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# *Ex Vivo* Lung Perfusion Provides New Insights into Human Lung-Resident Immune Cell Localization and Functional Interactions

Our immune system functions from the level of the whole organism down to the complex assortment of cells permanently or transiently residing within individual tissues. Peripheral tissues are constantly exposed to pathogens, requiring a specialized set of immune cells to recognize these threats. Tissue-resident memory T ($T_{RM}$) cells, a noncirculating population of effector memory T cells, are considered to be a major player for protection from infection and cancer and participate in autoimmunity, allergy, and organ transplant rejection (1, 2). How $T_{RM}$ cells are recruited, maintained, and activated or reactivated are critical concepts in the development of successful preventative and therapeutic measures. Ongoing generation and maintenance of $T_{RM}$ cells in the lung is of particular interest, as the harsh environment of the lung may not allow for extended persistence of T-cell populations (3).

Much of what we currently understand about $T_{RM}$ cells comes from the use of animal models (2). The ability to perform techniques like intravascular staining in mouse models has transformed our understanding of this cell population, particularly in highly vascularized tissues such as the lungs (2, 4). Although the use of animal models is instrumental to our understanding of immune responses, there are nonetheless substantial differences between human and animal immune systems, particularly rodent models (5). Nonhuman primate and porcine animal models, although more representative of humans, are limited by cost and availability of facilities (6, 7). Numerous two- and three-dimensional models such as air–liquid interfaces (8) and organoids (9) have been established with human cells; however, they cannot model complex interactions between cells in the context of the whole organism. In this issue of the *Journal*, Snyder and colleagues (pp. 1230–1244) use *ex vivo* lung perfusion (EVLP) of human lungs in a novel way to explore interactions between $T_{RM}$ cells and lung-resident macrophages ($M_{LR}$s) within a whole organ (10).

EVLP has been used in transplantation after a clinical trial reported positive outcomes in 2011 (11). Since this time, EVLP has been used in translational research as it closely models the *in vivo* environment of the lung. For example, EVLP has been used in the pretransplantation treatment of massive pulmonary embolism and reduction of the antimicrobial burden, and to test chemotherapeutic and other anticancer drugs on lesions (reviewed in Reference 12). Much of the research using EVLP is aimed at whole lung processes, and by and large, has a goal of improving lung function in the broad context of transplantation and disease. There is little evidence for the use of EVLP to study how individual immune cell populations function in relationship to each other. In a recent article, Dumigan and colleagues performed EVLP experiments with porcine lungs, demonstrating that the EVLP model results in tissue pathology that resembles that seen *in vivo*, and further used EVLP to better understand the role of macrophage polarization in the immune response and pathology associated with *Klebsiella pneumoniae* infection (13), demonstrating the potential of EVLP for this purpose.

The results presented by Snyder and colleagues (10) support the innovative use of EVLP for the study of complex human immune cell interactions and provide unexpected findings about tissue-resident immune cell populations in human lungs. To demonstrate the utility of EVLP for this purpose, the group first used *ex vivo* CD45 staining to distinguish immune cells in circulation from those in the parenchyma, which they dubbed "protected" cells. As expected, the phenotype of protected $CD4^+$ and $CD8^+$ tissue cells include the expression of CD103 and CD69. For myeloid origin cells, protected $M_{LR}$s closely resembled alveolar macrophages, expressing CD64 and CD206. These protected $T_{RM}$ cells and $M_{LR}$s were colocalized in the airways.

Interestingly, they found two major distinct protected $T_{RM}$ cell populations: all $T_{RM}$ cells were $CD45^+CD14^-CD206^-CD4/CD8^+CD69^+$, and either $PD1^{hi}$ or $PD1^{lo}$, with different expression of the transcriptional factors HOBIT and TBET. Particularly in the $CD8^+$ $T_{RM}$ cell compartment, $PD1^{hi}HOBIT^{lo}$ ($PD1^{hi}$) and HOBIT/$TBET^{hi}PD1^{lo}$ ($HOBIT^{hi}$) were functionally distinct subsets, preferentially producing higher effector cytokines or higher baseline granzyme B, respectively. In previous reports, $CD103^+$ $T_{RM}$ cells in human lung did not express TBET and eomesodermin (14, 15), HOBIT and Blimp-1 were not to be expressed by $CD103^+$ lung $T_{RM}$ cells compared with their expression by circulating $T_{EM}$ cells in mice (16). Moreover, tumor-infiltrating $CD8^+$ $CD103^+$ T cells have increased expression of TBET (14), suggesting that human lung $T_{RM}$ cells have a capacity to adapt to the microenvironment. It was also reported $CD4^+$ cells and IFN-γ are required for generating $CD8^+$ lung $T_{RM}$ cells in mice (17). Given that both $CD4^+$ and $CD8^+$ have a unique transcriptional profile in human subjects with EVLP compared with animal models and other sampling methods with human subjects, further experimentation with $CD4^+$ and $CD8^+$ interactions is required.

Lung-resident myeloid cells are believed to participate in the development and activation of $T_{RM}$ cells based on several animal models. For example, lung-resident dendritic cells (DCs) that present antigen and express TGF-β are believed to be crucial to the development of lung $T_{RM}$ cells in mice (18). In the nonhuman

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primate model, interstitial macrophages differ from alveolar macrophages in that they have high turnover in the lung and increased expression of TNF-α in response to IFN-γ plus LPS treatment (19). Interestingly, Snyder and colleagues (10) found that human lung parenchyma was enriched $CD64^+CD206^+$ (alveolar) macrophages compared with DCs. Furthermore, $M_{LR}$ could induce cytokine response from $CD4^+$ or $CD8^+$ $PD1^{hi}$ cells, suggesting potential differences between the murine and EVLP human model. The minor population of DCs could also have the potential to influence $T_{RM}$ cell function, however, and further study of the $T_{RM}$ cell, $M_{LR}$, and DC interaction in the development and maintenance of $T_{RM}$ cells is necessary.

Although these results provide exciting evidence for the use of EVLP of human lungs for immunological studies, there are nonetheless caveats that should be considered. EVLP provides an ability to look at complex cell interactions within the context of the whole lung, however, it is not feasible to evaluate long-terms kinetics in infection or disease models. Although these studies would be limited to those cells already present in the lung, the ability to obtain lungs from subjects with existing disease conditions could allow for short-term studies of cellular interactions and pathogenesis early in infection. There are also potential concerns with EVLP-induced inflammation or altered cellular metabolism that should be considered. Finally, it remains to be seen whether EVLP will be translatable to a larger number of laboratory groups.

Despite these limitations, EVLP should be considered as a promising model by which to study numerous human lung-resident cell populations *in situ*, as well as to specifically isolate tissue-resident populations of these cells for additional phenotypic and functional analyses. ■

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