Title: Preventing the NET negative in PGD

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Primary graft dysfunction (PGD) is a form of acute lung injury triggered by ischemia–reperfusion injury after lung transplantation. It affects 10 to 35% of lung allograft recipients and is the major cause of early morbidity and mortality after lung transplant (1). Additionally, PGD has been associated with an increased risk of chronic lung allograft dysfunction, which is the major cause of late mortality after lung transplantation (2). Prior studies have proposed several risk factors for the development of PGD based on the donor, recipient, and surgical variables (3). The pathogenesis of PGD is not well understood. An inflammatory cascade initiated by ischemia-reperfusion injury post-lung transplant, which ultimately leads to an influx of neutrophils into the lungs, has been suggested as the underlying etiology of the development of PGD (4). Given the unclear pathogenesis, and lack of any established therapy for PGD, it is crucial to characterize the cellular and molecular pathways leading to PGD in order to develop targeted therapies. In the current issue of the journal Sayah et al (pp xxx), present evidence that neutrophil extracellular traps (NETs) develop after ischemia-reperfusion injury and recruitment is dependent on platelets. Further, they find increased NETs in the bronchoalveolar lavage of human lung transplant recipients with PGD compared to subjects without PGD. Interestingly, the prevention of NET formation using an anti-platelet agent or intra-alveolar disruption of NETs using DNase I protects against PGD in a mouse model of orthotopic lung transplant (5).

Previous studies in animal models have suggested a role for neutrophils in the inflammatory response after graft reperfusion and found that disrupting neutrophil infiltration can reduce lung injury post-transplant (4, 6). One of the consequences of neutrophil infiltration and activation can be the formation of NETs, extrusions of neutrophil DNA-protein complexes generated by cell death in a process called NETosis (7). Prior work by Looney and colleagues found that NETs were involved in lung injury in a mouse model of transfusion-related acute lung injury (TRALI) (8). Disruption of platelet function with aspirin decreased NET formation and the severity of lung injury (9). The group has extended their work by investigating the contribution of NETs to lung injury using two mouse models of ischemia-reperfusion pathology, a hilar clamp

model and an orthotopic left lung transplant model with prolonged cold ischemia time. The models provide complementary results on the involvement of NETs in ischemia-reperfusion injury, as both models resulted in NET formation. However, the hilar clamp model had a significant increase in NETs in the plasma and not the BAL, while the transplant model had an increase in NETs in the BAL and not the plasma. These data suggest the two models are not equivalent in the compartmentalization of NET formation initiated after lung ischemia and may provide different insights into the impact of ischemia-reperfusion on the lung.

The findings in the orthotopic left lung transplant model are particularly interesting as they reflected the clinical scenario with elevated levels of NETs detected in patients with PGD grades 2 or 3 in the BAL and not the plasma. Disruption of NETs with administration of DNase I markedly reduced lung injury after transplant supporting a pathogenic role for NETs. In addition to reducing the indices of lung injury, DNase I treatment was also associated with a reduction in neutrophils in the BAL. These data are interesting and support a role for NETs not only in promoting lung injury, but also in augmenting the recruitment or expansion of neutrophils. To address the mechanism of NET formation after transplant, the authors drew on their experience with the TRALI model. They postulated that platelet-induced thrombosis may promote NET formation and used aspirin to inhibit platelet aggregation. Remarkably, the use of aspirin significantly decreased the formation of NETs and the severity of lung injury. A role for pro-thrombotic factors in ischemia-reperfusion injury has been found by previous studies, but the authors are the first to find that aspirin diminishes platelet-induced thrombosis and post-engraftment acute lung injury in a lung transplant model (10). They were unable to directly test the depletion of platelets due to the bleeding complications associated with transplantation. Nevertheless, the studies with aspirin inhibition of platelets and the impact on NETs are compelling and provide novel targets for therapies.

The authors readily acknowledge several caveats of the studies. While the orthotopic lung transplant model provides a relevant model to study transplant-related injury and

differences were found compared to the more commonly used hilar clamp model, the model involved 18 hours of prolonged cold ischemia time. The timing was apparently chosen as it had been used by others as a model of PGD and it reproducibly induced lung injury (11). Cold ischemia is a known contributor to graft dysfunction, but clinical lung transplantation does not involve using tissue that is beyond 4 to 8 hours of cold ischemia time (10). Whether this longer ischemia time induces mechanisms of injury that are not relevant to clinical transplantation remains to be investigated. However, the identification of NETs in the human BAL samples from subjects with PGD lends support for the findings and enthusiasm for further studies on thrombosis and NET formation after lung transplant.

Perhaps most exciting is the potential to determine the donor factors that promote thrombosis and NET formation and modify those factors prior to transplant. With the advent of ex vivo lung perfusion, therapies aimed at preventing pro-thrombogenic factors or conditions may have significant impact on organ availability and outcomes. In addition, biomarkers of pro-thrombotic conditions may be found in the donor lung perfusate which identify lungs that are at higher risk of PGD. The pro-thrombotic factors may either be modified peri-transplant or the lungs refused if the risk of injury is too high. The mouse model of orthotopic lung transplant is technically challenging but does provide an exciting and relevant new tool to begin to tackle the pathogenic mechanisms arising from both donors and recipients that promote allograft dysfunction.

Over the past decade several strategies targeting putative pathways in PGD have been studied, such as the use of inhaled nitric oxide, modulation of the complement cascade, and instillation of surfactant (12-14). More recently Diamond et al, provided evidence that the prostaglandin E2 (PGE2) pathway is implicated in pathogenesis of PGD, providing another possible target for therapeutics (15). Given the current evidence supporting multiple factors involved in PGD, it may be unlikely to find one single approach or one effective therapy for PGD. The management of PGD will likely continue to require a multidisciplinary approach

starting with optimizing the modifiable risk factors for development of PGD followed by a combination of target therapies based on well-designed clinical trials. The current study provides exciting new data on the role of NETs and platelets in PGD and lends support to trials of the simple interventions of DNase I or aspirin in clinical lung transplantation.

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