A Need for Targeted Immunosuppression after Lung Transplantation

Since the first reported success of lung transplantation over three decades ago, chronic allograft rejection has carried the highest burden of long-term mortality. Chronic rejection affects ~10% of patients each year, with the highest prevalence by the fifth year (1). Unfortunately, although the current immunosuppressive regimens have been effective in reducing acute rejection episodes, the incidence of chronic rejection has remained relatively unaltered. In this issue of the *Journal*, Takahagi and colleagues (pp. 355–366) report a novel use of the drug trametinib in delaying chronic lung allograft rejection after rodent major histocompatibility complex (MHC)-mismatched allotransplantation (2). Trametinib is an inhibitor of the mitogen-activated extracellular signal-regulated kinase (MEK) pathway and is already approved for clinical use in patients with melanoma. The authors suggest that the beneficial effects of trametinib are mediated through suppression of the indirect pathway of allorecognition when administered after initial conditioning with a calcineurin inhibitor such as cyclosporine. Preconditioning with cyclosporine served to suppress direct allorecognition, which allegedly is not affected by trametinib. The authors also show that trametinib by itself could delay chronic rejection in the minor MHC-mismatched model, which is predominantly mediated by indirect allorecognition. Furthermore, differentiation of $CD4^+$ and $CD8^+$ T cells in the thymus, as well as thymus function, was preserved in recipients treated with trametinib in lieu of cyclosporine. The authors reference seminal papers that demonstrated that thymus atrophy induced by calcineurin inhibition can increase infectious complications after transplantation. Nevertheless, the role of a functioning thymus in solid organ transplantation remains unclear, with preclinical studies suggesting that the thymus might be dispensable for long-term acceptance of MHC-disparate lung allografts (3).

The immunosuppressive regimens used for lung transplantation are mostly extrapolated from studies in renal, liver, and heart transplantation, and typically consist of a three-drug combination including a calcineurin inhibitor (such as tacrolimus or cyclosporine), an antimetabolite (such as mycophenolate mofetil or azathioprine), and a glucocorticoid (prednisone). However, solid organ transplantation has lagged in the introduction of novel immunosuppressive agents. In particular, targeted therapies to inhibit immune pathways relevant to allograft rejection need to be evaluated. Hence, this study may have a great clinical impact because it introduces the possibility of inhibiting the RAS/MEK/ERK pathway, using agents such as trametinib, after lung transplantation to suppress indirect allorecognition and reduce the incidence of chronic rejection. However, why MEK inhibition would preferentially suppress indirect rather than direct allorecognition remains unclear given that the proposed T-cell receptor activation plays a role in both (4). As such, the precise mechanism through which trametinib leads to the observed delay in chronic rejection in this study remains unknown. Further preclinical and clinical studies are required to validate these findings and determine the safety of this drug class in transplant recipients. It is also noteworthy that, in contrast to rodent models, the transition of direct to indirect allorecognition after human lung transplantation is neither linear nor predictable. Professional antigen-presenting cells of donor origin (such as alveolar macrophages) that are capable of direct allopresentation can persist in the allograft for prolonged periods of time in human recipients (5). Hence, replacement of calcineurin inhibitors with a drug such as trametinib in human lung transplant recipients may lead to acute allograft rejection even at late time points after transplantation, assuming that its mechanism of action is suppression of indirect allorecognition. Nevertheless, if replacement of calcineurin inhibitor with trametinib is found to be clinically effective, it would be impactful given the other major adverse effects associated with calcineurin inhibitors, such as renal failure. Alternatively, the possibility of using calcineurin inhibition with trametinib is also intriguing. Recent data show that the MEK pathway might play a role in the development of ischemia-reperfusion injury after solid organ transplantation (6), which has emerged as the predominant risk factor for short-term mortality as well as the development of chronic lung allograft rejection (7). Accordingly, early introduction of MEK inhibitors may ameliorate both ischemia-reperfusion injury and chronic rejection.

The immunopathogenesis of chronic human allograft rejection is multifactorial, and tissue-restricted autoimmunity in particular is increasingly recognized as the final terminal pathway leading to chronic lung allograft rejection (8). We previously found that regulatory T cells (Tregs) are reduced over time in lung transplant recipients (9), and their dysfunction leads to the development of *de novo* lung-restricted autoimmunity after transplantation and chronic rejection (10). It is known that the survival of Tregs is dependent on IL-2, which is suppressed by calcineurin inhibition, the same mechanism that suppresses effector T cells (11). Hence, long-term use of calcineurin inhibitors can lead to the loss of Tregs, a mechanism that has been proposed to prevent the development of allotolerance. The preservation of Tregs in the trametinib-treated recipients observed in this study is highly significant and could be of great clinical benefit. Using cell-surface markers, the authors also showed suppression of B cells in the graft, peripheral blood, and spleen. This may have clinical benefits, as this strategy could potentially suppress the development of lung-restricted autoantibodies, which are causally linked to chronic allograft rejection in both human and rodent models [12, 13], after transplantation.

The role of the RAS/MEK/ERK pathway in mediating human disease is being recognized beyond the context of cancer biology. Although it was not the focus of the present paper, a potential benefit

of drugs such as trametinib is the inhibition of the pathogenic effects of both donor-specific MHC and lung-restricted antibodies. The conventional treatment for allograft rejection mediated by these antibodies includes antibody-directed therapies such as intravenous immunoglobulins and plasmapheresis; however, they remain ineffective due to the lack of agents to target the downstream signaling pathways triggered by the ligation of these antibodies with the Fc receptors on the immune cells. Cross-linking of Fc receptors on immune cells can trigger the production of inflammatory mediators by signaling pathways via activation of MEK/ERK or the PI3K pathway (14, 15). Unpublished data from our lab show that stimulation of the recently identified pulmonary intravascular nonclassical monocytes with lung-restricted autoantibodies can produce neutrophil chemoattractants through the MEK/ERK pathway, which is detrimental to the function of the allograft.

In summary, the work by Takahagi and colleagues highlights several potential mechanisms by which the benefits observed with MEK inhibition can be translated to human subjects, and strongly argues for future studies to explore the role of this strategy in improving outcomes after human lung transplantation. ■

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References

1. Chambers DC, Cherikh WS, Goldfarb SB, Hayes D Jr, Kucheryavaya AY, Toll AE, *et al*.; International Society for Heart and Lung Transplantation. The International Thoracic Organ Transplant Registry of the International Society for Heart and Lung Transplantation: thirty-fifth adult lung and heart-lung transplant report-2018; focus theme: multiorgan transplantation. *J Heart Lung Transplant* 2018;37:1169–1183.
2. Takahagi A, Shindo T, Chen-Yoshikawa TF, Yoshizawa A, Gochi F, Miyamoto E, *et al*. Trametinib attenuates delayed rejection and preserves thymic function in rat lung transplantation. *Am J Respir Cell Mol Biol* 2019;61:355–366.
3. Sahara H, Weiss MJ, Ng CY, Houser SL, Pujara AC, Sayre JK, *et al*. Thymectomy does not abrogate long-term acceptance of MHC class I-disparate lung allografts in miniature swine. *Transplant Proc* 2006;38:3253–3255.
4. Gauthier JM, Li W, Hsiao HM, Takahashi T, Arefanian S, Krupnick AS, *et al*. Mechanisms of graft rejection and immune regulation after lung transplant. *Ann Am Thorac Soc* 2017;14(Suppl 3):S216–S219.
5. Nayak DK, Zhou F, Xu M, Huang J, Tsuji M, Hachem R, *et al*. Long-term persistence of donor alveolar macrophages in human lung transplant recipients that influences donor-specific immune responses. *Am J Transplant* 2016;16:2300–2311.
6. Kaizu T, Ikeda A, Nakao A, Tsung A, Toyokawa H, Ueki S, *et al*. Protection of transplant-induced hepatic ischemia/reperfusion injury with carbon monoxide via MEK/ERK1/2 pathway downregulation. *Am J Physiol Gastrointest Liver Physiol* 2008;294:G236–G244.
7. Bharat A, Kuo E, Steward N, Aloush A, Hachem R, Trulock EP, *et al*. Immunological link between primary graft dysfunction and chronic lung allograft rejection. *Ann Thorac Surg* 2008;86:189–195. [Discussion, pp. 196–197.]
8. Hachem RR, Tiriveedhi V, Patterson GA, Aloush A, Trulock EP, Mohanakumar T. Antibodies to K-α 1 tubulin and collagen V are associated with chronic rejection after lung transplantation. *Am J Transplant* 2012;12:2164–2171.
9. Bharat A, Fields RC, Steward N, Trulock EP, Patterson GA, Mohanakumar T. CD4+25+ regulatory T cells limit Th1-autoimmunity by inducing IL-10 producing T cells following human lung transplantation. *Am J Transplant* 2006;6:1799–1808.
10. Chiu S, Fernandez R, Subramanian V, Sun H, DeCamp MM, Kreisel D, *et al*. Lung injury combined with loss of regulatory T cells leads to de novo lung-restricted autoimmunity. *J Immunol* 2016;197:51–57.
11. Chinen T, Kannan AK, Levine AG, Fan X, Klein U, Zheng Y, *et al*. An essential role for the IL-2 receptor in T_{reg} cell function. *Nat Immunol* 2016;17:1322–1333.
12. Bharat A, Chiu S, Zheng Z, Sun H, Yeldandi A, DeCamp MM, *et al*. Lung-restricted antibodies mediate primary graft dysfunction and prevent allotolerance after murine lung transplantation. *Am J Respir Cell Mol Biol* 2016;55:532–541.
13. Fernandez R, Chiu S, Raparia K, Garcha P, Farver C, Budev M, *et al*. Humoral human lung allograft rejection by tissue-restricted non-HLA antibodies. *Ann Thorac Surg* 2016;102:e339–e341.
14. García-García E, Sánchez-Mejorada G, Rosales C. Phosphatidylinositol 3-kinase and ERK are required for NF-kappaB activation but not for phagocytosis. *J Leukoc Biol* 2001;70:649–658.
15. Rosales C. Fcγ receptor heterogeneity in leukocyte functional responses. *Front Immunol* 2017;8:280.