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# Toll-like receptors: molecular mechanisms of the mammalian immune response

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## INTRODUCTION

The unrelenting bombardment by microbial pathogens from the environment and their increasing resistance to medical treatments are a constant threat to the survival of all organisms on earth. Microbes are covered by molecular patterns that are common among a broad range of pathogens. These include the lipopolysaccharides (LPS) of Gram-negative bacteria, lipoteichoic acids of Gram-positive bacteria, lipoproteins of bacteria and parasites, glycolipids of mycobacteria, mannans of yeast and double-stranded RNAs of viruses.[1,2] Recognition of and responses to these molecules are controlled by a wide variety of cellular receptors. The best characterized receptors are the T-cell receptor and B-cell antibody receptor of the adaptive immune response, the specificity of which is randomly generated and clonally selected during the development of T and B lymphocytes.[3] Unlike the receptors of the adaptive immune system, the pattern recognition receptors of the innate immune system have predetermined specificity generated early on in evolution and play an essential role in the determination of self versus non-self during the initial rapid responses to infection.[1,2] As described in this review, a family of cell surface receptors, termed Toll-like receptors, are emerging as key regulators of host responses to infection.

## TOLL AND THE *DROSOPHILA* HOST DEFENCE

The origins of the mammalian innate immune response are clearly seen with the involvement of the Toll protein in *Drosophila* development and host defence. Prior to the discovery of its involvement in *Drosophila* host defence, Toll was identified by a genetic screen for a defect in the establishment of dorsal–ventral polarity. This *toll* mutant was a dominant-ventralizing mutation in contrast to the recessive-dorsalizing mutants *dorsal* and *gastrulation defective*. Further mutant screens and double-mutant strategies characterized the order of other genes involved in this developmental pathway.[4] The best characterized being the Rel signalling pathway involving the activation of family members *dorsal*, *dif and relish*.[5–8]

The factors involved in the embryonic Toll signalling pathway have been identified and characterized.[9] Activation of Toll requires the endogenous ligand Spaetzle, which is proteolytically cleaved to its active form by a series of serine proteases genetically upstream of *spaetzle* and *toll*. Stimulation of Toll leads to the activation of Rel proteins, such as Dorsal, through signalling factors Tube and Pelle, resulting in the degradation of Cactus and release of Dorsal. Cactus is the *Drosophila* homologue of the mammalian I-κB family that sequesters and inhibits Rel family members in the cytoplasm prior to activation. Once released, Rel proteins travel to the nucleus where they positively or negatively regulate target genes involved in many cellular events. In fact, recent studies have placed Toll signalling pathways at the centre of mediating the up-regulation of *Drosophila* host responses to infection.[4,10,11]

The *Drosophila* immune response relies on the production of antimicrobial peptides, and each peptide has inherent specificity for its particular class of pathogen.[12] In an analysis of the regulation of these peptides it was found that they were all highly regulated by κB sites in their promoters. In fact, the Rel family members Dorsal, Dif and Relish are all activated in response to infection.[5–8] Genetic analysis identified the Spaetzle–Toll–Pelle–Tube–Cactus signalling cascade as an important regulator not only of Rel protein activation, but also in the induction of antimicrobial peptides. As a result, differential responses to yeast and bacterial infection have been observed between Toll family members. Toll and Spaetzle mutant flies suffer severe fungal infection and are unable to induce the expression of the antifungal peptide, Drosomycin. Although many of the same factors involved in the embryonic pathway are utilized, the Toll–antifungal pathway leads to the activation of Dif, but not Dorsal, in the regulation of the *drosomycin* gene.[10] In addition, there are mechanisms in place to respond to bacterial infection. The Toll family member, 18-wheeler (18w), has been linked to the production of Attacin, an antibacterial peptide.[13,14] Mutants for *ird and imd* have also been linked to antibacterial responses.[6] In contrast to mutants biased to specific pathogens (*toll*, *spaetzle*, *18w*, *ird and imd*), Cactus is required for both antifungal and antibacterial responses. This suggests that Cactus is a common player in both kinds of responses and may therefore involve separate Cactus/Rel complexes activated by independent upstream pathways.[8] This is a potential mechanism for the differential regulation of Rel family target genes. Strikingly, similar receptors and signalling pathways have been observed recently in other species, ranging from plants to mammals, a fact which

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emphasizes the conservation of molecules throughout evolution.

## TOLL-LIKE RECEPTORS

The link between *Drosophila* Toll (dToll) and mammalian Toll-like receptors exemplifies the evolutionary connection between *Drosophila* host defence and the mammalian innate immune response, which has been considered for many years to be the ancient arm of the mammalian immune system. Mammalian Toll homologues have been termed Toll-like receptors. Nine have been published.[15] The first functional analysis of mammalian Toll-like receptor was the study of hToll4 by Medzhitov *et al.*[16] The hToll4 mRNA was isolated from dendritic cells, γδ T cells, T helper type 1 (Th1) and 2 (Th2) αβ T cells and B cells. A dominant active form of the receptor induced cytokine expression and NF-κB activation when expressed in monocyte cell lines.[16] These findings and the homology with dToll suggest that Toll-like receptors may play an important role in mammalian host responses to infection.

Toll-like receptors have moderate homology with dToll, but more importantly share a common structure. Toll-like receptor structure includes leucine-rich repeats in the extracellular domain[15,17,18] and a cytoplasmic domain which shares significant homology with the interleukin-1 receptor (IL-1R) signalling domain, termed the Toll/IL-1 receptor homologous region.[19] The leucine-rich repeats of the extracellular domain mediate the response to conserved pathogen-associated molecular patterns commonly found among microbial organisms. A number of pattern recognition receptors recognize these molecules, including mannose and scavenger receptors, Toll-like receptors and CD14.[1,2] There is considerably more diversity found in the extracellular domains of Toll-like receptors, when compared to the intracellular signalling domain.[20] This has also been observed in the Toll-like receptors of plant species.[21] Analysis of the leucine-rich repeats of plants suggests that the extracellular domain plays an important role in the specificity of host responses to infection.[22] Although leucine-rich repeats play a role in the recognition of microbial ligands, their involvement in Toll-like receptor function has yet to be fully addressed.

## TOLL-LIKE RECEPTOR GENETICS AND THE 'LPS RECEPTOR'

Responses to LPS can cause septic shock in extreme Gram-negative bacterial infections, causing a severe health risk to the population every year. The search for the 'LPS receptor' has a long history. Three strains of mice, C3H/HeJ, C57BL/10ScCr and C57BL/10ScN, are naturally hyporesponsive to high doses of LPS. These mutations are referred to as the *lps*$^{d}$ gene.[23,24] These strains of mice showed inherent specificity by their increased susceptibility to Gram-negative infection, and normal responses to Gram-positive bacteria. Furthermore, LPS hyporesponsive macrophages from *lps*$^{d}$ mutant mouse strains respond normally to microbial lipoproteins.[23–25] This suggested that different receptors were involved in responding to different pathogens. Following the identification of the mouse *tlr4* gene and considerable mapping of the *lps*$^{d}$ gene locus, *lps*$^{d}$ was identified as the *lps* gene.[26–28] In a 1999 review, Samuel D. Wright described Toll-like receptors as, 'the missing piece of the puzzle'.[29] In fact, the mutations that occurred in all three mouse strains occurred in the *tlr4* gene. The C3H/HeJ hyporesponsiveness is caused by a dominant negative mutation[30] owing to a proline to histidine substitution mutation at amino acid 712. C57BL/10ScCr and C57BL/10ScN strains carry a recessive *tlr4* null mutation.[26–28]

Another Toll-like receptor, TLR2, was also implicated in responses to LPS. TLR2 conferred LPS responsiveness to a normally non-responsive cell line, HEK293. TLR2 activated NF-κB in response to LPS and an anti-TLR2 antibody inhibited the production of tumour necrosis factor-α (TNF-α) and IL-12 p40 from monocytes stimulated with LPS.[31–33] (Please see Note Added in Proof at end of Reference list.) Hamster macrophages and the Chinese hamster ovary CHO cell line carry a TLR2 null mutation, but are still responsive to LPS.[34] While TLR2 may be LPS responsive in stably transfected cell lines, genetic evidence supports TLR4 as the LPS receptor. Toll-like receptor-deficient (TLR$^{-/-}$) mice support this model. TLR4$^{-/-}$ mice are hyporesponsive to LPS, while TLR2$^{-/-}$ mice still respond normally. The results from the Toll-like receptor knockouts also suggest that there is clear specificity for microbial ligands between Toll-like receptor family members.[35,36] Although the evidence supports TLR4 as a key component of the LPS receptor, TLR4 mutant mice have been observed to be responsive to LPS under some conditions. Responsiveness has been observed with LPS from different species of Gram-negative bacteria[37] as well as with extremely high doses of LPS.[38] In addition, activation can be restored to normal levels by treatment with interferon-γ (IFN-γ).[39] Although there appear to be clear differences in specificity between TLR4 and TLR2 in mouse, the ability of human TLR2 to be activated by LPS and the inhibition of LPS-induced cytokine activation by an anti-human TLR2 blocking antibody suggest that there may either be some factors in common between TLR4 and TLR2 specificities or species differences between human and mouse Toll-like receptors.[31–33] Clearly, the mechanisms underlying responses to microbial ligands are very complex and may involve multiple factors that determine the specificity of Toll-like receptor responsiveness. Much like the specificity of *Drosophila* Toll family members, mammalian Toll-like receptor family members are emerging as having their own specificity for different classes of microbial molecules.

## MICROBIAL LIGANDS (PATHOGEN-ASSOCIATED MOLECULAR PATTERNS) AND TOLL-LIKE RECEPTOR SPECIFICITY

Microbial molecules which elicit strong responses from the innate immune system are quite common among a broad range of pathogens. These pathogen-associated molecular patterns are generally repetitive in nature and are distinct from the molecules found in the host. In addition, pathogen-associated molecular patterns are not subject to antigenic variation, but are essential for the survival and pathogenicity of the organism.[1,2] Microbial carbohydrates are a good example of this, which are a common structure that decorate the cell walls of many species of bacteria.[40–42] Lipid modifications are also common among microbial molecules.[43–48] It is thought that pathogen-associated molecular patterns are the target of pattern recognition receptors, such as Toll-like receptors;

however, the exact mechanism of this interaction is under much debate.

While much of the focus of Toll-like receptor biology has centred on its role in mediating LPS responsiveness, there has been much interest in identifying other pathogens and molecules that activate TLRs. Although a growing number of Toll-like receptors have been identified, TLR2 and TLR4 are the only Toll-like receptors that have been shown to be responsive to microbial ligands. TLR4 is clearly the main LPS receptor, but what role do TLR4 and TLR2 play in the responses to other antigens? Microbial lipoproteins are very common among bacteria including mycobacteria, Gram-positive and Gram-negative bacteria, such as the spirochaetes *Borrelia burgdorferi* and *Treponema pallidum*. These microbial lipoproteins share a common triacyl motif at their N-termini that is responsible for their stimulatory properties.[43–47] Much like many of these bacterial ligands, microbial lipoproteins are strong stimulators of macrophage activation, including the production of cytokines and the up-regulation of inflammatory events.[48–52] TLR2 was found to mediate responsiveness to lipoproteins from mycobacteria,[33] mycoplasma[53] and spirochaete species.[54] TLR2 was found to be the primary mediator of lipoprotein activation, while TLR4/MD2 were found not to be involved.[33,53] An anti-TLR2 antibody inhibited lipoprotein stimulation of cytokine production from macrophages stimulated with lipoproteins.[33] Transfection experiments, as well as the Toll-like receptor-deficient mice, demonstrate that TLR2 is the primary receptor for microbial lipoproteins.[33,53,54] While TLR4 has been shown to also mediate responses to some forms lipoteichoic acids and LPS, TLR2 responds to a broad range of molecules from a wide variety of pathogens. TLR2 has been shown to be involved in the phagocytosis of yeast,[55] as well as in responses to mycobacteria,[33,49,55] specifically to the 19 000 MW lipoprotein[33] and lipoarabinomannan from *Mycobacterium tuberculosis*.[49] TLR2 appears to be the primary regulator of Gram-positive responses. Interestingly, *lps*$^{d}$ mice respond normally to Gram-positive infection while being more susceptible to Gram-negative infection.[26] Gram-positive bacterial ligands that stimulate through TLR2 include *Listeria monocytogenes*,[56] *Staphylococcus aureus* cell walls, peptidoglycan, and lipoteichoic acids.[36,49,53,57–59] Differential responsiveness to pathogens by multiple receptors is reminiscent of *Drosophila* host defence. *Drosophila* Toll receptors mediate responses to a specific class of pathogen resulting in the production of target genes that specifically fight that infection. It appears that this has been conserved at some level with mammalian Toll-like receptors which have an ever growing array of ligands. This could lead to the activation of Toll-like receptor- specific target genes. With the vast range of microbial ligands, these innate receptors provide a mechanism by which the immune system can respond specifically to distinct classes of molecules. Further studies with Toll-like receptor-deficient mice will be essential to verify TLR2 transfection experiments in addition to understanding the specificity of responses and the susceptibility to infection.

## MECHANISM OF TOLL-LIKE RECEPTOR ACTIVATION

The parallels between *Drosophila* host defence and mammalian innate immunity are striking. Although the data suggest that TLR2 and TLR4 have clear specificity for different microbial ligands, the actual mechanism of TLR activation is still unclear. Is there a direct interaction between Toll-like receptors and their putative ligands, or are there endogenous ligands, similar to the *Drosophila* Spaetzle, that activate the receptors in response to infection? Furthermore, what role do Toll-like receptor co-receptors, such as CD14 and MD2, play in this process?

One protein which may play a critical role in Toll-like receptor function is CD14. CD14 was the first protein to be identified as an LPS receptor. CD14 binds many lipid-containing molecules, including LPS, microbial lipoproteins, Streptococcal cell walls and *M. tuberculosis* lipoarabinomannan.[50,60–68] Once shed from the surface of the bacterium, LPS is brought to membrane-bound or soluble forms of CD14 by serum protein LPS-binding protein (LBP). The presence of a LPS/CD14/LBP complex at the membrane results in cellular activation of LPS-responsive cell types.[69] CD14 expression correlates with increased sensitivity of many cell types to LPS and other microbial molecules in their ability to activate downstream signalling events and cytokine production.[69–77] CD14 is attached to the outer leaflet of the membrane by a glycosylphosphatidyl inositol (GPI) anchor. Since CD14 does not traverse the membrane into the cytoplasm, it cannot mediate LPS signalling events alone, but may require a co-receptor to activate intracellular signalling pathways.[78] Despite this inability to signal, CD14-deficient mice are hyporesponsive to LPS, suggesting that CD14 plays a critical role in this process.[79] TLRs seem to fit this requirement for a co-receptor. CD14 expression alone does not confer LPS responsiveness to cell lines. However, when CD14 is co-expressed with TLR2 or TLR4, activation of NF-κB by LPS, microbial lipoproteins, or lipoarabinomannan is enhanced.[31–33,54,58] These observations are consistent with the role of CD14 in cellular activation and cytokine production.

Other co-receptors may be involved in regulating TLR responsiveness. Initial studies of TLR4 suggested that it was not responsive to LPS activation when stably expressed in HEK293 cells. However, subsequent studies identified a novel secreted protein, MD2, which was shown to associate with TLR4 and enhance its responsiveness to LPS. Furthermore, MD2 may be required for TLR4 function.[80] Therefore, the question remains, do other Toll-like receptors require similar co-factors to function? To date, other Toll-like receptor family members have not been shown to be responsive to microbial ligands.[32,54,59,81] Therefore, they may require MD2-like co-factors or heterodimerization with other Toll-like receptor family members to function.

Is there direct interaction between Toll-like receptors and their putative microbial ligands? The specificity demonstrated in Toll-like receptor-transfected cell lines, as well as the knockout mice, suggests that direct interaction may be involved. However, the biochemistry of this interaction is lagging behind the molecular biology. Unlike the strong association of CD14 with ligands LPS and microbial lipoproteins, the binding affinity of TLRs with LPS is much lower than would be expected for this kind of interaction.[31] However, other proteins, such as CD14 and MD2, may enhance this interaction.[20] Recent studies suggest that there is a direct TLR4–LPS interaction in a comparison of species-specific responsiveness to different forms of LPS–lipid A by

genetic complementation. Human and mouse TLR4 were able to discriminate between different forms of LPS–lipid A when overexpressed in C3H/HeJ mice.[82] However, there have yet to be studies published demonstrating a strong biochemical association between Toll-like receptors and their putative ligands.

The activation of *Drosophila* Toll during infection requires the endogenous ligand Spaetzle. Much like the processing of Spaetzle in the developmental Toll pathway, Pro-Spaeztle is proteolytically cleaved to its active form, Spaetzle, following stimulation by fungal spores.[10] The serine proteases Snake and Easter involved in the developmental pathway, however, are not involved in the response to fungal infection.[10] Cleavage of Spaetzle is required for induced expression of antimicrobial peptides via Toll in *Drosophila*, but the protease(s) involved in the immune response has yet to be identified.[9,12,83] Although, Spaetzle and Toll are required for antifungal responses in *Drosophila*, no endogenous ligand has been identified for antimicrobial responses upstream of the Toll family member, 18-wheeler.[10]

Similar protease cascades have been observed in other species and are thought to play a role in mammalian immune response, as well. For example, serine proteases have been observed in the horseshoe crab clotting cascade. These proteases share sequence similarities with the *Drosophila* Easter serine protease.[84,85] The limulus proteases factor B and proclotting enzyme, which cleave pro-coagulogen, are homologues of Snake and Easter, respectively. Interestingly, coagulogen contains cysteine knots similar to those found in Spaetzle.[86] The activation of the mammalian complement cascade involves a pathway of proteases activated during infection.[87] These parallel mechanisms clearly demonstrate factors in common between widely diverse organisms.

Is there a protease cascade and endogenous ligand upstream of Toll-like receptor activation? Many have hypothesized that Toll-like receptors are activated by an endogenous ligand. However, studies of endogenous ligands for Toll-like receptors have only recently started to emerge. A study by Ohashi *et al.* implicated the heat-shock protein (hsp 60) chaperone as an endogenous TLR4 ligand, suggesting that it acts as a 'danger antigen to the innate immune response'.[88] In fact, hsp 60 is normally sequestered in the cell interior, but is rapidly translocated to the membrane in response to cell stress. Responses to LPS and hsp 60 are very similar. Interestingly, C3H/HeJ mice are not responsive to hsp 60, suggesting that like LPS, TLR4 mediates stimulatory effects of hsp 60. Although this is the first example of an endogenous protein activating Toll-like receptor signalling pathways, a direct interaction of TLR4 with hsp 60 has yet to be demonstrated. A role for protease cascades in the activation of responses to LPS is not unprecedented. Jin *et al.* identified 'epithelial cell-derived inhibitor of leucocyte serine proteases' (SLPI) from LPS-stimulated epithelial cells.[89] Overexpression of SLPI suppressed LPS-induced activation of NF-κB, and the production of nitric oxide and TNF-α. SLPI renders macrophage cell lines hyporesponsiveness to LPS. Again, there are interesting parallels between organisms. *Drosophila* cleavage of pro-Spaetzle is regulated by the serine protease inhibitor, Spn43Ac, suggesting that there may be multiple levels of regulation in LPS response mechanisms.[90] Interestingly, SLPI expression is inhibited by IFN-γ and thus enhances LPS responsiveness. This responsiveness is consistent with IFN-γ enhancement of LPS stimulation, and may serve to prolong activation by LPS. Further exploration of an endogenous Toll-like receptor ligand will require more thorough biochemistry and genetic screening to identify molecules involved in Toll-like receptor activation.

## TOLL-LIKE RECEPTOR SIGNALLING

The immune responses of *Drosophila and* mammals not only share common receptors, but also share evolutionarily conserved downstream signalling pathways involved in the activation of host defences. The Toll-like receptors share significant homology with the Toll/IL-1R intracellular signalling domain.[19] In fact, they share common downstream signalling molecules of the Rel/NF-κB pathway.[16,91] Activation of NF-κB occurs downstream of a number of receptors including TNF receptors, CD40, the IL-1 receptor, dToll and Toll-like receptors.[92] Activation of the Toll/IL-1R receptor domain results in sequential recruitment of the adapter molecule MyD88, through its own Toll/IL-1R receptor domain.[93] Furthermore, MyD88-deficient mice do not respond to either IL-1 or LPS, thus demonstrating its bifunctional role in these signalling pathways.[94,95] MyD88 recruits IRAK (IL-1 receptor accessory protein kinase) through death domain interactions of both proteins resulting in the autophosphorylation of IRAK.[93,95–99] TRAF6 (TNF-receptor-associated factor) is then recruited leading to the activation of MAP kinase kinase kinases (MAPKKK), such as NF-κB-inducing kinase (NIK).[100] The NF-κB-inducing kinase activates I-κB-alpha and -beta kinases which are involved in the phosphorylation of members of the NF-κB inhibitory family, I-κB.[101–103] I-κB family members are ubiquitinated following phosphorylation, resulting in I-κB degradation. NF-κB then translocates to the nucleus where the active dimer can transactivate gene expression of such cytokines as IL-1β, IL-6, IL-8, IL-12 p40, and co-stimulatory molecules CD80 and CD86.[104–107]

A number of signalling pathways have been implicated downstream of microbial ligands, such as LPS and lipoproteins. In fact, the regulation of many immunomodulatory genes requires more than one signalling pathway. For example, the IL-12 p40 gene is regulated by NF-κB and CCAAT/enhancer binding protein (C/EBP) family members.[108,109] In addition to the NF-κB signalling pathway, other signalling pathways have been linked to Toll-like receptor signalling pathways. Overexpression of the dominant active form of TLR4 activates not only NF-κB but also Ap1 and Jun N-terminal kinase (JNK).[16,97,98] In fact NF-κB, C/EBP and Ap1 are prominently involved in the regulation of many pro-inflammatory and immunomodulatory genes.[109,110] The mitogen-activated protein (MAP) kinase pathway is also activated in response to microbial ligands, possibly downstream of Toll-like receptor activation as well. The MAPKKK, TAK1, appears to be involved in LPS-induced NF-κB activation downstream of TLR2 and TLR4 in transfected cell lines and in the murine macrophage cell line RAW264.7.[111] A TAK1-dominant negative mutant blocked NF-κB activation in both of these cell types, and TAK1 is phosphorylated in response to LPS. The specific interactions leading to the activation of MAP kinases downstream of Toll-like receptors are unclear; however, the MyD88 knockout suggests that while MyD88 is

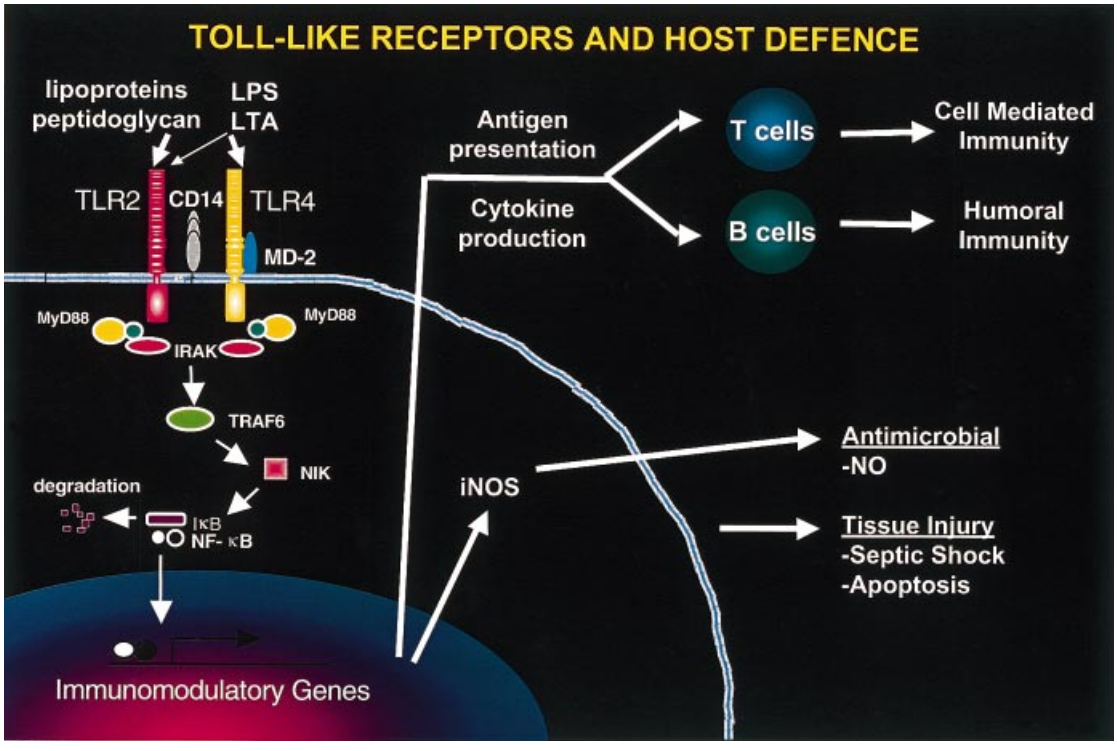


**Figure 1.** Toll-like receptors and host defence: LPS, lipopolysaccharide; LTA, lipoteichoic acid; TLR, Toll-like receptors; IRAK, interleukin-1 receptor accessory protein kinase; TRAF6, tumour necrosis factor-receptor-associated factor; NIK, NF-κB-inducing kinase: iNOS, inducible nitric oxide synthase; NO, nitric oxide.

essential for responses to LPS, MyD88 alone is not required for the activation of NF-κB or MAP kinase pathways.[94] The B-cell-specific Toll-like receptor, RP105, activates Src-family tyrosine kinase Lyn, protein kinase C beta and MAP kinase pathways.[112] There is growing evidence that Toll-like receptors lead to the activation of a number of different signalling pathways that contribute to the specificity of target gene activation and cellular responses.

## TOLL-LIKE RECEPTOR AND HOST RESPONSES TO INFECTION

Toll-like receptors appear to be involved in the activation of a growing list of mammalian host responses to infection. The signalling pathways and target genes downstream of Toll-like receptors have drastic effects on how the host responses are activated. The innate immune response is rapid, allowing for the early detection of microbial pathogens and the control of infection. In contrast, the adaptive immune response is delayed and involves immunological memory. Not only do innate and adaptive immune responses complement each other, they are interactive. The innate immune response influences the type of adaptive immune response elicited through the production of immunomodulatory genes, which are regulated in large part by Toll-like receptor activation.[1,2,16,31,33]

Activation of lymphocytes is not preprogrammed, but often depends on activation signals generated by cells of the innate immune response.[1,2,113,114] Cells of the innate immune system, antigen-presenting cells (APC) that have been in contact with the pathogen can polarize lymphocyte responses through the production of cytokines and co-stimulatory molecules.[115,116] Toll-like receptors are clearly involved in the activation of cytokines by microbial ligands. Overexpression of a dominant active TLR4 in macrophage cell lines induces cytokine production and the expression of co-stimulatory molecules.[16] IL-12 is a critical regulator of cell-mediated immune responses influencing the induction of Th1-type responses from T and B lymphocytes.[117] Microbial lipoproteins activate the IL-12 p40 promoter through a TLR2-dependent mechanism. A TLR2-dominant negative mutant inhibits the induction of the IL-12 p40 and inducible nitric oxide synthase (iNOS) promoter following stimulation by microbial lipoproteins. Furthermore, an anti-TLR2 inhibitory antibody blocks the production of IL-12 and other pro-inflammatory cytokines, such as TNF-α, from primary monocytes.[33] Activation of Toll-like receptors may also lead to the induction of down-regulatory cytokines, such as IL-10. As a result, the balance of cell-mediated (Th1) and humoral (Th2) immune responses can be directly influenced by Toll-like receptor target genes. Previous studies have suggested that pro- and anti-inflammatory cytokines are differentially regulated in response to the same microbial ligands.[110,118–123] In fact, the differential expression of IL-12 versus IL-10 has been observed in disease models, such as leprosy, where the dysregulation of these two cytokines can have drastic effects on the state of disease.[124,125] The involvement of TLRs in this differential regulation of immunomodulatory genes will be an intriguing avenue to pursue in future studies.

Toll-like receptors are clearly activated by mycobacterial components.[33,49,53,57] Mycobacteria are strong stimulators

of IL-12 production and cell-mediated immune responses.[33,120,126–128] The *M. tuberculosis* 19 000 MW lipoprotein can activate the iNOS promoter and nitric oxide production from murine macrophages in a TLR2-dependent manner.[33] Inducible NOS is critical for the production of nitric oxide from macrophages. Nitric oxide represents a powerful antimicrobial pathway. In particular, nitric oxide generation is required for efficient host defence against *M. tuberculosis*, since iNOS-deficient mice are highly susceptible to infection.[129] Therefore, activation of mammalian Toll-like receptors, by induction of nitric oxide, can directly contribute to an antimicrobial response. Since a main function of *Drosophila* Toll is the induction of antimicrobial peptides, the function of Toll in host defence has been conserved throughout evolution.[127,128]

In addition to macrophage activation and pro-inflammatory responses attributed to Toll-like receptor activation, the activation of down-modulatory mechanisms has also been observed. Aliprantis *et al.* demonstrated that microbial lipoproteins induced features of apoptosis in human monocytes, including cell shrinkage and membrane blebbing, by a TLR2-dependent mechanism.[130] Induction of apoptosis was confirmed by TdT-mediated dUTP nick-end labelling (TUNEL) assay and cell lysis was demonstrated according to lactate dehydrogenase release. Thus, microbial lipoproteins have the ability to induce both TLR-dependent activation of host defence and tissue pathology. This dual signalling pathway is similar to TNF receptor and CD40 signalling, which can induce both NF-κB activation and apoptosis.[131] In this manner, it is possible for the immune system to activate host defence mechanisms, then by apoptosis down-regulate the response from causing tissue injury, such as in sepsis. Alternatively, by leading to apoptosis, activation of TLRs may also contribute to tissue damage during the course of inflammation.

## CONCLUSION

In a recent review, K. Anderson suggested that the *Drosophila* immune response is 'genetically hardwired' to respond specifically to pathogenic infection.[20] *Drosophila* Toll receptors have clear specificity for the class of antimicrobial peptides that they activate. Toll induces the antifungal peptide gene, drosomycin, and 18-wheeler induces the antibacterial peptide, attacin. Interestingly, Rel family members have been found to influence the specificity of target gene activation as well; for example, *dif* mutants exhibit reduced production of drosomycin and defensin.[11,132] Similarly, *relish* mutants fail to produce cecropin A and diptericin, in addition to having reduced production of other antimicrobial peptides.[133]

Is there a similar hardwired genetic mechanism in the mammalian immune response? The complexity of the mammalian Toll-like receptor responses involves multiple levels of regulation. Although individual target genes for each Toll-like receptor have not been identified yet, the Toll-like receptors clearly have individual specificity for certain classes of microbial ligands (Figure 1). Toll-like receptors may be able to heterodimerize, thus increasing the combinatorial diversity of ligands that these receptors could respond to, as well as, the diversity of target gene activation downstream. NF-κB-deficient mice have been generated and are currently being characterized for NF-κB family member involvement in the activation of specific target genes. Altered expression of NF-κB family members has critical effects on the production of cytokines and immune responses to infection.[134–136] Therefore, as more studies emerge, the effects of NF-κB family members on host responses will become more evident. A wide variety of cell types are responsive to microbial ligands and express a battery of Toll-like receptors. The combination of these receptors may be critical to a number of cell-type-specific host responses ranging from the production of macrophage cytokines to B-cell proliferation and antibody production. The future of the Toll-like receptor field appears to be rich with many avenues to traverse.

The presence of Toll in *Drosophila* indicates that Toll proteins represent a host defence mechanism that has been conserved over hundreds of millions of years of evolution. In mammals, Toll-like receptors provide the innate immune system with the ability to recognize and react to a wide spectrum of microbial pathogens expressing lipoproteins and lipopolysaccharides. Toll-like receptors can activate innate immune responses including iNOS, a direct microbicidal mechanism, and IL-12, which functions as a biological adjuvant amplifying acquired T-cell responses to pathogens. Thus microbial ligand activation of Toll-like receptors is probably an early signal for the induction of IL-12 and hence the generation of Th1 cytokine responses. It will be important to determine whether by induction of nitric oxide or other mechanisms, activation of TLRs leads to a direct antimicrobial effector pathway, as is evident in *Drosophila*. Under certain conditions, the Toll-like receptor signalling pathway can lead to the activation of down-modulatory responses, including apoptosis of the target cells resulting in down-regulation of the immune response or pathology to the host[130] (Fig. 1). It should be possible, however, to develop strategies to stimulate Toll-like receptors in order to activate host defence mechanisms and to enhance immune responses to a wide variety of antigens in vaccines.

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## NOTE ADDED IN PROOF

A recent study suggests that LPS preparations contain a mix of microbial ligands with distinct TLR specificities.

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