Balancing Act: PD-1, PD-L1, and the Inflammatory Tightrope of Acute Respiratory Distress Syndrome

PD-1 (programmed cell death protein 1) and its cognate ligands (PD-L1 and PD-L2) are cell surface proteins that act as "checkpoints" of the adaptive immune system and promote tolerance to self-antigen. PD-L1 expressed by antigen-presenting cells binds PD-1 receptor on T cells inhibiting proliferation, production of cytokines, and other antigen-specific responses. Soluble forms of the proteins generated by splice variants or proteinase cleavage likely function similarly to cell-bound forms. The proteins have been best studied in malignancy, where expression of PD-L1 by cancer cells allows them to evade the immune system. Monoclonal antibody antagonists effectively target this axis, stimulate T-cell–mediated killing of cancer cells, and improve survival in many cancer types. In comparison, the role of PD and PD-L signaling in diseases of exuberant or "unchecked" inflammation is poorly understood. Theoretically, stimulation of the PD and PD-L axis in diseases such as sepsis or acute respiratory distress syndrome (ARDS) would be expected to decrease inflammation and promote adaptive immune responses, whereas blockade could help stimulate the immune system to combat secondary infections.

Several lines of evidence suggest that the PD and PD-L axis is a major regulator of inflammation in the lung, yet a significant knowledge gap exists regarding expression patterns and downstream signaling consequences of these proteins during acute lung inflammation. In this issue of the *Journal*, Morell and colleagues (pp. 534–545) address this gap by performing in-depth characterization of soluble and membrane-bound forms of PD1, PD-L1, and PD-L2 in the blood and lungs of patients with ARDS and control subjects without ARDS and correlating their findings with clinical outcomes (1).

To begin, the authors measured soluble ligand concentrations in blood and respiratory specimens of patients with ARDS and control subjects without ARDS from three large clinical cohorts. They compared protein amounts with relevant ARDS outcomes, including 28-day mortality and ventilator-free days, and found that increased concentrations of soluble PD-L1 (sPD-L1) were associated with death in all cohorts and decreased ventilator-free days in two of three cohorts. sPD-1 and sPD-L2 were detected in all samples but did not associate with ARDS outcomes. Interestingly, lung sPD-L1 measurements exhibited a wide range of concentrations, likely because of the diluting technique of BAL, and did not correlate with outcomes or were slightly protective, depending on the cohort.

Next, the authors explored cell surface expression of PD-1 and PD-L1 on cells obtained from paired BAL and blood from patients with ARDS or matched control subjects. Using mass cytometry, they determined that ARDS is associated with decreased proportions of airspace macrophages (AMs) expressing PD-L1 and increased proportions of airspace T cells expressing PD-1. Because absolute numbers of cells were similar between conditions, they conclude that these findings likely represent differences in absolute numbers of each cell type within the lung. Notably, these differences were not present in blood monocytes and T cells, highlighting the unique microenvironment and cellular dynamics within the lung.

In an attempt to ascribe functional context to their findings, the authors next stimulated AMs and T cells in cocultures and found that IFNγ and TNF-α expression was higher in PD-1–expressing $CD4^+$ T cells than PD-1–negative cells.

Concordantly, PD-L1–expressing AMs expressed less IL-8 than PD-L1–negative AMs. Last, addition of PD- L1 blocking antibodies to AM and T-cell cocultures led to slight decreases in IFNγ and TNF-α.

Taken together, the study provides a comprehensive assessment of checkpoint protein expression patterns and several potential cellular sources during ARDS. Furthermore, the data provide convincing evidence of an imbalance between high numbers of PD-1–expressing T cells and decreased numbers of PD-L1–expressing macrophages in the lungs of patients with ARDS and an association between elevated sPD-L1 in the blood and poor patient outcomes.

In addition to the tested hypotheses, several other important insights emerge from the study. First, the study adds to a growing body of evidence showing that the bloodstream serves as a poor surrogate for understanding the unique microenvironment of the airspaces. Because immune cells and signaling molecules may passively or actively move between compartments and may be influenced by factors such as the degree of destruction of the alveolar/capillary barrier (2) and the BAL technique (3), inferring correlation is difficult. Morell and colleagues should be commended for measuring molecules of interest by compartment and cell type, across multiple cohorts from multiple institutions.

Opposing associations of sPD-L1 concentrations in blood versus BAL with mortality suggests different cellular sources or mechanisms of action in these compartments. For instance, although T cells and AMs were the focus of the study, endothelial cells, innate lymphoid cells, dendritic cells, and neutrophils (4) may express PD-1 and/or PD-L1, and their expression patterns may be differentially induced by various cytokines and pathogen-associated molecules, many of which do not correlate between blood and BAL. Hence, it is possible that AMs are the main sources of sPD-L1 in the lung and that higher amounts of sPD-L1 reflect a more balanced AM:T-cell repertoire predictive of recovery. Conversely, sPD-L1 may derive from endothelial cells in the blood, and higher concentrations may indicate endothelial damage, predicting poor patient outcome.

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In addition, PD-1 and sPD-L1 may have roles in regulating the immune response beyond their effects on T cells. In murine models, sPD-L1 can induce macrophage apoptosis (5) and neutrophil extracellular trap activation (6), which may induce downstream effects in various cell types.

Given these considerations, additional studies are needed to understand the potentially unique roles of sPD-L1 in the blood versus lung.

Interestingly, the current findings provide a sharp contrast to a prior study in which sPD-L1 measured in blood was associated with improved survival in ARDS (5). Although an explanation for these disparate findings is not readily apparent, several possibilities, including ARDS etiology, patient characteristics, and clinical variables, may be posited. Each of these likely fuels the increasingly recognized heterogeneity of ARDS and suggests that a personalized approach to understanding PD-1 and PD-L1 expression in ARDS may be needed to identify patients for whom manipulation of PD-1 or PD-L1 may be beneficial.

The author team should be commended for their collaborative efforts, yielding data from three unique, large cohorts of patients across multiple institutions. Although the populations differ in several characteristics, the complementary data across cohorts provides validity to the findings and suggests conserved biologic mechanisms across etiologies of ARDS. In sum, the manuscript provides a strong impetus for future studies aimed at identifying the cellular sources and precise functions of checkpoint molecules in ARDS to determine whether targeted intervention on this important pathway may, indeed, provide therapeutic benefit. ■

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