Innate Immunity and Organ Transplantation: The Potential Role of Toll-like Receptors

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Traditionally, the recognition and tolerance of transplanted grafts has been considered to be within the realm of the adaptive immune system. Innate immunity, on the other hand, as the first line of host defense, plays a role in fighting against invading microorganisms. Recently, with the discovery of the Toll-like receptors (TLRs), the role of innate immune responses in the control of adaptive immunity has become a new area of interest. Emerging evidence suggests that in addition to responding to pathogen-associated molecular patterns of microorganisms, TLRs can be activated by endogenous ligands, expressed by mammalian cells. These ‘danger signals’ may participate in ischemia-reperfusion related organ damage and subsequently influence function and survival of transplanted grafts. Furthermore, it has been suggested that adaptive immune responses can enhance the acute inflammatory responses controlled by innate immunity in organ transplantation. This review addresses the potential involvement of TLRs in different stages of organ transplantation. Intriguing and controversial findings are presented and discussed in order to stimulate more attention to this emerging and potentially important area of research in organ transplantation.

Key words: Adaptive immunity, ‘danger signals’, ischemia-reperfusion, rejection

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Introduction

With the development and application of immunosuppressive strategies, organ transplantation has become a common and effective therapy for patients with end-stage organ failure. To achieve long-term survival we must prevent the rejection of the transplanted grafts. Our general understanding of transplantation immunology is based on the concept that the immune system is able to distinguish self and non-self signals. However, many interesting observations remain unexplained by this theory; for example, why kidney transplants from major histocompatibility complex (MHC)-mismatched living donors often perform better than MHC-compatible kidneys from cadavers, or why overall survival in lung transplant is lower than that of kidney transplantation (1). Triggering immune responses relies on the specific recognition of pathogens or antigens. For long time, it has been believed that this function is achieved by the adaptive immune system. This classical concept was challenged by Medzhitov and Janeway, who proposed that innate immunity might participate in decoding the patterns of self and non-self signals (2). The ‘danger signal’ model proposed by Matzinger suggests that the immune system recognizes and reacts to damage signals in the body regardless of their origin (1). With the discovery of Toll-like receptors (TLRs) and their ligands endogenously expressed by mammalian cells, the role of innate immunity in the regulation of the adaptive immunity has become an area of heightened interest. The role of TLRs has begun to be investigated in all the major aspects of organ transplantation: ischemia-reperfusion injury, acute and chronic rejection and post-transplant infection. This line of investigation may lead to revolutionary changes in our understanding and management of organ transplantation. In this review, we focus on studies related to both TLRs and organ transplantation. We apologize that many excellent articles related to TLRs are not cited as references.

Toll-Like Receptors and Innate Immunity

In mammals, the immune system consists of innate and adaptive immunity. The adaptive immune system is a highly sophisticated system observed only in vertebrates, which is mediated by antigen-specific T cells and B cells. The role of adaptive immunity in organ transplantation has been the center of investigation for decades. The innate immune system is phylogenetically conserved and
Figure 1: Toll-like receptors and intracellular signal transduction pathways. TLRs present large extracellular domains that recognize specific pathogen-associated molecular patterns in different microorganisms. A variety of molecules from mammalian origin have been proposed as endogenous ligands for TLRs. The intracellular domains of TLRs are similar to that of IL-1 receptor, and thus named as Toll/IL-1 receptor (TIR) domains. Herein, TLR4 and TLR3 are used as an example to demonstrate the complexity of intracellular signal transduction mechanisms. After activation, TLR4 can trigger intracellular signals by recruiting the adaptor protein MyD88, eventually resulting in the activation of transcriptional factors such as NF-κB and AP-1. Increased expression of cytokines and other inflammatory mediators is an important component of innate immune responses. Examples of MyD88 independent mechanisms are also given.

Toll, a transmembrane protein originally identified as a receptor required for the establishment of dorso-ventral polarity in Drosophila embryo, was found to be crucial for the defense of Drosophila against fungus and other microorganisms. The cytoplasmic domains of Drosophila Toll and the mammalian IL-1 receptor show remarkable similarity and are referred to as the Toll/IL-1 receptor (TIR) domain. Both share similar intracellular signal transduction pathways, leading to the activation of transcriptional factors such as NF-κB, and the expression of genes related to inflammatory and immune responses (4) (Figure 1). Following the identification of Drosophila Toll, a search on the National Center for Biotechnology Information database led to the discovery of a homologous Toll in human. This receptor, currently known as TLR4, has been shown to be involved in the recognition of lipopolysaccharide (LPS). Subsequent studies have identified many other proteins that are structurally related to TLR4 (3). To date, the ‘TLR family’ consists of 11 members. They are a special form of pattern recognition receptors (PRRs) that can recognize specific classes of molecules known as pathogen-associated molecular patterns (PAMP) in different microorganisms (3).

TLRs are present in different cell types and tissues with varying levels of mRNA and protein. TLRs are present on the surface of antigen presenting cells (APC), including macrophages, monocytes, dendritic cells and natural killer (NK) cells, as well as on other cells related to the innate immune system such as neutrophils, mast cells, basophils and eosinophils (5). Different subsets of human dendritic cell precursors, or the same cell type at different stages of maturation, can express different levels of TLRs (5). TLRs are also expressed in endothelial and epithelial cells. These tissue cells may detect the PAMP locally, and thus participate in the regulation of immune responses (1).

Most TLRs are transmembrane proteins with a large extracellular domain and an intracellular TIR domain. One of the major signal transduction pathways of TLRs is mediated through an adaptor protein, MyD88, which can bind to TIR domain and further recruit downstream signaling proteins ultimately leading to activation of transcriptional factors,
such as NF-κB and AP-1, resulting in the expression of genes related to inflammatory responses (4). Others adaptors (TIRAP, TRIF, TRAM) have been implicated in providing specificity for TLR signaling. For instance, TIRAP is involved in the MyD88-dependent pathway via TLR2 and TLR4. A MyD88-independent pathway through TRIF and TRAM has also been described for TLR4 and is associated with the induction of IFN-inducible genes through IRF3. TRIF is also involved in TLR3 signaling (4) (Figure 1).

**Ischemia-Reperfusion Injury and TLRs**

The first clue that innate immunity may affect adaptive immunity in organ transplantation comes from clinical observation. It has been noted that early graft dysfunction due to ischemia-reperfusion related injury can contribute to acute rejection as well as long-term graft outcome (6). Administration of a free radical scavenger (superoxide dismutase) during surgery in order to reduce ischemia-reperfusion related injury, led to a significant reduction of acute and chronic rejection in kidney transplant patients (6). During reperfusion, ischemic organs are infiltrated early on by activated CD4+ T cells and monocytes/macrophages, which can secrete cytokines, chemokines and other inflammatory mediators. These inflammatory responses are very similar to those observed when the innate immune system is activated by PAMPs via the TLRs (6). Using a quantitative real-time RT-PCR technique, Andrade et al. examined mRNA expression levels of TLRs in lung tissues collected during ischemia and reperfusion in human lung transplantation (7). In hypothermically preserved donor lungs, mRNA levels of most TLRs correlated with the mRNA levels of cytokines (IL-1β, IL-6, IL-8, IL-10 and IFNγ) (7). These observations again suggest that the inflammatory responses experienced in the donor organ may affect TLR gene expression and activity; alternatively, the TLR expression levels and activation may contribute to the regulation of cytokine gene expression.

There is increasing evidence to suggest that TLRs may be involved in ischemia-reperfusion induced organ injury. In a study of myocardial ischemia-reperfusion injury, two strains of TLR4 deficient mice (C57/BL10 ScCr and C3H/HeJ) showed significantly smaller areas of myocardial infarction than that in control strains (C57/BL10 ScSn and C3H/OuJ) (8). TLR4 deficient animals also demonstrated reduced neutrophil infiltration, less lipid peroxidation and less complement deposition in the cardiac tissues (8). In a murine model of renal ischemia-reperfusion, expression of TLR2 and TLR4 mRNA was significantly increased after 5 h of reperfusion in various cell types, including epithelial cells in the kidney (9). In a model of murine ischemia-reperfusion in the liver, injury with cellular damage was found to be mediated by TLR4 through a MyD88 independent pathway, with increased production of proinflammatory cytokines (TNFα, IL-6, IFN-inducible protein 10) (10).

In the setting of transplantation, most donors have suffered from trauma, intracranial hemorrhage, major surgical procedure or other severely stressful conditions. Paterson et al. have shown that major injury can prime the innate immune system for enhanced TLR reactivity: spleen cells challenged with ligands for TLR2 and TLR4 significantly increased IL-1β, IL-6 and tumor necrosis factor α (TNFα) production (11). Increased TLR4 reactivity was also found in vivo, primarily in the lung, liver and spleen, with macrophages and dendritic cells being the major sources of these cytokines (11). In a rat liver transplantation model, Tsoufas et al. reported that LPS levels were elevated shortly after reperfusion, and a group of molecules related to LPS detection, including LPS binding protein, CD14 and TLR2, were also increased at mRNA and/or protein levels (12). The induction of CD14 mRNA correlated with the length of cold ischemic time (12). These intriguing observations suggest that TLRs potentially serve a pro-inflammatory role in ischemia-reperfusion injury.

**‘Danger Signals’**

Ischemia-reperfusion may create a milieu of inflammation that may act as a ‘danger signal’ in organ transplantation; it may regulate an activated state of innate immunity by activating host TLRs (6). What are the ‘danger signals’ that might activate TLRs during ischemia-reperfusion? Currently, there is no definite answer. However, Li and coworkers have demonstrated that necrotic cells, but not apoptotic cells, induce activation of NF-κB and neutrophil-specific chemokine genes KC and macrophage-inflammatory protein-2 (MIP-2) in fibroblasts, macrophages and dendritic cells. These effects were mediated through the TLR-related MyD88-TRAF6 pathway. They also found that NF-κB activation by necrotic cells was dependent on TLR2, and could be further enhanced by the presence of TLR6 (13). During organ transplantation, the significant inflammatory response associated with the process of ischemia-reperfusion may lead to both necrosis and apoptosis (14). Although reperfusion after short periods of cold ischemia is usually well tolerated, tissue and cell damage is generally progressively increased during prolonged hypothermic organ preservation. For example, it has been shown that freshly isolated isogenic donor lungs provide excellent oxygenation function after transplantation without significant acute inflammation and cell death. In contrast reperfusion induces apoptotic cell death in lungs preserved between 6 h and 12 h, whereas further prolongation of hypothermic preservation to 18 h and 24 h results in necrotic cell death even before reperfusion (14). Necrotic cellular contents could trigger innate immunity via TLRs. The presence of apoptotic cells per se, may not activate the immune response through TLRs. However, the presence of infection and apoptotic cells may have a combined effect in the regulation of macrophage cytokine secretion (15). Therefore, these dead cells may be considered as a potential source of ‘danger signals’.
How necrotic cell contents activate TLRs remains unclear. However, a number of molecules of mammalian origin have been proposed as putative endogenous ligands of TLRs (16,17). Extensive work has focused on heat shock proteins (HSPs). Other TLR ligand candidates include fibrinogen, extra domain A of fibronectin, heparin sulfate, soluble hyaluronan, surfactant protein-A, β-defensin 2-lymphoma antigen idiotype sFv fusion protein and high-mobility group box 1 protein (16,17). Interestingly, most of these molecules have been proposed to be the ligands of TLR4 and/or TLR2. Recently, it has been found that some of the reported activation of TLR2 and TLR4 by HSPs may be a result of contaminating LPS and LPS associated molecules (17). Thus, the roles of HSPs and other molecules as TLR ligands need to be further clarified.

In addition to proteins, heterologous RNA released from or associated with necrotic cells or generated by in vitro transcription have been suggested to be endogenous ligands for TLR3 (18). TLR9 directly recognizes viral and bacterial CpG-DNA motifs, whereas murine TLR7 and human TLR8 sense viral single stranded RNA motifs as ligands (19). These motifs are also present in DNA and RNA of mammals. These facts reinforce once more the concept that products escaping from damaged tissues and cells may activate the innate immune response.

**TLRs and Rejection**

Long-term graft survival is obviously the desired outcome of organ transplantation. Despite the use of new immunosuppressive agents, rejection remains the main cause of graft failure and mortality. Acute rejection generally occurs during the first 6 months after transplantation and can compromise long-term graft survival of the kidney, lung and heart. The cause of late graft loss is usually chronic graft dysfunction or chronic rejection, an incompletely understood condition that may be mediated by both alloantigen-dependent and alloantigen-independent mechanisms. Clinically, chronic rejection is manifested by gradually progressive graft dysfunction, ultimately leading to organ failure. Although likely similar in underlying pathophysiology, the principal pathologic manifestations of chronic allograft rejection are somewhat organ specific: glomerular sclerosis/tubular atrophy in the kidney, coronary arteriosclerosis in the heart, oblitative vasculopathy and disappearing bile ducts in the liver and bronchiolitis obliterans in the lung. These pathologic processes currently represent the most important factors limiting the long-term function of transplanted organs.

Efficient priming of adaptive immune responses involves not only the presentation of antigen in the context of MHC, but also the induction of accessory signals on APCs (Figure 2). Schnare et al. hypothesized that TLRs expressed on APCs may regulate these accessory signals (co-stimulators and cytokines), and in this fashion consequently control activation of antigen-specific adaptive immune responses (20). Most TLRs signal through a common adaptor protein, MyD88. When MyD88 knockout mice were immunized with ovalbumin (OVA), and then challenged with the same antigen, the antigen-specific T cells responses (proliferation, production of IFNγ and TGF-1 immune responses) were impaired (20). In contrast, TGF-2 immune responses (production of total IgE, OVA-specific IgE and TGF-2 immunoglobulin - IgG1) were not affected (20). MyD88 is also required for IL-1 and IL-18 signaling. The protease caspase-1 (also called interleukin-1β converting enzyme, ICE) is required for processing of IL-1β and IL-18 from their precursors to mature and biologically active forms. Using ICE knockout mice as controls, it has been demonstrated that impaired antigen-specific TGF-1 immune responses are only seen in MyD88-deficient, but not in ICE-deficient mice (20). This strongly supports the contention that MyD88, a key adaptor protein for TLR signaling, is a link connecting innate and adaptive immunity.

To determine whether TLR/MyD88 play a similar role linking innate and adaptive immunity in graft rejection, Goldstein et al. used a skin transplantation model and demonstrated that minor antigen-mismatched (HY-mismatched) allograft rejection does not occur in MyD88 knockout mice (21). The rejection response can be restored by infusion of primed wild type (WT) spleen cells or by the provision of either WT donor or recipient APCs (21). They further demonstrated that the inability to reject allografts in MyD88-deficient mice was associated with a reduced number of mature and immature dendritic cells in the draining lymph nodes 2 weeks after transplantation, and with impaired generation of antigraft T cells (21). Additionally, MyD88-/mice demonstrated impaired TGF-1 immunity to the allograft that correlated with an inability to reject HY-incompatible allografts (21). This study provided key evidence thatadaptive alloimmunity could be controlled by the innate immune system and that TLRs, through MyD88, may play an important role in rejection of minor antigen-mismatched allografts.

To determine the importance of the TLR-MyD88 pathway in rejection of major MHC-mismatched allografts, Goldstein and coworkers further studied the rejection of skin and cardiac grafts in mice. They showed that there was a slight delay in cardiac rejection when MyD88 was absent from the recipient alone or from both recipient and donor, and no detectable difference in skin allograft rejection in MyD88-deficient mice (22). Absence of MyD88 did not impair the ability of dendritic cells to express costimulatory molecules during acute allograft rejection or the ability of allogeneic APCs and APCs derived from rejecting transplant recipients to stimulate alloprimed T cells (22). On the other hand, MyD88 signaling was important for the generation of mature dendritic cells post-transplantation and for the ability of allogeneic dendritic cells to prime naive recipient T cells (22). The lack of protection of fully MHC-mismatched grafts in MyD88 deficient mice indicates that...
Figure 2: Potential role of Toll-like receptors in the regulation of adaptive immune responses. TLRs on immature dendritic cells recognize conserved pathogen-associated molecular patterns in microorganisms. Additionally, TLRs also may recognize endogenous ligands released from damaged cells or tissues. Activation of TLRs and phagocytosis of these ligands may stimulate dendritic cell maturation. The mature dendritic cells present the antigen to naïve T-cells, in association with the induction of co-stimulatory molecules as well as cytokines. IL-12 is an important cytokine in the differentiation of naïve T-cells, and it induces the activation of T\(\text{H}_{1}\) cells and production of cytokines such as IFN-\(\gamma\). Differentiation of naïve T cells into T\(\text{H}_{2}\) leads to the production of cytokines such as IL-4, IL-5, IL-10 and IL-13. Both T\(\text{H}_{1}\) and T\(\text{H}_{2}\) responses may participate in the rejection of transplanted grafts.

Are TLRs Involved in Organ Rejection?

Although MyD88-deficiency is sufficient to prevent minor MHC-mismatch related graft rejection, the exact types of TLRs involved in this process remain to be elucidated. Goldstein et al. showed that TLR2-/- mice had a small but significant, prolongation of survival of their HY-mismatched skin allografts, but TLR4-/- mice had no such difference when compared to WT mice (21). Necrotic cell content induces NF-\(\kappa\)B activation mainly through TLR2, however, in the presence of both TLR2 and TLR6, the NF-\(\kappa\)B activity was further significantly enhanced (13). Therefore, endogenous ligand-induced signal transduction may be mediated and augmented through the actions of multiple TLRs—which may also be true for the role of TLRs in mediating the connection between innate and adaptive immunity in organ transplantation.

Samstein et al. used a skin graft transplantation model to examine the role of TLR4 in graft rejection. Using two TLR4-deficient strains of mice (C3H/HeJ and C57/BL10ScNcr), they demonstrated that dysfunction of TLR4 did not in fact delay the rejection of either major or minor MHC-mismatched skin allografts (24). Two loss-of-function polymorphisms of human TLR4 associated with blunted responsiveness to LPS have been described.
Adaptive immune responses may also in turn participate in the control of innate immune responses in the setting of transplantation. To determine the interactions between innate and adaptive immune responses, He et al. used a model of cardiac transplantation to compare syngeneic with allogeneic transplants (26). They also included an alymphoid group using mice with recombinase activating gene (RAG) deleted, which blocks the production of both T cell and B cell antigen receptors and thus blocks adaptive immune responses (26). These investigators observed that when adaptive immunity was abolished there was a robust immune response 1 day after the transplantation, featuring macrophage infiltration and up-regulation of multiple cytokines, chemokines and chemokine receptors in all groups (26). The similarity of gene profile response of these three groups indicates that these early responses are primarily mediated through the innate immune system. When the grafts were extended to 7 days, up-regulation of a subset of markers by innate mechanisms was confirmed. In addition, a subset of genes were up-regulated only in the context of an adaptive response (27). More interestingly, they found that the expression of markers associated with the innate response was markedly amplified in the allogeneic, but not in syngeneic or alymphoid groups. These results suggest that the significant inflammatory response seen during transplant organ injury involves both adaptive and innate immunity, and that the adaptive component of the rejection process may enhance the innate response via a positive feedback regulation. In these studies, genes for TLRs were not specifically reported upon. With the development of microarray, proteomics and bioinformatics, the overall interaction of innate and adaptive immune responses during the early and later stages of organ transplantation can be further explored.

Conclusion

Interactions between innate and adaptive immune responses have been implicated in many stages of organ transplantation, from ischemia-reperfusion to chronic rejection. The specific molecular mechanisms may vary according to different organs and clinical settings. However, the role of TLRs and their endogenous ligands in mediating injury of transplanted grafts represents an emerging area of significant interest and investigation. Further studies using newly developed transgenic animals, molecular tools and new therapeutic strategies, will enhance our knowledge of the overall host response to transplants and hopefully lead to the development of strategies to modify this response and to improve the outcome of organ transplantation.

References