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Toll-like receptors, innate immunity and lung transplantation

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1. ABSTRACT

Lung transplant allografts have the highest rate of rejection and shortest graft survival time among the commonly transplanted solid organs despite high levels of immunosuppression. This observation strongly indicates that mechanisms unique to the lung allograft contribute to rejection post lung transplant. Unlike most other solid organ recipients, the lung allograft is exposed to both the external environment and a significant amount of donor-derived lymphatic and structural tissue. For these reasons, the recipient's innate immune system may be critically involved in the initiation and maintenance of rejection after lung transplant. The strongest evidence for innate immune activation participating in lung allograft rejection is based upon genetic studies which demonstrate that variation in toll-like receptors and the related molecule CD14 modulate posttransplant lung allograft rejection. However, secreted pathogen recognition receptors, including defensins and collectins, and complement are parts of the innate pulmonary host defense and may be important in lung transplant rejection. This report will review the current understanding of innate immunity in lung allograft rejection in both murine and human studies.

2. INTRODUCTION

Lung transplant (LTx). has exponentially grown since the first successful lung transplant operation fifteen years ago. However, lung allografts have one of the shortest survival times of solid organ transplants with chronic rejection (CR). the main limiting factor for long term lung allograft survival. Chronic rejection involves the obliteration of the small airways with fibrous tissue. Clinically, chronic rejection is manifest by bronchiolitis obliterans syndrome (BOS). with a progressive airflow obstruction. The strongest risk factor for CR and BOS is the number and severity of acute rejection (AR). episodes (1). AR is defined by a mononuclear cellular infiltrate around blood vessels and small bronchioles, suggesting that CR is an extension of immunological injury to the small airways.

The lung allograft has a significant amount of donor derived vasculature and lymphatic tissue in direct contact with recipient derived immune cells. Through both direct and indirect antigen presentation of donor antigens, recipient antigen presenting cells (APCs). can trigger an immune response. In direct antigen presentation, recipient

APCs present intact donor antigens to recipient T cells. In indirect antigen presentation, recipient APCs present processed donor antigens to recipient T cells. Both of these pathways activate an adaptive immune response and have been the focus of the majority of research. However, innate immunity is increasing being recognized as an important facet of the immune response in transplant rejection.

Unlike most other solid organ allografts, the lung allograft has direct contact with the environment. Consequently, the lung is designed to respond to environmental insults with multiple receptors that can trigger the innate immune response. Toll-like receptors (TLRs). are primarily cell surface receptors found on macrophages, dendritic cells, epithelial cells and circulating leukocytes (2). Secreted receptors, including collectins and defensins can also bind pathogens. The innate immune response may promote a more specific adaptive immune response evident as acute rejection. Consistent with this theory, environmental exposures such as viral pulmonary illnesses have been linked to lung allograft rejection (3-5). In this review, we will explore the recent research into aspects of the innate immune systems that contribute to lung allograft rejection in humans and animal models.

3. TOLL-LIKE RECEPTORS

The strongest evidence for the importance of innate immunity in LTx rejection is based on toll-like receptor 4 (TLR4).. Our laboratory has been particularly interested in the role of TLR4 and its associated ligand, lipopolysaccharide (LPS)., a potent trigger of innate immune activation. LPS is part of the cellular membrane of gram-negative bacteria and certain gram-positive bacteria and thus ubiquitous in the environment. In addition, there are TLR endogenous ligands released at the time of transplant, including heparin sulfate, heat shock proteins (HSP)., and hyaluronan and fibrinogen degradation products. An analysis of human lung allografts before and after reperfusion demonstrated upregulation of HSP70 mRNA with reperfusion (6). We hypothesize that activation of the innate immune response by endogenous or exogenous stimuli through TLR4 and related receptors augments the adaptive response leading to rejection.

In support of this idea, we have developed a mouse model of lung transplant rejection by inducing a local activation of innate immunity. Briefly, we expose fully mismatched murine bone marrow transplant recipients to inhaled LPS. Repeated local pulmonary exposures with LPS induce histological and biological features of obliterans bronchiolitis and lymphocytic bronchiolitis similar to that observed after lung transplantation without systemic graft-versus-host disease (7). This mouse model provides a clear link between innate immune activation and pulmonary alloimmune injury.

Arbour, *et al* described individuals with TLR4 missense mutations Asp299Gly and Thr399Ile were hyporesponsive to inhaled LPS (8). We initially evaluated 147 LTx recipients for these polymorphisms and found that those with these polymorphisms had reduced severity, frequency and onset of acute rejection in the first 6 months compared to those recipients with the wild-type genotype (9). Importantly, only the recipient genotype was important in predicting AR, not the donor (or structural). TLR4 polymorphisms. This finding suggests a role for TLR4 in *recipient* cells such as macrophages, dendritic cells and leukocytes. Expanding our cohort and extending our followup time, we reported a delay and overall lower incidence of AR and trend to delay of onset and lower incidence of BOS (10). We believe these findings indicate that a decrease in the activation of the innate immune system through TLR4 polymorphisms is protective for AR and likely BOS.

The Asp299Gly and Thr399Ile TLR4 missense mutations are relatively rare in the general population. We have explored other innate immune polymorphisms that may promote activation of the TLR4 as well. CD14 is a receptor that also binds LPS and promotes the binding of TLR4 and LPS. Soluble CD14 is detectable in the bronchoalveolar lavage fluid (BALF). of stable LTx subjects and elevated compared to nontransplant control subjects, perhaps reflecting an overall heightened state of innate immune activation in the transplant allograft (11). We have evaluated a common promotor polymorphism of CD14 (-159 TT). that increases transcription and thus increases signaling through the CD14-TLR4 pathway. In lung transplant recipients, the CD14 -159 TT promotor genotype is associated with higher levels of soluble CD14, TNF-α and IFN-γ, confirming increased innate immune activation. Importantly, these lung transplant recipients with this CD14 promotor genotype -159 TT had an earlier onset of AR and BOS as well as a decrease in survival posttransplant (12).

Another indication of the potential importance of TLR4 is the upregulation of a downstream modifier, IL-12p40 and its homodimer p80 in a mouse model of allograft rejection. In this tracheal transplant model, a major histocompatibility complex (MHC). mismatched trachea is subcutaneously inserted heterotopically. The trachea undergoes epithelial injury and progressive fibrosis thought to be similar to lung allograft chronic rejection. Using this model combined with viral inoculation with the Sendai virus, Mickols *et al* established that IL-12p40 expression in the epithelial cells was upregulated and led to an increase in macrophages and p80 expression. Utilizing selective transgenic mice, this group was further able to confirm that the p80 increase was the initial signal leading to increased epithelial injury and collagen deposition. In human lung transplant recipients with lymphocytic bronchitis, a type of acute rejection, p80 was markedly increased in the BALF compared to transplant recipients without lymphocytic bronchitis (13).

TLRs may be activated through fragments of extracellular hyaluronan (14). Through an elegant series of experiments, Tesar, *et al* were able to determine that hyaluronan degradation products may activate dendritic cells via toll-interleukin 1 receptor domain containing

adaptor protein (TIRAP). independently of MyD88 or toll-interleukin 1 receptor domain containing adaptor inducing interferon beta (TRIF). (15). Importantly, fragments of hyaluronan were significantly elevated in the BALF from LTx recipients with BOS compared to those without BOS (15).

TLR activation in both animal and human studies of lung allograft rejection appears to be a critical component to initiate allograft rejection. Further studies of TLRs and their ligands will add to the growing body of literature on this subject.

4. COLLECTINS

Although less well studied, collectins may play a role in alloimmune injury in the lung. Collectins are part of the host defense systems and are particularly interesting as they cross innate and adaptive immunity. In the lung, collectins include surfactant proteins which act as important modulators of the innate immune system. Surfactant A and D (SP-A and SP-D). is directly degraded by *Pseudomonas aeruginosa (*16).. Furthermore, SP-A degradation products have been identified in BALF of lung transplant recipients with cystic fibrosis, a disease often complicated by *P. aeruginosa (*16).

In a study of 60 LTx recipients, the small surfactant aggregate to large surfactant aggregate ratio was noted to be significantly decreased compared to nontransplant healthy controls. Furthermore, the LTx group had impaired surfactant properties (17). This finding may be due to decreased levels of SP-A in lung transplant recipients (18).

One potential mechanism for surfactant dysfunction in lung transplant recipients was recently identified. Gastroesophageal reflux (GER). and pulmonary aspiration are recognized as important non-alloimmune triggers of acute and chronic rejection (19, 20). In a careful analysis of the BALF from 50 lung transplant recipients, SP-A and SP-D were reduced in individuals with high levels of bile acids in the BALF liked related to GER. This study suggests that bile acid aspiration may lead to impaired innate immunity through decreased surfactant protein levels. This observation may partially explain why gastroesophageal reflux has been linked to lung allograft rejection.

5. DEFENSINS

Like collectins, defensins are particularly interesting in the lung allograft as they cross innate and adaptive immune responses. Defensins are important secreted pathogen recognition receptors found in the lung. Defensins can bind and permeabilize the lipid bilayers of pathogens (21).

Human α defensins, alternatively called human neutrophil peptides (HNPs)., have antimicrobial activity against bacteria. Nelsestuen, *et al* reported higher levels of HNPs in the BALF of LTx recipients compared to normal controls. Furthermore, those with the highest levels of HNPs beyond the immediate posttransplant period developed advanced BOS in the next several months (22).

Human β defensins (HBDs). bind yeast and gram-negative bacteria. HBD-2 is an important link between adaptive and innate immune as it recruits dendritic cells and T lymphocytes through CCR6 (23). Supporting a role for HBD-2 in lung rejection was the finding of elevated levels of HBD-2 in the BALF of lung transplant recipients with BOS. Importantly, HBD-2 was not elevated before or after lung transplant in individuals without infection or rejection (24).

Interestingly, mouse β defensin-2 can act as a ligand for TLR4 on dendritic cells (25). Furthermore, mucoid *P. aeruginosa,* certain cytokines and LPS can induce HBD-2 mRNA expression in cultured epithelial cells (26). This finding may be particularly relevant to lung transplant recipients with native septic lung disease, such as cystic fibrosis, as these recipients can harbor *P. aeruginosa* chronically.

6. COMPLEMENT

Activation of the complement system is the major component of host's humoral nonspecific defense system. As a result, complement may cross adaptive and innate immunity. Humoral rejection has gained increasing interest in solid organ rejection in the last several years. In lung transplant, septal capillary necrosis with complement protein deposition has been described as a type of humoral rejection in the lung allograft (27). In this report, complement proteins, including C3, C4d, C5b-9, as well as immunoglobulin G were noted in lung allografts (27). Specifically, complement deposition has been reported in the endothelium, epithelium, chondrocytes, and basement membrane of bronchial walls associated with BOS (28-30). The variety of deposition sites suggests multiple antigenic stimuli, not just human leukocyte antigens (31). Detection of complement is difficult and standardizing the nuances of septal capillary necrosis makes the confirmation of humoral rejection challenging. Complement and humoral immunity is an area of active research and future reports will expand the understanding of this area in lung transplant rejection.

7. SUMMARY

In summary, endogenous and exogenous ligands for TLRs and other related innate immune receptors are in constant contact with the lung allograft. This chronic exposure promotes innate immune activation which augments the adaptive immune response. Based on the current studies, activation of TLRs appears to be a critical component of lung allograft rejection although collectins, defensins and complement may also contribute to innate and adaptive immune activation. Additional research is needed to more precisely define the mechanisms of innate and adaptive immune cross-talk in lung transplantation. The role of innate immune activation in other solid organ allografts in both human transplants and animal models of transplant may identify common mechanisms of rejection.

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