prkg1	Platelet aggregation	rs1881597	0.6	0.4–0.8	[29]
Ftcd	Folate metabolism	rs17004504	2.9	1.6–5.2	[29]
Tollip	Toll interacting protein	rs3168046	1.4	1.1–1.8	[30]
Il17R	IL-17 receptor	rs882643	2.2	1.1–4.5	[31]
		rs2241049	2.3	1.3–3.9	[31]
Nfe2l2 (Nrf2)	Nuclear factor (erythroid-derived 2)-like 2	rs6726395	0.8	0.6–1.0	[32]
		rs1806649	0.8	0.6–1.0	[32]
Gpx1	Glutathione peroxidase	rs9818758	0.7	0.5–1.0	[32]
		rs1800668	0.7	0.6–0.9	[32]

Gene	Protein or pathway	SNP	Odds ratio	95% confidence interval	Reference
		rs3811699	0.7	0.6–0.9	[32]
DONOR GENETICS					
Nox3	NAPDH oxidase 3	rs3749930	0.4	0.2–0.8	[32]
		rs231956	1.5	1.1–2.1	[32]
		rs13207865	1.6	1.1–2.3	[32]
		rs231948	0.6	0.3–1.0	[32]
		rs1546894	0.3	0.1–0.9	[32]

Table 3

Potential targets for new therapeutic approaches for prevention of primary graft dysfunction.

Pathway or Process	Therapeutic agent	Approved for human use
Reactive oxygen species production	N-acetylcysteine	Yes
ROS production	A2A receptor agonist	Yes
ROS production	A3A receptor agonist	No
Glutathione utilization	N-acetylcysteine	Yes
Apoptosis	Diannexin	No
Protease / Anti-protease	Alpha-1 anti-trypsin	Yes
Endothelial permeability	Cingulin	No
Lipid peroxidation	Acetaminophen	Yes
Cell-free hemoglobin	Acetaminophen	Yes
Sphinoglipids	S1P	No
Epithelial injury	Soluble RAGE	No
Neutrophil extracellular traps	DNaseI	Yes
Neutrophil extracellular traps	Aspirin	Yes
Tissue repair	Mesenchymal stem cells	No
ATP release	Apyrase	No
Inflammasome activation	IL1R antagonist	Yes
Innate immune suppression	Corticosteroids	Yes
Anticoagulation	Streptokinase	Yes