DNase to the Rescue! Clearing Mitochondrial DNA May Have NET Benefits in Lung Transplantation

Survival after lung transplant is inferior compared with that after other solid organ transplants, owing to the constant exposure of the lung to the environment and the rich immune milieu within the allograft. Innate immune stimuli trigger injury in transplanted lungs, increasing the risk of acute and chronic lung allograft dysfunction.

Pattern recognition receptors (PRRs), which protect the host against infection by binding to pathogen-associated molecular patterns, are instrumental in activating innate immunity. PRRs are also triggered by damage-associated molecular patterns (DAMPs), which are released during sterile tissue injury, including ischemia–reperfusion injury (IRI) (1). The first study that reported a role for PRRs in lung transplant showed that recipient Toll-like receptor (TLR) 4 loss-of-function polymorphisms correlated with lower rates of acute rejection (2). Endotoxin-driven TLR4 signaling plays a role in murine models of alloimmune lung injury and inflammation (3, 4). In addition, pulmonary DAMPs, such as HMGB1 (high mobility group protein B1), heat shock proteins, hyaluronan, tenascin C, and nucleic acids, including mitochondrial DNA (mtDNA), augment rejection or fibrosis in murine models and have been associated with acute and chronic rejection in human lung transplant recipients (1).

We believe that the earliest events in the life of the pulmonary allograft are critical determinants of long-term outcome. Primary graft dysfunction (PGD), the clinical correlate of IRI, is an important risk factor for chronic lung allograft dysfunction in humans (5, 6). In addition, IRI augments chronic rejection in a mouse orthotopic lung transplant model (7). The assumption is that DAMPs, released during IRI, increase T-cell priming (8), thus augmenting acute rejection and potentiating injurious and profibrotic pathways. However, the specific DAMPs involved and their downstream mechanisms in lung PGD are unclear.

In this issue of the *Journal*, Mallavia and colleagues (pp. 364–372) report on the role of mtDNA in driving TLR9-mediated neutrophil extracellular trap (NET) formation in a mouse model of PGD (9). Their group had previously demonstrated NET accumulation in experimental and human PGD and showed that NET eradication with intrabronchial deoxyribonuclease (DNase) I in mice improved graft function (10). The mechanisms of NET production, however, remained obscure.

Using a syngeneic mouse orthotopic lung transplant model, Mallavia and colleagues now show (9) that mtDNA is elevated in the BAL after prolonged lung allograft cold ischemia compared with minimal ischemia. Importantly, purified mtDNA is sufficient to release NETs and recapitulate PGD after minimal ischemia. Supporting the potential clinical relevance of these findings, the authors demonstrate higher levels of mtDNA and NETs in the BAL of patients with severe PGD than in patients with no or minimal PGD.

To assess the role of TLR9, the authors first confirm prior findings that mtDNA triggers TLR9-dependent NET formation by neutrophils *in vitro*. They then show that TLR9 deficiency in either the lung donor or the recipient decreases NET formation and injury in the mouse prolonged preservation model.

The authors describe two potential strategies to reduce NET-driven PGD. Administration of DNase I reduces both mtDNA and NETs and improves graft function (9, 10). In addition, histone citrullination by PAD4 (peptidyl arginine deiminase 4), which is required for NET formation, is needed for mtDNA-driven PGD in the model. Because prevention of PGD is a major clinical goal, these are crucial observations.

This is an elegant and important study that reveals new PGD mechanisms. Nevertheless, it also has important limitations. First, an experiment using exogenous mtDNA in the setting of TLR9 deficiency would have provided further confirmation that TLR9 mediates mtDNA-induced PGD *in vivo*. Second, a syngeneic mouse model was employed, but it is well recognized that lung IRI is more severe in the allogeneic setting (7). It therefore remains uncertain whether the mtDNA–TLR9–NET pathway would have the same prominence in alloantigen-mismatched lung transplants. Third, other TLR9 ligands might be playing a role. Examples include complexes of other (nonmitochondrial) nucleic acids and binding proteins such as HMGB1 (11) and defensins, as well as microbial DNA. The latter is an important consideration because it is commonplace for donor lung allografts to be colonized with microorganisms. Fourth, the human data should be interpreted with caution. The cohort is small, and BAL samples were obtained from two institutions using divergent methods: 20 ml instilled once versus 60 ml instilled twice. The authors do not discuss how this discrepancy could have affected the results, nor do they show whether resulting data differ between centers. If PGD severity varies by center, this could have significantly biased the results. Finally, given the associative nature of human observational data, it remains unproven whether mtDNA and NETs have the same mechanistic importance in humans as they have in mice. It is possible that other DAMPs are more important in humans and that intervening in the mtDNA and NET pathway will have no clinical effect.

This study raises two clinically relevant concepts. First, mtDNA and NETs could be useful biomarkers of severe PGD and allograft injury at the time of transplant. Indeed, a recent study showed that elevated perfusate NET levels during *ex vivo* lung perfusion correlate with adverse recipient outcomes (12). Second, the authors propose mtDNA and NETs as therapeutic targets. Administration of DNase I would be the most straightforward strategy; other approaches could include TLR9 blockade or PAD4 inhibition, although these could pose an increased risk of infection.

Originally Published in Press as DOI: 10.1165/rcmb.2019-0390ED on November 14, 2019

The possibility of administering DNase I to donor lungs during transplant is attractive, but its route of delivery requires further consideration. In studies by the Looney group, mtDNA and NETs have been identified primarily in the bronchoalveolar spaces and not in the vasculature (9, 10, 13). In other lung injury settings, NETs have been detected in interstitial and intravascular spaces (12, 14). Would intrabronchial, intravenous, or both routes of administration therefore be needed to prevent or treat PGD? Nebulized DNase I has been used extensively and safely in patients with cystic fibrosis (15); a clinical trial in lung transplantation would be quite feasible. In contrast, we could find only one small pilot study of intravenous DNase I in lupus, which demonstrated safety but no therapeutic effect (16).

In summary, the study by Mallavia and colleagues represents an important advance in our understanding of how DAMPs trigger lung allograft injury, and it proposes actionable diagnostic, prophylactic, and therapeutic strategies for PGD, focused on mtDNA and NETs at the time of transplant. We hope that further research will move these findings to the clinical arena. ■

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

Stephen Juvet, M.D., Ph.D.
Tereza Martinu, M.D., M.H.S.
Toronto Lung Transplant Program
University Health Network
Toronto, Ontario, Canada

References

1. Todd JL, Palmer SM. Danger signals in regulating the immune response to solid organ transplantation. *J Clin Invest* 2017;127:2464–2472.
2. Palmer SM, Burch LH, Davis RD, Herczyk WF, Howell DN, Reinsmoen NL, *et al*. The role of innate immunity in acute allograft rejection after lung transplantation. *Am J Respir Crit Care Med* 2003;168:628–632.
3. Martinu T, Kinnier CV, Gowdy KM, Kelly FL, Snyder LD, Jiang D, *et al*. Innate immune activation potentiates alloimmune lung disease independent of chemokine (C-X-C motif) receptor 3. *J Heart Lung Transplant* 2011;30:717–725.
4. Garantziotis S, Palmer SM, Snyder LD, Ganous T, Chen BJ, Wang T, *et al*. Alloimmune lung injury induced by local innate immune activation through inhaled lipopolysaccharide. *Transplantation* 2007; 84:1012–1019.
5. Fiser SM, Tribble CG, Long SM, Kaza AK, Kern JA, Jones DR, *et al*. Ischemia–reperfusion injury after lung transplantation increases risk of late bronchiolitis obliterans syndrome. *Ann Thorac Surg* 2002;73: 1041–1047. [Discussion, pp. 1047–1048.]
6. Whitson BA, Prekker ME, Herrington CS, Whelan TP, Radosevich DM, Hertz MI, *et al*. Primary graft dysfunction and long-term pulmonary function after lung transplantation. *J Heart Lung Transplant* 2007;26: 1004–1011.
7. Watanabe T, Martinu T, Chruscinski A, Boonstra K, Joe B, Horie M, *et al*. A B cell–dependent pathway drives chronic lung allograft rejection after ischemia–reperfusion injury in mice. *Am J Transplant* 2019;19: 3377–3389.
8. Land WG, Agostinis P, Gasser S, Garg AD, Linkermann A. DAMP-induced allograft and tumor rejection: the circle is closing. *Am J Transplant* 2016;16:3322–3337.
9. Mallavia B, Liu F, Lefrançais E, Cleary SJ, Kwaan N, Tian JJ, *et al*. Mitochondrial DNA stimulates TLR9-dependent neutrophil extracellular trap formation in primary graft dysfunction. *Am J Respir Cell Mol Biol* 2020;62:364–372.
10. Sayah DM, Mallavia B, Liu F, Ortiz-Muñoz G, Caudrillier A, DerHovanessian A, *et al*.; Lung Transplant Outcomes Group Investigators. Neutrophil extracellular traps are pathogenic in primary graft dysfunction after lung transplantation. *Am J Respir Crit Care Med* 2015;191: 455–463.
11. Tian J, Avalos AM, Mao SY, Chen B, Senthil K, Wu H, *et al*. Toll-like receptor 9-dependent activation by DNA-containing immune complexes is mediated by HMGB1 and RAGE. *Nat Immunol* 2007;8: 487–496.
12. Caldarone L, Mariscal A, Sage A, Khan M, Juvet S, Martinu T, *et al*. Neutrophil extracellular traps in *ex vivo* lung perfusion perfusate predict the clinical outcome of lung transplant recipients. *Eur Respir J* 2019;53:1801736.
13. Sayah DM, Mallavia B, Looney MR. Reply: neutrophil extracellular traps in primary graft dysfunction after lung transplantation [letter]. *Am J Respir Crit Care Med* 2015;191:1089.
14. Tanaka K, Toiyama Y, Inoue Y, Araki T, Mohri Y, Mizoguchi A, *et al*. Imaging neutrophil extracellular traps in the alveolar space and pulmonary capillaries of a murine sepsis model by multiphoton microscopy [letter]. *Am J Respir Crit Care Med* 2015;191:1088–1089.
15. Yang C, Chilvers M, Montgomery M, Nolan SJ. Dornase alfa for cystic fibrosis. *Cochrane Database Syst Rev* 2016;4:CD001127.
16. Davis JC Jr, Manzi S, Yarboro C, Rairie J, Mcinnes I, Averthelyi D, *et al*. Recombinant human Dnase I (rhDNase) in patients with lupus nephritis. *Lupus* 1999;8:68–76.