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Autoimmune Responses to Grafted Lungs: Immune Responses to a Native Collagen—Type V Collagen

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Lung transplantation is the only definitive treatment modality for many forms of end-stage lung disease. However, the lung is rejected more often than any other type of solid organ allograft due to chronic rejection known as bronchiolitis obliterans (BO). Indeed, BO is the primary reason why the 5- and 7-year survival rates are worse than any other transplanted organs. Alloimmunity to donor antigens is established as the primary mechanism that mediates rejection responses. However, newer immunosuppressive regimens designed to abrogate alloimmune activation have not improved survival. Therefore, these data suggest that other antigens, unrelated to donor transplantation antigens, are involved in rejection. Utilizing human and rodent studies of lung transplantation, our laboratory has documented that a native collagen, type V collagen (col(V)), is a target of the rejection response. Since col(V) is highly conserved, these data indicate that transplant rejection involves both alloimmune and autoimmune responses. The role of col(V) in lung transplant rejection is described in this review article.

Keywords: autoimmunity, type V collagen, allore cognition, lung transplantation

Introduction

Lung transplantation is the only definitive treatment for many forms of end-stage lung disease such as emphysema, idiopathic pulmonary fibrosis, and cystic fibrosis. The first lung transplants were performed nearly 40 years ago, and currently, more than 1400 lung transplants are performed annually. However, the survival of the transplant recipient is limited by the development of chronic rejection known as bronchiolitis obliterans (BO), the leading cause of death in lung allograft recipients. Indeed, BO is the primary reason why the 5- and 7-year survival rates of lung allograft recipients are less than 50% and 35%, respectively, posttransplantation, the worst survival data for all recipients of solid organ allografts (Fig. 1). The poor survival statistics take on a new importance when considered in the context of advancements in surgical techniques, immunosuppression, and other supportive measures developed for the care of these patients over the past 20 years. In sum, the current sophistication in treatment regimens has not been translated into improved survival of lung transplant recipients.

Repeated acute rejection episodes are believed to be the main risk factor for the development of BO. Rejection episodes are initiated by recipient T cells recognizing polymorphisms in donor major histocompatibility complex (MHC) antigens. Alloreactive T cells induce cellular immune responses that culminate in graft destruction. Accordingly, therapies to prevent rejection have focused on downregulating alloimmune responses. However, the incidence of BO in patients has remained constant for the past several years despite the development of newer therapeutic agents that prevent alloimmunity. This observation suggests that other antigens, unrelated to MHC molecules, may be involved in the rejection process.
Similar to reports in this forum from Drs. Benichou and Heeger describing immune responses to self-antigens in heart and renal allografts, respectively, our laboratory has determined that immunity during lung allograft rejection involves an immune response to another self-antigen, type V collagen (col(V)).

All collagen molecules are triple helices composed of $\alpha$ chains. Col(V) is a 116 kD heterodimer composed of $\alpha_1$ and $\alpha_2$ chains. In the lung, col(V) is considered a minor collagen, located within the perivascular and peribronchiolar connective tissues, which are the same sites of rejection activity. Data showing that col(V) is a target of the immune response during lung allograft rejection and that recognition of polymorphisms in donor MHC antigens stimulate rejection activity suggested that col(V) may have partial sequence homology to MHC proteins. Interestingly, the immune response to col(V) in lung transplantation is directed primarily against the $\alpha_1$ chain of col(V)($\alpha_1(V)$). $\alpha_1(V)$ is nearly 80% homologous to the $\alpha_2$ chain of type XI collagen ($\alpha_2(XI)$), and the gene for $\alpha_2(XI)$ maps within the MHC class II loci in humans and mice. Although these data suggest col(V) peptides may have sequence homology to MHC antigens, analysis of amino acid sequences did not reveal any primary homology between col(V) and MHC molecules. However, primary se-

Key Points

1. Lung allograft rejection involves both alloimmune and autoimmune responses.
2. Autoimmunity to col(V) may perpetuate lung allograft rejection and abrogate tolerance induction.
3. Col(V) antigens are recognized by indirect recognition during lung allograft rejection.
4. Col(V)-induced oral tolerance results in the activation of regulatory T cells that suppress alloimmune responses.

Figure 1. Survival data for recipients of solid organ allografts (2002). Data adapted from the Collaborative Transplant Study Group with permission.
quence homology to alloantigens alone may not be required to induce alloimmunity. For example, Luz et al. recently reported that a single amino acid substitution in a peptide bound to MHC molecules that alters the affinity of the MHC-peptide complex to the T cell receptors may determine the difference between autoreactivity or alloreactivity. These data suggest that secondary or tertiary characteristics of the peptide, affinity of peptide for the T cell receptor, or other factors may explain the phenomenon of col(V)-induced immunity during lung allograft rejection.

Immune Response to Col(V) Contribute to the Rejection Response

The first evidence showing that col(V) was involved in local immune responses to lung alloantigens was obtained from our murine model in which repeated intrapulmonary instillations of allogeneic lung macrophages and dendritic cells reproduce the immunology and pathology analogous to acute rejection in recipient lungs. After 4 weekly instillations of allogeneic lung cells, recipient mice develop lymphocytic perivascular and peribronchior infiltrates analogous to grade 1–2 acute rejection and IgG2a antibody deposits in perivascular and peribronchior tissues. Our ongoing studies in human lung allograft recipients undergoing rejection show similar antibody deposits in the transplanted lung and that col(V) is the antigen recognized by these antibodies (Wilkes and Burlingham, manuscript in preparation).

During ontogeny of the immune system, autoreactive T cells, that is, cells that express T cell receptors with high affinity for self-antigens, are deleted by the process of negative selection. However, under normal conditions, T cells with low affinity for self-antigens circulate in the periphery or reside in various organs. Therefore, unless there are perturbations involving immune homeostasis or exposure of sequestered self-antigens, then it is unlikely that autoreactive T cells will become activated. The immune response that occurs during lung allograft rejection may explain the development of autoreactive T cells. As mentioned above, col(V) is located beneath the basement membrane within bronchiolar and vascular tissues in the lung and possibly intercalated within type 1 collagen, the major collagen in the lung. The inflammatory responses and architectural remodeling that occur in these tissues during the rejection response may expose graft-infiltrating lymphocytes to fragments of col(V). Indeed, we reported that lung allograft rejection is associated with the release of col(V) fragments in bronchoalveolar lavage fluid (BAL). Collagen molecules may be degraded by a class of enzymes known as metalloproteinases (MMPs). MMP-2 and MMP-9 are capable of degrading col(V), and Trello et al. reported activity of MMP-2 and MMP-9 in lungs of human transplant recipients during rejection. To support the role of MMPs in the release of col(V) fragments during the rejection response, Figure 2 shows MMP-2 and MMP-9 are active in rat lung allografts during acute rejection. These data support the theory that inflammation and remodeling that occurs during the rejection response may lead to release of potentially antigenic col(V) peptides.

However, the aforementioned data are indirect evidence that immune responses to col(V) are involved in the pathogenesis of lung allograft rejection. Since rejection is mediated by T cells, we...
sought evidence of col(V)-specific cellular immune activity during the rejection response. T cells isolated from the lungs of mice that receive instillations of allogeneic antigen-presenting cells (APCs) proliferate in response to col(V) but not type II collagen (col(II)), a collagen found in cartilage and not the lung. Similarly, rats develop strong delayed-type hypersensitivity responses, and index of cellular immune responses, to col(V) but not other collagens during lung allograft rejection. Moreover, col(V)-specific T cells are present in the lungs of rats undergoing acute and chronic rejection. These T cells proliferate strongly in response to col(V) and produce copious amounts of the Th1 cytokines, interferon gamma, and TNF-α in response to col(V) and have oligoclonal expression of specific Vβ regions in their T cell receptors. Although adoptive transfer of the col(V)-specific T cells did not induce pathology in lungs of normal rats, transfer of these same cells induced severe acute rejection-like pathology in isograft lungs. The disparity between the ability of these cells to induce disease in normal lungs compared to isograft lungs is likely due to ischemia-reperfusion injury that occurs during transplantation. Indeed, our studies confirm that harvesting and transplantation of isograft lungs, a process that involves ischemia reperfusion, is associated with disruption of the perivascular and peribronchiolar tissues. We hypothesized that this type of injury exposes col(V) to immune cells infiltrating the graft. This hypothesis is supported by data showing release of col(V) fragments in BAL from isografts comparable to that observed in allograft lungs.

Alloimmune responses may occur directly (direct allorecognition) or indirectly (indirect allorecognition). The direct pathway involves presentation of allogeneic MHC class I and II antigens expressed on donor APCs, such as dendritic cells, in the transplanted lung to recipient T cells. The indirect pathway involves processing and presentation of donor MHC antigens by recipient dendritic cells to recipient T cells. The direct pathway is believed to be the primary mechanism of allorecognition in the early transplant period, a time when the transplanted lung is rich in donor APCs. Conversely, indirect allorecognition is believed to be the major pathway of alloimmunity later in the posttransplant period coincident with the replacement of the majority of donor APCs by those of the recipient. Although described classically as a pathway for the presentation of alloantigens, autoantigens involved in the rejection response are presented by the indirect pathway. Furthermore, although the direct pathway may prime alloreactive T cells, epitope spreading that occurs during alloimmune responses can lead to indirect recognition of self-antigens during rejection. For example, direct allorecognition is the mechanism by which allogeneic APCs induce rejection-like responses when instilled into lungs of normal mice. In contrast, col(V)-pulsed autologous APCs do not induce immunologic or histologic alterations when instilled into lungs of normal mice.

However, intrapulmonary instillation of col(V)-pulsed autologous APCs into alloantigen-primed lungs perpetuates the immunology and pathology of the rejection response. The contribution of indirect allorecognition to col(V) reactivity during lung transplant rejection is also exemplified by the rejection response that results from transplanting lungs into recipients mismatched at MHC class I but matched at MHC class II loci. For example, transplantation of F344 rat lungs (RT1b) into WKY rats (RT1), a strain combination matched at MHC class II but mismatched at MHC class I, results in CD4+ col(V)-specific T cells. Since the MHC mismatch occurs at the class I locus, and class I presents antigens to CD8+ T cells, then the development of CD4+ col(V)-specific T cells in this model must occur via indirect allorecognition. These data are similar to those reported by Dr. Heeger and colleagues examining mechanisms of allorecognition in skin graft rejection and Dr. Benichou investigating cardiac allograft rejection. Collectively, these data show that the direct pathway may initiate the rejection response and that the indirect pathway has a key role in autoimmunity triggered by alloimmune responses.

Use of Col(V) to Induce Immune Tolerance to Lung Allografts

Although contributing in the pathogenesis of the rejection response, indirect allorecognition may be used to induce immune tolerance to organ allografts. Non-pharmacologic-induced immune tolerance to solid organ allografts may result from different techniques. These include injection of
donor-derived MHC peptides into the thymus of the recipient prior to transplantation of the allograft or by oral tolerance that refers to feeding donor-derived MHC antigens to the host prior to transplantation. In either setting, donor antigens are believed to be presented indirectly by immature dendritic cells to recipient T cells. Depending on the dose of antigen used, these techniques induce anergy in alloreactive T cells, eliminate alloreactive T cells by clonal deletion, or induce activity of regulatory T cells that actively suppress alloimmune responses.

Data from our studies showing col(V) is an antigen during lung allograft rejection and that col(V)-reactive T cells perpetuated the rejection response suggested col(V) could be used as a tolerogen to prevent lung allograft rejection. To examine this possibility, we used col(V)-induced oral tolerance to determine its effect on acute and chronic lung allograft rejection. WKY (RT1\textsuperscript{lv1}) rats were fed several doses of col(V) prior to transplantation of lung allografts from F344 rats (RT1\textsuperscript{l}). In the absence of any immunosuppression, feeding col(V) prevented the onset of acute lung allograft rejection (Fig. 3) and, most important, abrogated the development of BO (Fig. 4). The ability of col(V)-induced tolerance to prevent rejection was not haplotype specific in that feeding col(V) prevented the onset of acute lung allograft rejection in another unrelated rat strain undergoing lung transplantation.\textsuperscript{5} Importantly, tolerance induced by col(V) did not induce global immune hyporesponsiveness as cellular immune responses to nominal antigens were not suppressed in recipients made tolerant to col(V).\textsuperscript{1}
Examination of the immune mechanisms that mediated suppression of alloreactivity revealed that feeding col(V) followed by lung transplantation resulted in systemic activity of TGF-β that suppressed alloimmune responses during acute and chronic rejection. Clonal deletion of alloreactive T cells was not the mechanism of col(V)-induced oral tolerance as neutralizing TGF-β recovered activity of alloreactive T cells. These data suggested that regulatory T cells that produce TGF-β may have a key role in col(V)-induced oral tolerance. Indeed, data showing that tolerance to lung allografts may be adoptively transferred to naive rats (Wilkes, manuscript in preparation) confirm a role for regulatory T cells in col(V)-induced tolerance. The critical role of presentation of alloantigens in the de-
development of col(V)-induced oral tolerance is exemplified by data showing tolerance could be adoptively transferred only by T cells isolated from lung allograft recipients made tolerant by feeding col(V) and not by T cells isolated from rats fed col(V) that did not receive lung allografts. Furthermore, the overlap of autoreactivity with alloreactivity was also shown by experiments in which adoptive transfer of col(V)-specific T cells abrogated col(V)-induced immune tolerance to lung allografts.  

APC-induced immune activation of T cells is dependent on bi-directional signaling between APC and T cells. Since oral tolerance could affect T cells, as well as APC function, then defective antigen presentation could have contributed to the inability of T cells from tolerant rats to respond to alloantigens. However, data showing that APCs isolated from tolerant allograft recipients were comparable to APCs from normal rats in stimulating proliferation in donor-derived T cells indicated that col(V)-induced oral tolerance affected function of T cells and not APCs.  

Future Directions  
Data showing that col(V) is an antigen during lung allograft rejection and that col(V)-induced oral tolerance prevents lung allograft rejection raise several issues. Perhaps the most important is determination of the epitopes of col(V) that are either tolerogenic or antigenic. This is intriguing in that not all col(V)-reactive T cells induce pathology after adoptive transfer. This suggests that there may be different antigenic regions within col(V) that are recognized as antigens during the rejection response. The difference between antigenic and potentially tolerogenic peptides of col(V) could be related to their primary sequence or affinity for T cell receptors. Data showing that not all col(V) reactive T cells induce disease could also be related to differential expression of co-stimulatory molecules on these cells rendering them less susceptible to activation or more resistant to active suppression by regulatory T cells. These questions are currently under investigation.

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