



Review

The Role of Autoimmunity in the Pathogenesis of Lung Allograft Rejection

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Abstract. For many patients, lung transplantation is the only definitive treatment modality for different forms of end-stage lung disease. However, the lung is rejected more often than any other type of solid organ allografts, and the 5-year survival rate is less than that of other transplanted organs. While alloimmunity directed against donor transplantation antigens is believed to be the key mechanism that mediates rejection responses, newer immunosuppressive regimens designed to abrogate alloimmune activation have not improved survival. Accordingly, these data suggest that other antigens are involved in rejection. Autoimmune responses, reported to occur during allograft rejection, could participate in graft destruction. This review article discusses the role of autoimmune responses to type V collagen, a minor collagen in the lung, in the pathogenesis of lung allograft rejection. By recognizing that lung transplant rejection involves both alloimmune and autoimmune responses, scientific investigation may uncover novel targets for therapeutic intervention that could prolong the life of the lung transplant recipient.

Key words: lung transplantation; alloimmune response; autoimmune response; transplant rejection.

Introduction

Lung transplantation is the only definitive treatment for many forms of end-stage lung disease. Although over 1400 lung transplants are performed annually⁵, survival is limited by the development of chronic rejection, known as bronchiolitis obliterans (BO)⁵. 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, post-transplantation, the worst survival data for all recipients of solid organ allografts. The poor survival statistics take on a new importance when considered in the context of advancements in surgical techniques, immunosuppression, and other sup-

portive measures developed for the care of these patients over that last 20 years. In sum, the current sophistication in treatment regimens have not translated into improved survival of lung transplant recipients.

Repeated acute rejection episodes are believed to be the main risk factor for the development of BO⁶. 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 down-regulating alloimmune responses. However, the incidence of BO in patients has remained constant for the last several years despite

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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.

Our laboratory has determined that immunity during lung allograft rejection involves an immune response to a self antigen, type V collagen (col(V))^{4, 11, 18, 19}. All collagen molecules are triple helices composed of α -chains¹⁴. Col(V) is a 116 kDa heterodimer composed of α 1 and α 2 chains^{7, 14}. 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^{7, 9, 10}. Data showing that col(V) is a target of the immune response during lung allograft rejection^{4, 11, 18, 19} and that recognition of polymorphisms in donor MHC antigens stimulates 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 α 1 chain of col(V) (α 1(V)). α 1(V) is nearly 80% homologous to the α 2 chain of type XI collagen (α 2(XI))¹, and the gene for α 2(XI) maps within the MHC class II loci in humans and mice³. 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 sequence homology to alloantigens alone may not be required to induce alloimmunity. For example, LUZ et al.⁸ recently reported 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, which may determine the difference between autoreactivity or alloreactivity. These data suggest that secondary or tertiary characteristics of the peptide, affinity of the peptide for the T cell receptor, or other factors may explain the phenomenon of col(V)-induced immunity during lung allograft rejection.

Col(V) and Alloimmunity

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 reproduced the immunology and pathology analogous to acute rejection in recipient lungs¹⁷. In these studies, 4 weekly instillations of allogeneic lung cells induced lymphocytic perivascular and peribronchiolar infiltrates analogous to grade 1–2 acute rejection in the lungs of recipient mice and IgG2a

antibody deposits in perivascular and peribronchiolar tissues¹⁷. 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). Moreover, early studies suggest that reactivity to col(V) may be associated with poor outcome.

During ontogeny of the immune system, autoreactive T cells, i.e. 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 col(I), the major collagen in the lung^{9, 10}. The inflammatory responses and architectural remodeling that occurs 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)⁴. Collagen molecules may be degraded by a class of enzymes known as matrix metalloproteinases (MMPs)²¹. MMP-2 and MMP-9 are capable of degrading col(V), and TRELLO et al.¹³ reported activity of MMP-2 and MMP-9 in lungs of human transplant recipients during rejection. Our studies have detected activity of MMP-2 and MMP-9 in lung allografts during rejection. These data support the theory that the inflammation and remodeling that occurs during the rejection response may lead to release of potentially antigenic col(V) peptides.

However, the data described above 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 (col(II)), a collagen found in cartilage and not the lung^{4, 18, 19}. Similarly, rats develop strong delayed type hypersensitivity responses, an index of cellular immune responses, to col(V) but not other collagens during lung

allograft rejection^{18, 19}. Moreover, col(V)-specific T cells are present in the lungs of rats undergoing acute and chronic rejection⁴. These T cells proliferate strongly in response to col(V) and produce copious amounts of the T helper 1 cytokines, interferon γ and tumor necrosis factor α , in response to col(V) and have oligoclonal expression of specific V β regions in their T cell receptors⁴. 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 lungs⁴. The disparity between the ability of these cells to induce disease in normal lungs compared with isograft lungs is likely due to the ischemia-reperfusion injury that occurs during transplantation. Indeed, our studies confirm that the 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 a release of col(V) fragments in BAL from isografts comparable to that observed in allograft lungs⁴. Furthermore, not all col(V)-reactive T cell lines induced lung pathology⁴. These data may be explained by differential expression of V β regions in the T cell receptors of pathogenic cells compared with cells that do not induce disease after adoptive transfer. Differential V β expression suggests that these col(V)-reactive T cells detect different epitopes on col(V). Epitope mapping for the antigenic regions of col(V) molecule is under investigation.

Col(V)-Induced Oral Tolerance to Lung Allografts

Non-pharmacologically 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, which refers to feeding donor-derived MHC antigens to the host prior to transplantation. In either setting, donor antigens are believed to be presented by indirect allorecognition 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^{2, 15, 16, 20}. Recent studies highly the role of oral tolerance in the generation of regulatory T cells¹².

Data from our studies showing that col(V) is an antigen during lung allograft rejection and that col(V)-reactive T cells perpetuate the rejection response suggest that col(V) could be utilized as a tolerogen to prevent lung allograft rejection. To examine this possibility, we utilized col(V)-induced oral tolerance to determine its effect on acute and chronic lung allograft rejection. WKY (RT1^{lv1}) rats were fed several doses of col(V) prior to transplantation of lung allografts from F344 rats (RT1^l). In the absence of any immunosuppression, feeding col(V) prevented the onset of acute lung allograft rejection and, most importantly, abrogated the development of chronic rejection – BO. The ability of col(V)-induced tolerance to prevent rejection was not haplotype specific in that feeding col(V) was effective in suppressing acute rejection in another, unrelated rat strain combination undergoing lung transplantation¹⁸. Importantly, tolerance induced by col(V) did not induce global immune hyporesponsiveness, as cellular immune responses to bovine serum albumin, a nominal antigen, was not suppressed in recipients made tolerant to col(V)¹⁸.

Examination of the immune mechanisms that mediated suppression of alloreactivity revealed that feeding col(V) followed by lung transplantation resulted in systemic activity of transforming growth factor β (TGF- β) that suppressed alloimmune responses during acute and chronic rejection¹⁸. Clonal deletion of alloreactive T cells was not the mechanism of col(V)-induced oral tolerance, as neutralizing TGF- β , but not interleukin (IL)-10 or IL-4, recovered the activity of alloreactive T cells¹⁸. These data suggest 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 naïve rats (WILKES, manuscript in preparation) confirms a role for regulatory T cells in col(V)-induced tolerance. The critical role of presentation of alloantigens in the development of col(V)-induced oral tolerance is exemplified by data showing the tolerance could only be adoptively transferred 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 allografts⁴.

APCs-induced immune activation of T cells is dependent on bi-directional signaling between APCs and T cells. Since oral tolerance could affect T cell as well as APC function, defective antigen presentation could

have contributed to the inability of the 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 the function of T cells, and not APCs¹⁹.

Conclusion

Lung allograft rejection involves both alloimmune and autoimmune responses. Understanding how alloimmunity triggers autoimmune activation against the graft will be key to developing new techniques to suppress this response and prolong the lives of lung transplant recipients.

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