Three Is Better than One: An Improved Multiple-Hit Model of Primary Graft Dysfunction

To date, we are unable to cure or reverse most end-stage lung diseases, including chronic obstructive pulmonary disease, idiopathic pulmonary fibrosis, pulmonary hypertension, and cystic fibrosis. Therefore, lung transplantation (LTx) is the only therapeutic option for many patients with end-stage lung disease. In recent years, the number of LTxs has significantly increased: in 2014, the International Society for Heart and Lung Transplantation reported a total of 4,122 adult LTxs (in 140 participating centers) (1, 2). Although short-term survival after LTx has improved over the past few decades due to advances in, e.g., surgical procedures, the median and long-term survival of individuals who have undergone LTx remains severely limited, primarily due to chronic rejection of the allograft (chronic lung allograft dysfunction [CLAD]). Current unadjusted survival rates for lung transplant recipients are 80%, 54%, and 32% at 1, 5, and 10 years, respectively, and are thus the worst for any solid organ transplantation (2). A major risk factor for CLAD is primary graft dysfunction (PGD), which is defined as the syndrome of acute lung injury occurring within the first few days after lung allograft reperfusion in lung transplant recipients (3). PGD is a major cause of early mortality and morbidity of LTx (its incidence varies between 10% and 30%) (2–4). PGD is graded based on the presence of pulmonary edema on chest X-ray and the arterial oxygen tension (Pa_{O_2})/fraction of inspired oxygen (FI_{O_2}) ratio, as outlined in the most recent report of the International Society for Heart and Lung Transplantation Working Group on PGD (4). Grade 3 PGD is present when the Pa_{O_2}/FI_{O_2} ratio is <200 and diffuse allograft infiltration/pulmonary edema is evident on chest X-ray. Histologically, PGD is characterized by acute macrophage- and neutrophil-dependent inflammation associated with diffuse alveolar damage and intragraft edema (4). Our knowledge about the mechanisms and potential therapeutic approaches to PGD and CLAD has been severely limited due to the paucity of clinically relevant animal models that reflect the entirety of PGD or CLAD.

In this issue of the *Journal*, Wang and colleagues (pp. 244–256) present a novel and potentially impactful extension of previous PGD models that incorporates multiple hits (5) to which the lung graft is exposed before LTx, such as ischemia-reperfusion injury (IRI), hemorrhagic shock, and preceding brain death of the donor (3). Thus far, most animal studies of PGD have only induced IRI and cold ischemia in rodent models (6–8). Wang and colleagues investigated several combinations of IRI, brain death, and hemorrhagic shock in a mouse model of syngeneic LTx-based PGD. The authors report that this combination of preoperative risk factors markedly worsens PGD, as evidenced by an enhanced activation of necroptosis.

Although apoptosis and necrosis have been observed in animal models of lung IRI (9, 10), this is the first study to detect necroptosis *in vivo* in PGD after LTx. Necroptosis is a programmed type of necrosis that allows a cell to commit suicide in critical circumstances, such as viral infection, or in disease (e.g., Crohn's disease, pancreatitis, and myocardial infarction) (11). Necroptosis is initiated by the interaction of TNF with its receptor, which leads to the activation of receptor interacting serine/threonine kinase 1 (RIPK1), which in turn recruits RIPK3 to form the necrosome. The necrosome or ripoptosome recruits MLKL, which provokes cell membrane and organelle permeabilization upon oligomerization and insertion into biological lipid layers. A major difference between apoptosis and necroptosis is that apoptotic cells are rapidly cleared and thus pose only a limited risk for promoting inflammation, whereas necroptosis potentially perpetuates inflammation, in particular via the release of damage-associated molecular patterns (DAMPs) into the extracellular space. Necroptosis is often referred to as pathological cell death, whereas apoptosis appears to be a controlled and silent cell death that is involved in various processes, including development (12).

The discovery of the contribution of necroptosis to multiple-hit–induced PGD is clinically rather important because *1*) available data from this model and human PGD samples have highlighted necroptosis as a conserved mechanism in PGD (5, 13), and *2*) although inhibitors of necroptosis are currently being revisited and refined in preclinical models, some of them are indeed available for use in clinical trials and could be a valuable addition to current immunosuppressive regimens in LTx (14, 15). Wang and colleagues determined that the RIPK1 inhibitor Nec1 successfully prevented the onset of necroptosis in their multiple-hit mouse model of PGD. This suggests that preclinical investigations to determine whether and the extent to which necroptosis inhibitors prevent the functional deterioration of early graft function are now needed. Such investigations could include assessments of functional readouts (e.g., the Pa_{O_2}/FI_{O_2} ratio and the wet/dry ratio) and long-term outcomes (e.g., survival) after mouse LTx.

The contributions of neutrophil-derived secretory proteins (8), such as proteases, have recently been demonstrated in PGD after LTx. DAMPs are potent neutrophil chemoattractants. Another interesting finding is that preexisting autoantibodies against collagen V (ColV) are likely to target the lung epithelium in PGD (16). How does necroptosis affect these known mechanisms of PGD, or is affected by them? Necroptosis potentially contributes to DAMP-induced inflammation, in particular via the recruitment of additional neutrophils. It is also possible that epithelial cell death by necroptosis favors the exposure of ColV and therefore aggravates epithelial injury in PGD via the autoantibody-mediated destruction of epithelial cells. Although Wang and colleagues present evidence that epithelial cells undergo necroptosis, it is not clear whether necroptosis is restricted to this cell type. In a number of pathological contexts, neutrophils have been reported to undergo

necroptosis and in turn release neutrophil extracellular traps (17), which are known to promote PGD after LTx (7). One potential way to address compartmental restriction of necroptosis would be to use *Ripk1*-floxed mice crossed to diverse Cre drivers and determine whether necroptosis plays a more important role in structural/resident versus recruited immune cells. This would theoretically inform treatment: should the recipient be treated pre- or postoperatively, or is it possible to precondition the graft? Another important issue to address is the timing of the onset of necroptosis: is it initiated after reperfusion or during graft preservation? In the latter case, it would be sensible to supplement the *ex vivo* conditioning medium with necroptosis inhibitors before implanting the graft.

In conclusion, PGD remains a major limitation to the survival and quality of life of patients who have undergone LTx, in particular because there is no consensus on the potential treatment options to prevent PGD. The current study by Wang and colleagues highlights a new mechanism that may aid in the development of novel therapeutic targets for PGD. The authors should be applauded for establishing and interrogating a novel and complex multiple-hit model of PGD. Their study constitutes a solid foundation for future investigations to assess the effects of necroptosis inhibitors on long-term outcomes of PGD in preclinical and potentially clinical studies. ■

Author disclosures are available with the text of this article at www.atsjournals.org.

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