Mechanisms of Graft Rejection and Immune Regulation after Lung Transplant

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Abstract

Outcomes after lung transplant lag behind those of other solid-organ transplants. A better understanding of the pathways that contribute to rejection and tolerance after lung transplant will be required to develop new therapeutic strategies that take into account the unique immunological features of lungs. Mechanistic immunological investigations in an orthotopic transplant model in the mouse have

shed new light on immune responses after lung transplant. Here, we highlight that interactions between immune cells within pulmonary grafts shape their fate. These observations set lungs apart from other organs and help provide the conceptual framework for the development of lung-specific immunosuppression.

Keywords: lung transplant; acute rejection; tolerance; lymphoid neogenesis

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Advances in surgical techniques and perioperative management have resulted in a significant change in the early mortality rate after lung transplant. Initial reports of lung transplant outcomes in 1990 described 1-year survival rates of around 40%, while modern day 1-year survival rates have risen to 83% (1, 2). Nevertheless, lung transplant recipients are plagued by poor long-term outcomes compared with other solid-organ transplant recipients (3). In the modern era of lung transplantation, the 5-year survival rate is approximately 50%, a figure that has not changed substantially over the past 2 decades (1). Investigations by our group and others have suggested that these relatively disappointing outcomes may be related at least in part to the use of immunosuppressive regimens that do not take into account the unique immunological properties of lungs (4–8). In fact, currently used immunosuppression for pulmonary transplant recipients is based largely on

strategies that have been developed and proven efficacious for other organs.

Long-term survival after lung transplant is limited by chronic lung allograft dysfunction, a condition for which no effective medical treatment exists. One of the main risk factors for the development of chronic lung allograft dysfunction is acute rejection (9). Approximately 60% of lung recipients experience acute rejection during the first year after transplant. Even a single episode of mild acute rejection has been shown to be an independent risk factor for the development of chronic lung allograft dysfunction (10). A mechanistic understanding of acute lung allograft rejection has stemmed largely from small-animal models of pulmonary transplant. For example, experiments in the 1980s using orthotopic pulmonary transplant models in rats suggested that the pathways leading to acute rejection of lungs may differ from those of other organs (11). In 2007, our group reported the development of an

orthotopic lung transplant model in the mouse (12). Importantly, when lungs are transplanted across major histocompatibility complex (MHC) barriers in nonimmunosuppressed mice, histological characteristics of acute rejection resemble those observed in human lung grafts (13). Taking advantage of transgenic and knockout strains, we and others have been able to gain new mechanistic insight into the immunological pathways that regulate acute rejection and tolerance after lung transplant (4,14–16). Here, we review three observations that set the lung apart immunologically from other organ grafts and suggest that immunosuppressive strategies must be tailored specifically toward lungs (Figure 1).

Location of Alloimmune Response

Recognition of alloantigens by the host immune system after transplant can occur

Figure 1. Mechanisms of rejection and immune regulation in lung allografts. (*Left panel*) During acute rejection, recipient T cells cluster around donor-derived CD11c⁺ cells with a dendritic morphology in the lung allograft, a process that occurs independently of secondary lymphoid organs. (*Middle panel*) C-C chemokine receptor type 7 (CCR7)-expressing central memory CD8⁺ T cells (CD8 CM) interact with CD11c⁺ cells and facilitate immune tolerance induction; this process triggers the release of nitric oxide (NO) in an IFN- γ dependent manner. (*Right panel*) Allograft tolerance is associated with induction of bronchus-associated lymphoid tissue (BALT) in the graft that is rich in Foxp3⁺ regulatory T cells.

through two distinct pathways, known as the direct and indirect allorecognition pathways. The direct pathway occurs when recipient T cells recognize intact donor major histocompatibility complexes on donor antigen-presenting cells, whereas the indirect pathway refers to the presentation of processed donor alloantigen by recipient antigen-presenting cells in a self-MHC-restricted fashion. Of note, a “semidirect pathway” has also been described, in which recipient antigen-presenting cells acquire intact donor MHC molecules, which they present on their surface to directly alloreactive T cells (17). For decades, it had been the accepted paradigm that directly or indirectly alloreactive T cells could be activated only in secondary lymphoid organs, such as draining lymph nodes or the spleen. This notion was established on the basis of landmark studies in which the rejection of skin grafts in guinea pigs was prevented by disrupting the afferent

lymphatic drainage of the tissue bed (18). Furthermore, it should be noted that splenectomized alymphoplastic mice that lack peripheral lymph nodes because of a genetic mutation are not able to reject allogeneic cardiac grafts (19).

On the basis of reports in models of asthma and respiratory viral infections suggesting that antigen-presenting cells can activate CD4⁺ and CD8⁺ T cells locally within the lung, our laboratory set out to examine whether cell trafficking to secondary lymphoid organs was necessary to trigger acute lung allograft rejection (20, 21). We transplanted lungs derived from B6 mice that expressed a yellow fluorescent protein under a CD11c promoter into allogeneic CBA mouse recipients (14). Using two-photon microscopy, we observed that, within 30 hours after transplant, recipient (CBA) T cells were clustering around donor-derived (B6) CD11c⁺ cells with a dendritic

morphology in allogeneic lung tissue, suggesting the rapid activation of direct allorecognition within the pulmonary graft (Figure 1). Perhaps more importantly, pulmonary allografts were acutely rejected after transplant into splenectomized alymphoplastic allogeneic hosts (mice that lack lymph nodes), demonstrating that, unlike the case for other organs, acute lung rejection is not dependent on secondary lymphoid organs. In subsequent work, we showed that recipient T cells can interact with recipient CD11c⁺ cells within lung allografts, many of which express donor MHC molecules on their surface (22). These observations suggest that the indirect and semidirect allorecognition pathways can also be activated locally. On the basis of these observations, lungs have been referred to as “lymph nodes with alveoli” (23). The fact that lungs provide a suitable environment for the activation of alloreactive T cells may explain the comparatively rapid onset of acute pulmonary rejection that has been observed previously in experimental models (24). These findings provide a framework for the development of local immunosuppressive strategies for lung transplant recipients.

Tolerance Induction

Having demonstrated that interactions between immune cells within lungs can shape the fate of grafts, we set out to further characterize early events of immune recognition after pulmonary transplant. We have shown that perioperative blockade of the B7-CD28 and CD40-CD40 ligand costimulatory pathways mediates long-term survival of fully MHC-mismatched lung allografts (5). It has been recognized in both experimental and clinical settings of organ transplant that memory CD8⁺ T cells promote alloimmunity and are relatively resistant to immunosuppression (25–27). In fact, memory CD8⁺ T cells are widely considered to represent a barrier to tolerance induction, and strategies have been developed to target this cell population to improve outcomes after transplant. Surprisingly, our work in the mouse lung transplant model revealed that graft-infiltrating central memory CD8⁺ T cells are critical in inducing tolerance in hosts that are treated with perioperative costimulatory blockade (4). We showed mechanistically that graft-infiltrating central memory CD8⁺ T cells trigger the

production of nitric oxide in an IFN- γ -dependent fashion (Figure 1). Infiltration of CD8⁺ T cells into other grafts including the kidney and heart depends on alloantigen recognition and occurs independent of chemokine receptor signaling (28). By contrast, migration of central memory CD8⁺ T cells into lung allografts depends on both alloantigen recognition and chemokine receptor signaling. Interestingly, the expression of CCR7 on CD8⁺ T cells played a critical role in tolerance induction. This chemokine receptor, which is expressed on central memory T lymphocytes and regulates trafficking of these cells into secondary lymphoid organs, mediated durable interactions between CD8⁺ T cells and CD11c⁺ antigen-presenting cells within lung allografts, which correlated with their production of IFN- γ . Thus, indiscriminate T-cell depletion at the time of lung transplant may be deleterious because it targets a potentially beneficial T-lymphocyte population (29). Clearly, our mouse data will have to be validated in preclinical large-animal models. However, it is important to point out that immunosuppressive agents that are currently in clinical use for lung transplant recipients interfere with the pathways that we have shown to be critical for tolerance induction in our mouse model.

Lymphoid Neogenesis and Tolerance

The accumulation of ectopic lymphoid tissue in a nonlymphoid tissue is known as lymphoid neogenesis; such foci are termed “tertiary lymphoid organs” and they are made up predominantly of T cells, B cells, CD11c⁺ antigen-presenting cells, and high-endothelial venules expressing peripheral node addressin (30). In 2005, Baddoura reported the results of an experiment in which 319 murine cardiac transplants were examined for evidence of tertiary lymphoid organs and acute or chronic rejection (31). These

investigators found evidence of lymphoid neogenesis, either organized lymphoid aggregates or peripheral nodal addressin-expressing high-endothelial venules, in 25% of heart allografts. These structures were generally associated with acute or chronic rejection. Randall’s group described inducible ectopic accumulations of lymphoid tissue in the airways of mice and humans in response to inflammation or infection; these lesions were termed inducible bronchus-associated lymphoid tissue (BALT) (21, 32). It was hypothesized that these BALT foci propagate cellular and humoral immune responses locally within the lung (33). On the basis of such observations, tertiary lymphoid organs within allografts have been referred to as the “murderer in the house,” because they represent a site in which alloimmune responses can be triggered and can contribute to chronic graft failure (34).

Interestingly, we observed the presence of tertiary lymphoid organs in tolerant lung allografts (5). These structures contained T cells, B cells, CD11c⁺ antigen-presenting cells, peripheral nodal addressin-expressing high-endothelial venules, and homeostatic chemokines (Figure 1). As in Randall’s reports, these organized structures were associated with airways and vessels (33). Remarkably, these tertiary lymphoid organs were rich in Foxp3⁺ regulatory T cells. We showed that the majority of recipient T cells that infiltrate tolerant lung grafts traffic preferentially to the tertiary lymphoid organs. Retransplant experiments suggested that inducible BALT is important in maintaining a tolerant state. We initially transplanted B6 lungs into allogeneic CBA recipients that were treated with perioperative costimulatory blockade, a regimen that we have shown can mediate graft survival in excess of 1 year (5). Thirty days after the initial transplant, we retransplanted these B6 lungs that now harbored BALT into nonimmunosuppressed secondary CBA recipients and observed that these grafts survived for at least 30 days. This is markedly different from the fate of

B6-naïve lungs that are transplanted into nonimmunosuppressed CBA hosts; these lungs undergo rejection-mediated necrosis by 30 days after engraftment. Even a time period of only 3 days in an immunosuppressed host was sufficient to protect lung allografts from immunological destruction after retransplant into a secondary nonimmunosuppressed recipient. These results indicate that immunoregulatory circuits are established in lung allografts within a short period of time when treated with immunosuppression. Analogous cardiac retransplant experiments showed that such immune regulation was not established locally within hearts, which is consistent with previous demonstrations that tolerance induction after heart transplant is dependent on cell trafficking to secondary lymphoid organs (35). Thus, interactions between immune cells within lung grafts early after transplant can regulate both rejection and tolerance.

Conclusions

Murine models of transplant have provided significant insight into the mechanisms of injury and immune regulation that occur during acute allograft rejection. The development of the mouse lung transplant model has enabled the discovery of immune pathways that differ between lungs and other organs. Lung grafts are active immunological sites, and our research thus far has shown that both deleterious and beneficial responses can be generated locally. Although these principles must be translated to preclinical models, our findings challenge the current practice of adopting immunosuppression that is efficacious for other organ recipients to patients undergoing a lung transplant. ■

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

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