REVIEW ARTICLE

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Advances in Innate Immunity: The Role of Toll-Like Receptor Signaling in Human Disease

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INTRODUCTION

The field of innate immunity has undergone an explosion over the past decade with the discovery of receptor families that are instrumental in the first line recognition of microbes by hosts, and the regulation of innate and adaptive immune responses. In particular, the Toll-like receptor (TLR) family acts to sense foreign microbial products, and initiates a cascade of events that include the secretion of proinflammatory cytokines, the maturation of dendritic cells, and the activation of B- and T- cells. Given their pivotal role in host defense, it is not surprising that recent studies have identified a role for TLR function in several important human diseases, including sepsis, immunodeficiencies, atherosclerosis and allergies.

The Toll receptor was initially identified in *Drosophila* as essential for the establishment of dorsoventral polarity in the developing fruit fly embryo (1). In 1996, the Toll protein was shown to be required for *Drosophila* to mount an effective immune response against *Aspergillus fumigatus* (2).

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In 1998, the mammalian TLR4 was positionally cloned and found to encode the lipopolysaccharide (LPS) receptor. This receptor was known to be necessary for an adequate immune response against gram negative bacteria, in which LPS is an integral part of the outer cell membrane (3). These observations documented a crucial role for this phylogenetically conserved signaling pathway in the recognition of pathogens, and the initiation of the subsequent host immune response to microbes.

TLR SIGNALING

The human TLR family consists of ten receptors, and each receptor senses a distinct repertoire of conserved microbial epitopes on pathogens-pathogen associated molecular patterns or PAMPs (Table 1). Collectively, the TLRs are thought to respond to most, if not all, microbes that a host might encounter. For example, gram positive organisms such as *Streptococcus pneumoniae,* are initially recognized by TLR1, 2 and 6, which in turn interact with a range of downstream signaling molecules to activate macrophages, neutrophils and dendritic cells. The extracellular domain of TLRs contain tandem copies of a leucine-rich repeat motif (LRR) that are

thought to be involved directly in the recognition of various pathogens (4,5). In addition, the TLRs share a region of homology in their cytoplasmic domain with the interleukin-1 receptor, termed the Toll-IL-1R (TIR) domain, which is crucial for signaling. Signaling through the various TLRs enable the innate immune system to initiate appropriate effector adaptive responses.

Following encounter with a pathogen, TLRs trigger a complex cascade of events that lead to the induction of a range of proinflammatory genes (5,6). Briefly, ligand binding results in the recruitment of several molecules to the receptor complex: an adaptor protein, MyD88 (which also contains a TIR domain), IL-1R-associated protein kinase 1 (IRAK-1), IRAK-4 and tumor necrosis factor receptor-associated factor 6 (TRAF6) (Figure 1). IRAK-1 and TRAF6 then dissociate from the complex, and bind another complex that consists of transforming growth factor β-activated kinase (TAK-1) and the TAK-1 binding proteins 1 and 2 (TAB1 and TAB2). TAK-1 then activates the IκB kinase kinase complex (IKK). The activity of this complex is regulated by the subunit, NF-κB essential modulator (NEMO). IKK-mediated phosphorylation of IκB leads to its degradation, allowing NF-κB to translocate to the nucleus and promote the transcription of multiple proinflammatory genes, including tumor necrosis factor, IL-1 and IL-6.

Table 1. Toll-like receptors and their ligands.

TLR	Ligands	Origin of Ligand	Possible Role in Disease
TLR1	Triacyl lipopeptides	Mycobacteria	
TLR2	Peptidoglycan	Gram positive bacteria	Sepsis, RA, IBD
	Lipotechoic acid	Gram positive bacteria	
	GPI-linked proteins	Trypanosomes	
	Atypical LPS	Gram negative bacteria	
	Lipoproteins	Mycobacteria	
	Zymosan	Fungi	
	Heat shock protein 70	Host	
TLR3	dsRNA	Viruses	
TLR4	LPS	Gram negative bacteria	Sepsis, RA, IBD
	Fusion protein	RSV	
	HSP 60?	Host	
	Fibrinogen fragments?	Host	
TLR5	Flagellin	Bacteria	IBD, Legionnaire's
TLR6	Diacyl lipopeptides	Mycobacteria	
	Zymosan	Fungi	
TLR7	ssRNA	Viruses	
	Imiquimod, R848	Synthetic	
	Loxiribine	Synthetic	
TLR8	ssRNA	Viruses	
	R848	Synthetic	
TLR9	CpG DNA	Bacteria and viruses	
	Herpes virus DNA	Virus	
	CpG ODNs	Synthetic	
TLR10	Not determined		

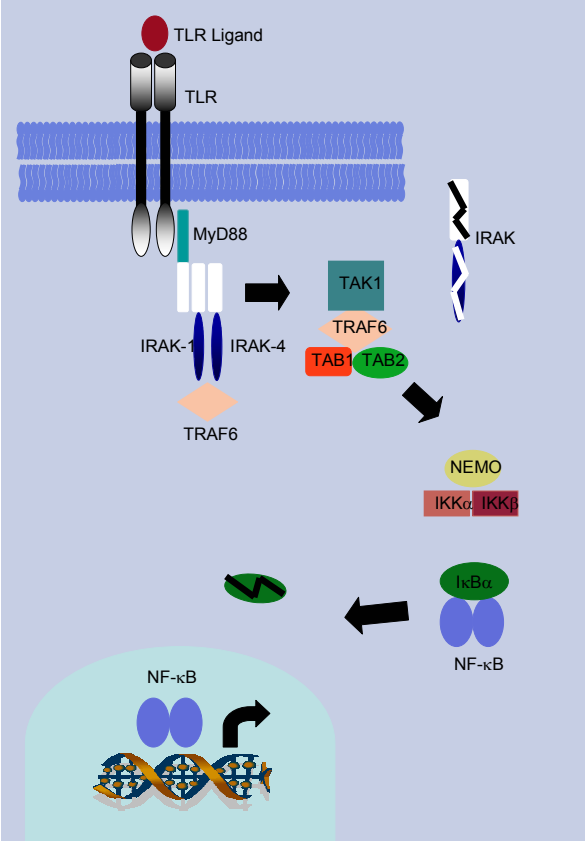

Figure 1. Overview of the Toll-Like Receptor Signaling Pathway

The mechanism by which TLR signaling tailors the immune response to individual pathogens is not entirely clear. The specificity of the TLR response is at least partly related to different adaptor molecule usage. Four adaptor molecules that all contain TIR domains have been identified to date: MyD88, TIR-domain-containing-adaptor protein (TIRAP), TIR-domain-containing-adaptor protein inducing IFN-β (TRIF) and TRIF-related adaptor molecule (TRAM) (5, 6). Different adaptor molecule usage results in the induction of different proinflammatory cytokines.

The inflammatory cytokines produced in response to TLR signaling can cause serious systemic illness if made in excess, and thus the regulation of TLR signaling is likely to be important in human disease as well. An illustrative example of this is endotoxic shock induced by the TLR4 ligand LPS. A number of molecules have been identified that negatively regulate TLR signaling: IRAK-M, suppressor of cytokine signaling 1 (SOCS1), MyD88 short (MyD88s), single immunoglobulin IL-1R-related molecule (SIGIRR) and ST2 (*5*).

IMMUNODEFICIENCY CAUSED BY ABNORMAL TLR SIGNALING

The intensive interest in the field of innate immunity in recent years has led to the identification of several mutations in genes encoding members of the TLR signaling pathway as the etiology of primary immunodeficiencies. A prime example is hypomorphic mutations within the *IKBKG* gene which have been found to account for some cases of X-linked recessive anhidrotic ectodermal dysplasia with immunodeficiency (EDA-ID) (*7*). The gene *IKBKG* encodes the IKKγ protein, also referred to as the NFκB essential modulator (NEMO). Mutations in this gene result in the inability of NF-κB to translocate to the nucleus, and therefore impairs many aspects of both innate and adaptive immunity (*8*). Although the impairment appears to exhibit a degree of variability, many affected patients have been found to display diminished production of pro-inflammatory cytokines in response to LPS, IL-1β and TNFα, as well as deficient NK cell cytotoxicity, and a decrease in the production of specific antibodies (*8-10*). These defects were manifested in the form of recurrent pyogenic bacterial infections including: *Streptococcus pneumoniae*, *Staphylococcus aureus*, *Haemophilus influenzae* and *Mycobacterium* (*9*). In addition to the defects in the immune system, such patients also exhibited developmental disorders, most commonly ectodermal dysplasia characterized by abnormal development of ectoderm-derived structures like sparse hair, abnormal or missing teeth, and absence of eccrine sweat glands (*7, 8*). These symptoms are not surprising given that NFκB also regulates a number

of diverse biological processes such as apoptosis (*11*), osteoclastogenesis (*12*) and ectodermal development through a number of pathways that converge upon IKK, in addition to its vital role in orchestrating inflammatory responses.

These developmental defects do not appear in patients with mutations in the gene encoding interleukin-1 receptor associated kinase 4 (IRAK4), a protein upstream of NFκB along the MyD88-dependent pathway. It has been shown that peripheral blood mononuclear cells from these affected patients have a deficient proinflammatory cytokine response to agonists of all known TLRs (*13-15*) (Turvey, unpublished data). However, unlike patients with the NEMO mutation, they predominantly suffered from recurrent infections caused by pyogenic gram-positive bacteria, particularly *Streptococcus pneumoniae* and *Staphylococcus aureus*. Currently, our group is investigating the role of IRAK4 in host defense against infections and the molecular basis of the remarkably narrow spectrum of infections experienced by these patients. These studies on immunodeficiency will not only be able to clarify the specific functions of IRAK4, but also expand our understanding of innate immunity garnered from studies obtained from immunodeficient mice and transfected human cell lines.

TLR POLYMORPHISM AND SUSCEPTIBILITY TO INFECTIONS

It is well known that genetic variation among individuals is one of the primary factors that contributes to variation in our immune responses, and subsequent successful defense against infections. Close examination of the gene sequences of TLRs in a number of studies have revealed polymorphisms which influence susceptibility to infection based on the specific pathogens recognized by the TLR. One of the earliest studies on TLR4 polymorphisms identified an allele with a common (approximately 5-10% in Caucasian populations) missense mutation D299G (Asp299Gly) in the extracellular domain of TLR4 (*16*). This study suggested the association of the D299G mutation with airway hyporesponsiveness to inhaled *E. coli* LPS in humans. Several subsequent reports have linked the D299G polymorphism to an increased incidence of gram-negative bacterial infection, LPS-induced septic shock and systemic inflammatory response syndrome (*17-19*). However, other studies have demonstrated that the D299G mutation does not appear to affect LPS-induced NFκB activation, with normal pro-inflammatory cytokine production in human primary leukocytes possessing the mutation (*20, 21*). These discrepancies indicate that the molecular basis of the observed correlation between altered susceptibility to diseases and polymorphisms in TLRs is not yet fully understood, and further investigation is required.

Genetic variation in individual *TLRs* is not restricted to *TLR4*. For example, polymorphisms in *TLR2* have been linked to increased susceptibility to leprosy (*22*). In a similar fashion, Hawn *et al* recently identified a common stop codon polymorphism in the ligand-binding domain of TLR5, which gives rise to a truncated version of the protein (*23*). This mutated protein was unable to transduce flagellin signaling and was shown to be associated with an increased susceptibility to Legionnaire's disease, caused by infection with *Legionella pneumophila*.

These examples reflect the increasing interest in studying the impact of polymorphisms on immune responses. However, it has become apparent that multiple factors other than genetic determinants contribute to disease outcomes in humans, and therefore it is often challenging to address these questions experimentally. Despite this, these studies will continue to improve our understanding of the

complexities of innate immunity, and eventually the knowledge gained will be integrated into a more coherent model of innate immune function.

ATHEROSCLEROSIS AND THE ROLE OF TLR SIGNALING

Studies have increasingly implicated inflammatory and immune mechanisms in the development and/or destabilization of atherosclerotic plaques. Since the initial report of an association between an unusual species of *Chlamydia pneumoniae* and coronary atherosclerosis in patients with myocardial infarction and coronary artery disease (*24*), a number of other infectious agents have also been associated with atherosclerotic cardiovascular disorders. More recently, the Bruneck study has provided evidence that circulating LPS levels and chronic infection constitute an important risk factor for the development of atherosclerosis (*25, 26*).

The expression of TLR1, TLR2 and TLR4 has been demonstrated in human atherosclerotic plaques, where they appear to be present mainly in macrophages and endothelial cells (*27*). The mechanism by which TLRs affect atherosclerosis is under active investigation (*28*). For example, immunologic cross-reactivity between bacterial (eg. chlamydial) and human hsp70 may exist, resulting in signaling through TLR4 and/or TLR2, and subsequent activation of NFκB-dependent proinflammatory gene targets (*29, 30*). *In vivo* evidence for the role of MyD88 and TLR4 in atherosclerosis was recently reported in mouse models. Mice that are deficient for MyD88 have significantly smaller plaques, lipid content, expression of proinflammatory genes and systemic expression of proinflammatory cytokines (*31, 32*). TLR4-deficient mice also showed a reduction in plaque size, lipid content and macrophage infiltration in atherosclerosis-prone apoE null mice (*32*).

One of the exciting possibilities is that human genomics will offer the ability to identify specific subpopulations at risk that would benefit from a particular intervention. The D299G polymorphism in TLR4 has been associated with a decreased risk of carotid artery atherosclerosis and acute coronary events (*33, 34*). This polymorphism results in lower circulating cytokines, such as IL-6, and in an increased susceptibility to infections. Thus, the protection from vascular inflammation afforded by the polymorphism appears to be balanced with the detrimental effect of more frequent infections. As we learn more about the effects of TLR polymorphisms in atherosclerosis and other diseases, this could allow us to tailor therapies based on genetic susceptibilities.

INNATE IMMUNE RESPONSES AND ALLERGIC DISEASE

The effect of the D299G TLR4 polymorphism has also been investigated in asthma. This link was initially made based on two lines of evidence: firstly, that exposure to LPS increases the severity of asthma (*35, 36*), and secondly, that exposure to LPS and other TLR ligands in early childhood may decrease the incidence of asthma (*37, 38*). The latter observation is one of a group of studies that suggest that there is a protective effect of childhood exposure to pathogens against the development of asthma. This has been postulated to be the result of an increase in the regulatory T cells that down-regulate immune responses following childhood exposure to pathogens.

The results of the studies on the D299G polymorphism have not consistently shown an effect on the incidence of asthma (*39-41*). One report of asthma that was associated with LPS in house dust showed that those with the polymorphism had a

decreased risk of bronchoreactivity (*40*), while another observed an increased severity of atopy in asthmatics with the D299G polymorphism. These are consistent with the previous observations that LPS can exacerbate existing asthma, and can also paradoxically decrease atopy, depending on the subset within the population that is studied.

CONCLUSION

The phylogenetically-ancient innate immune system provides the first line of host defense against infections, preceding and empowering the adaptive immune response. The innate immune system allows the host to differentiate itself from invading organisms and initiates a protective inflammatory response. In 1996, the *Drosophila* protein Toll was shown to be critical for defending the flies against fungal infections (*2*). This observation opened the way for the subsequent description of mammalian Toll-like receptors (TLRs).

The role of TLRs in human health and disease is now recognized to extend beyond resistance to infection and impacts other important areas of health, including risk of atherosclerosis and allergic disease. Now that TLRs are understood to play a central role in orchestrating the human immune response, this pathway will no doubt be targeted for therapeutic manipulation. For example, inhibiting TLR signalling may ameliorate some of the harmful effects of sepsis and autoimmunity, while boosting TLR activity may be a useful adjuvant to increase vaccine effectiveness. Overall, study of innate immunity and TLR function is certain to impact on clinical practice in the years to come.

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