Toll-Like Receptors in Health and Disease (13-Nov-2004)

D. A. Dean

Department of Molecular Biomedical Sciences, College of Veterinary Medicine, North Carolina State University, Raleigh, NC, USA.

Introduction

Host defense against pathogens in vertebrates depends on both innate and adaptive immunity. Although much of the investigative effort in the field of immunology over the last thirty years has focused on adaptive immunity, phylogenetic evolution of the immune system illustrates the necessity of the innate immune system. In fact, the vast majority of multicellular organisms (98.6%) cannot produce an adaptive immune response and are completely dependent on the innate immune system for elimination of pathogens. The innate immune system is a highly complex network of many cell types and soluble factors but has historically been considered to respond in an antigen-nonspecific manner. The recent identification of the Toll family of receptors and their pathogen-associated ligands has struck down the old paradigm of the non-specific innate response. The Toll receptor was originally identified in Drosophila as a developmentally critical receptor for the establishment of dorso-ventral polarity during embryogenesis. Flies with a mutation in the Toll gene were highly susceptible to fungal infection, suggesting the Toll receptor was also important in immunity. Highly conserved mammalian homologues were identified and were called Toll-like receptors (TLR). One of the mammalian receptors (TLR4) was subsequently shown to induce expression of inflammatory cytokines and mice with a missense mutation in the tlr4 gene were hyporesponsive to lipopolysaccharide (LPS) from Gram-negative bacteria. To date, 13 unique mammalian TLRs have been identified.

TLR Family and their Ligands

Toll-like receptors are germ line encoded and are categorized as pattern recognition receptors (PRRs) because they recognize pathogen associated molecular patterns (PAMPs). The TLRs are type I transmembrane glycoproteins characterized by a leucine-rich repeat (LRR) motif in the extracellular region. The LRR motifs form a horseshoe structure believed to be directly involved in the recognition of PAMPs. The ligands recognized by the TLRs are found on a wide range of pathogens including bacteria, viruses, protozoa and fungi. Table 1 lists the TLRs, the nature of the ligands and the source of the ligands. The TLR family also shares a cytoplasmic region of homology with the IL-1 receptor, and this Toll/IL-1 receptor (TIR) domain is of fundamental importance in signaling.
<table>
<thead>
<tr>
<th>Receptor</th>
<th>Ligand</th>
<th>Source of Ligand</th>
</tr>
</thead>
<tbody>
<tr>
<td>TLR1</td>
<td>Triacyl lipopeptides</td>
<td>Bacteria</td>
</tr>
<tr>
<td>TLR2</td>
<td>Lipoprotein, peptidoglycan, zymosan</td>
<td>Bacteria, protozoa, fungi</td>
</tr>
<tr>
<td>TLR3</td>
<td>Double-stranded RNA</td>
<td>Viruses</td>
</tr>
<tr>
<td>TLR4</td>
<td>Lipopolysaccharide</td>
<td>Bacteria</td>
</tr>
<tr>
<td>TLR5</td>
<td>Flagellin</td>
<td>Bacteria</td>
</tr>
<tr>
<td>TLR6</td>
<td>Diacyl lipopeptides, lipoteichoic acid, zymosan</td>
<td>Bacteria, fungi</td>
</tr>
<tr>
<td>TLR7</td>
<td>Single-stranded RNA</td>
<td>Viruses</td>
</tr>
<tr>
<td>TLR8</td>
<td>Single-stranded RNA</td>
<td>Viruses</td>
</tr>
<tr>
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<td>CpG- containing DNA</td>
<td>Bacteria, viruses</td>
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<tr>
<td>TLR10</td>
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<td>Unknown</td>
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**TLR Signaling**

There are several commonalities in the signaling pathways of the different TLRs. Generally speaking, ligand binding of TLRs triggers association of MyD88 (myeloid differentiation primary-response protein 88) with the cytoplasmic TIR domain. This allows binding of IRAK4 (IL1R associated kinase) that then phosphorylates IRAK1. TRAF6 (tumor necrosis factor receptor associated factor) is recruited to the receptor complex and then IRAK1 and TRAF6 disengage and associate with another preformed complex that includes TAK1 (transforming factor B activated kinase). TAK1 phosphorylates MAPK (mitogen activated protein kinases) and the IκK (inhibitor of nuclear factor-KB (κB) kinase complex. This leads to phosphorylation and degradation of IκB thus allowing translocation of NF-κB (nuclear factor −κB) to the nucleus where it activates transcription of its target genes. Research has been intensive on the signaling pathways of the TLRs and much more detail is known than is presented in this brief discussion. A MyD88-independent pathway has been described for TLR3 and TLR4 and several transcription factors in addition to NF-κB are known to be activated through TLR stimulation. There are also several negative regulators of TLR signaling. As more details of cell signaling emerge, it is clear that there are important differences in the signaling pathways among the TLRs and in the subsequent biological response. Activation of TLRs generally results in expression of inflammatory cytokines, type 1 cytokines and co-stimulatory molecules. These include TNF-α, IL-1, IL4, IL5, IL-6, IL-8, IL-12, IL-18, IFNa/β, SOCS, IP-10, COX2, NO, GARG16, IRG-1, CD80, CD86, CD150, and CD83. Regulation of the molecules expressed in response to TLR stimulation occurs at several levels. At the level of ligand binding, it has been shown that LPS from *Escherichia coli* induces IL-12 and IL-6 through TLR4 on macrophages while LPS from *Porphyromonas gingivalis* does not. Certainly, the different TLR themselves are associated with different responses. For example, ligation of TLRs 3, 7, 8, or 9 results in expression of type 1 interferons while ligation of TLRs 1, 2, or 4 does not. Lastly, different cells types expressing the same TLR respond in different ways. Taken together it is clear that although the TLRs have many structural and cell signaling features in common, the consequences of TLR stimulation depends on the TLR itself, the nature of the ligand, and the cell type expressing the TLR.

**TLR Distribution and Regulation of Expression**

The consequence of TLR activation is largely dependent on what cells express a given TLR and under what circumstances. The distribution of the TLRs has in no way been exhaustively investigated. TLR expression has been evaluated at the tissue level and on specific cell phenotypes but is also known to vary depending on maturational stage or activation state of a cell. There are also differences in TLR expression between species. In addition different TLRs are partitioned to different cellular locations. In general, those TLR recognizing microbial products (TLR1, TLR2, TLR4, TLR5, TLR6) are found on the cell surface while those recognizing nucleic acid are found in endosomes (TLR3, TLR7, TLR8, TLR9). Expression of TLRs is controlled at least in part at the transcriptional level. A variety of bacterial components induce TLR2 mRNA upregulation while down regulating TLR4 mRNA. Viral infection induces expression of TLR1, TLR2, TLR3, and TLR7 mRNA in macrophages and this may be secondary to IFN-α/β induction. TLR4 expression is positively regulated by macrophage migration inhibitory factor (MIF) and IFN-γ. Several cytokines including IL-2, IL-15, IL-1b, IFN-γ and TNF-α upregulate transcription of *tlr*2. Very little is known about post-translational regulation or recycling of TLRs.

**Link to Adaptive Immunity**

Given the information presented above, it is clear that a primary function of the TLRs is to directly activate acute anti-microbial defense systems. Binding of the TLRs by PAMPs induces anti-microbial and immune activating proteins and peptides by a variety of cells. TLR-induced macrophage activation results in direct killing of microbes, production of
cytokines and chemokines. Studies of knockout mice lacking expression of specific TLRs has proven the essential role of the TLRs in the innate immune response. While the innate response is highly effective against the majority of potential pathogens, the additional firepower of the adaptive immune response is necessary to combat highly evolved pathogens. Previously it was thought that the adaptive response emerged independent of the innate immune system. It is now clear that TLRs provide an essential link between the innate and adaptive immune response. Dendritic cells reside at the interface of innate and adaptive immunity. Dendritic cells are critically important antigen presenting cells necessary for T-cell activation. Immature dendritic cells have a high capacity for endocytosis that is necessary for antigen uptake. Stimulation of TLRs expressed by dendritic cells results in dendritic cell maturation characterized by expression of costimulatory molecules CD80/86 and the production of cytokines. Mature dendritic cells then migrate to the lymph node where they present antigen in the context of MHC class II to naïve T-cells while providing essential costimulation and a cytokine milieu that shapes the adaptive response. The role of TLRs in this process has been demonstrated using MyD88-deficient mice. It was shown that MyD88-deficient mice immunized with antigen and complete Freund’s adjuvant (CFA) were unable to generate antigen specific CD4+ T-cells or antibody. This and similar studies have also revealed the molecular mechanism of adjuvant activity. Exciting new avenues in vaccine research have opened whereby TLR-specific adjuvants may be employed to induce a specific type of immune response while mitigating the negative sequelae often associated with crude (although often highly antigenic) adjuvants.

TLRs in Disease

There is speculation that TLRs may be involved in “innate autoimmunity”. Just as with the adaptive immune system, it is critical that the innate immune system differentiate self from non-self. Given the role of TLRs as first responders and as a link to adaptive immunity, failure to prevent or control TLR responses could lead either to exuberant induction of detrimental cytokines such as TNF-α or to induction of adaptive immunity against self-antigen. There are several reports of TLRs (particularly TLR4) recognizing endogenous molecules including fibrinogen, heat-shock proteins, or DNA. While these observations remain to be definitively proven, the concept is not far fetched. Considering the condition of sterile inflammation, there must be stimulation of TLRs by endogenous molecules. There is data indicating that TLR binding (TLR9 on B-cells, in particular) by host DNA may drive antibody production from autoreactive B-cells. This may play a role in the immunopathogenesis of rheumatoid arthritis and systemic lupus erythematosus. What about autostimulation of other, non-nucleic acid binding TLRs? Some TLR ligands require binding of other host proteins before they will associate with the TLR. For example, LPS only achieves high affinity binding of TLR4 when complexed with CD14 and host LBP (LPS binding protein). Similarly, di-acylated bacterial proteins only bind TLR2 when associated with CD36. CD36 binds free fatty acids, thrombospondin, oxidized low-density lipoprotein and β-amyloid protein. Thus alterations of endogenous molecules whether genetic or acquired, may result in recognition by either the TLR or an accessory-binding molecule (such as CD14, LBP or CD36) leading to pathologic TLR stimulation. Such a mechanism may be involved in rheumatoid arthritis, Crohn’s disease, ankylosing spondylitis, and psoriasis. Viruses have been shown to interact with TLRs via their nucleic acids and through surface glycoproteins. While many of these interaction serve to initiate a productive immune response against the virus, some viruses have evolved to exploit or suppress TLR function. Vaccinia virus has been shown to suppress host immunity by interfering with TLR (particularly TLR3) signal transduction. The hemagglutinin of measles virus (MV) activates TLR2, which triggers an inflammatory immune response, but also upregulates the MV receptor on target dendritic cells. Inflammatory cytokines known to contribute to the pathogenesis of cytomegalovirus (CMV) have recently been associated with CMV/TLR2 interaction. In a series of elegant studies by Ross et al., mouse mammary tumor virus has been shown to interact with TLR4 as a means of upregulating viral receptors on target cells. Finally, ligation of TLR2, TLR4 or TLR9 has been shown to upregulate HIV replication and may be involved in reactivation of virus from latently infected cells.

Conclusion

The discovery of TLRs has dispelled the assumption that the innate immune response is not specific. This family of receptors has filled a gap in our understanding of how innate immunity is triggered, controlled, and linked to the adaptive response. However there remains a tremendous amount of work ahead to fully understand the role of TLRs in health and disease. Undoubtedly additional TLRs will be identified, as will their ligands. The specificity of signaling by each TLR also needs to be further resolved. The list of knowledge gaps in our understanding of normal TLR biology is lengthy, but the study of TLRs in disease pathogenesis is in its infancy. There are certainly many intriguing stories of the role TLRs play in disease to be told.
References


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