Toll-Like Receptors: Sentinels of Host Defence against Bacterial Infection

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Abstract
Innate immunity provides a first line of host defence against infection through microbial recognition and killing while simultaneously activating a definitive adaptive immune response. Toll-like receptors (TLRs) are principal mediators of rapid microbial recognition and function mainly by detection of structural patterns that do not exist in the host. TLR2 and TLR4 were the first members of this innate immune receptor family to be strongly implicated in antibacterial host defence. Following the initial description of the mammalian TLR family, susceptibility to infection with numerous human microbial pathogens has been intensively studied using mice with engineered deletions of each of these molecules. While it has become quite clear that TLR activation is necessary for optimal host defence, a comprehensive understanding of the mechanisms by which this family of pattern recognition receptors engages protective immunity, particularly the adaptive response, is still evolving.

Introduction
All multicellular eukaryotic organisms live in a potentially hostile environment. For example, upon emerging from a normally sterile environment at birth, humans are immediately confronted by ubiquitous micro-organisms, many of which have the capacity to establish themselves in large numbers on the skin, mucosal surfaces and the epithelial lining of various organs. Fortunately, the vast majority of these colonizing agents are not harmful, establishing a mutual relationship with their host that is either neutral (otherwise termed commensal) or symbiotic, in which at least one partner benefits [1]. This situation most clearly exists in the gastro-intestinal tract where the normal bacterial flora, despite vastly outnumbering the cells of the gut mucosa that it colonizes, contributes to the overall well-being of the host [2]. For example, intestinal bacteria facilitate the breakdown and absorption of nutrients, produce vitamin K, interfere with the growth of pathogenic bacteria through competition and production of toxic metabolites, and provide a low but constant level of stimulation to the immune system that contributes to the maintenance of epithelial integrity [3, 4]. Such a beneficial relationship is nevertheless quite fragile and prone to disruption. For example, many forms of gastro-intestinal tract injury may result in bacterial translocation across normal epithelial barriers or pro-
mote the secretion of microbial toxins that may potentially cause a wide spectrum of disease [5]. Accordingly, a primary goal of mammalian host defence is to ensure that the location and growth of these microbes positively contribute to homeostasis. Therefore, a series of mechanisms have evolved to ensure a productive relationship between host and microbe. Among the most fundamental roles of the innate immune system is the recognition of an abnormal microbial presence through a series of stably inherited molecules designated pattern recognition receptors (PRRs). Toll-like receptors (TLRs) are an important family of PRRs consisting of 11 well-characterized members [6]. Many of these receptors recognize structures that are unique to microbes and not expressed by the host (termed pathogen-associated molecular patterns or PAMPs), an efficient mechanism for self/non-self discrimination [7]. Comprehensive lists of the TLR ligands have been compiled [8] and it is likely that these will continue to grow in the future. The functions and ligands of TLR10, TLR12 and TLR13 are currently unknown, but, like all other mammalian TLRs, they are also most likely involved in innate immunity. TLR11, -12 and -13 are expressed as functional genes in the mouse but are pseudogenes in the human genome; conversely TLR10 is expressed in the human system but not in the mouse [9].

In this review, we summarize the current state of knowledge about members of the TLR family that play an important role in host defence against bacterial infection. The expression and function of TLR3, TLR7, TLR8 and TLR9, a subset that is confined to the intracellular compartment and principally involved in defence against viruses, will not be considered, but has been reviewed elsewhere [10–12]. Interestingly, TLR9 was first recognized as the major receptor for bacterial DNA [13]; the specific nucleic acid ligand is now recognized as either a natural or synthetic unmethylated CpG dinucleotide within a species-specific base context (commonly referred to as a CpG motif). Stimulation with CpG-motif-containing DNA has been shown to play a role in host defence against various bacteria [14–16] and fungi [17]; however, direct evidence for the role of TLR9 in host defence has been obtained only for viral infection [18–20].

**Ligands of TLRs**

Extensive studies have demonstrated that the mammalian TLRs principally recognize microbial structures, termed PAMPs, and trigger host cell activation. PAMPs are relatively invariant structures that are essential to microbial physiology and survival, widely distributed within a class of micro-organisms and not present in the host, attributes that make them reliable and economical targets for recognition by the immune system [21]. While it has become clear that activation of *Drosophila* Toll is triggered by infection through upstream inducible proteolytic cascades [22], the situation is different for mammals, and many of the molecular details of pathogen recognition by TLRs are unclear [23]. For example, it remains to be fully explained how conservation of the leucine-rich repeats that form the extracellular ligand recognition domain of all TLRs is compatible with recognition ofstructurally diverse microbial ligands. In certain cases, additional molecules may function directly as structural adaptors, for example CD14 for lipopolysaccharide (LPS)-TLR4, dramatically enhancing the cellular response [24]. Alternatively, certain molecules may participate in the localization of TLRs on the cell surface or stabilization of an established TLR ligand or macromolecular interaction, as in the case of MD-2 [25]. In support of the latter view is the limited amount of evidence for direct interaction between PAMPs and mammalian TLRs. Crystal structures of the TLRs may clarify the situation; for example, a recent report on the crystal structure of TLR3 demonstrates a compatible binding site for double-stranded RNA [26]. Finally, several reports have identified endogenous ligands that may activate TLRs, including the extra domain A of fibronectin, or heat shock proteins [27]. It is not clear whether these mechanisms induce protective responses to cellular injury or represent a mechanism for the development of auto-immunity.

**TLR Signalling**

In order to precisely control infection without inflicting self-injury, mammals must ensure that only pathogenic bacteria are recognized as harmful and use that information to selectively generate an appropriate innate immune response. The instructive signals from the innate immunity that stimulate a specific pattern of adaptive immune responses (for example Th1 vs. Th2) are only partly clear [28, 29]. Potential mechanisms for regulation of innate immunity have been described at several different levels, including distinct cellular expression patterns and intracellular localization of individual TLRs, use of different adaptor molecules for generation of activation signals in response to infection, soluble decoy receptors and intracellular negative regulators [30].
The first and most widely implicated adaptor protein in TLR signalling is myeloid differentiation factor 88 (MyD88), a cytoplasmic molecule consisting of a C-terminal TIR [Toll/interleukin 1 (IL-1) receptor homology] domain and an N-terminal death domain. MyD88 was first cloned as a myeloid differentiation primary response gene [31] and shown to mediate signalling by the IL-1 receptor (IL-1R) [32]. Based on the structural homology between the cytoplasmic TLR and IL-1R domain, its role in TLR signalling of innate immunity was also subsequently confirmed [33]. Certain TLRs, including TLR5, -7, -8, -9 and possibly TLR11, use this molecule exclusively to transmit their signals into the transcription of immune response genes. Through homology searches of genomic databases, several additional TIR-domain-containing molecules that are recruited to the TLRs have been identified including TIRAP/MAL (TIR-domain-containing adaptor protein/MyD88-adaptor-like), TRIF [TIR-domain-containing adaptor protein inducing β-interferon (IFN-β)] and TRAM (TRIF-related adaptor molecule) [34]. Each adaptor is essential to one or more individual TLR signalling cascades and contributes to the specificity of TLR responses. For example, it has been shown through the use of gene-deficient mice that both TLR3- and TLR4-dependent expression of IFN-inducible genes is dependent on TRIF but independent of MyD88/TIRAP [35]. The evidence to date suggests that TLR3 signals exclusively through TRIF following stimulation with double-stranded RNA [36]. In contrast, TLR4 has the most complex proximal signalling mechanism, using TRIF, TIRAP and TRAM for signal transduction in addition to MyD88 [8]. The TLR4 signalling pathway immediately bifurcates, utilizing either the combination of MyD88/TIRAP or TRIF/TRAM to link receptor activation with downstream signalling components. In response to the broadest range of microbial ligands, TLR2 engages the TIRAP/MyD88 signalling mechanism used by TLR4 but does not utilize TRIF or TRAM. Finally, two more sequences with significant homology to MyD88 (one termed SARM for sterile heat and armadillo motifs) have been identified, but their role in TLR signalling is not yet clear [37, 38].

**The Function of TLRs during Bacterial Infections**

The role of TLRs in the immune response against bacteria has been elucidated mainly by experimental challenge of mice lacking an important component in the TLR signalling pathway with various pathogens of relevance to human infectious diseases. Based on these studies, it is now clear that the most important function of mouse TLRs during an acute infection is through the activation and regulation of immediate effector responses by the innate immune system. In most bacterial infection models, mice with defective TLR signalling capacity succumb very rapidly, prior to the generation or evolution of a definitive adaptive immune response. TLRs regulate a variety of innate effector mechanisms, including the release of pro-inflammatory cytokines and chemokines, the oxidative burst of neutrophils and macrophages, and the release of reactive nitrogen intermediates [39–41]. Furthermore, the regulation and activation of cationic antimicrobial peptides such as the defensins have also been shown to depend on activation TLRs [42, 43]. Recently, mouse bactericidal/permeability-increasing protein, another very important antimicrobial protein, was found to be strongly inducible in granulocytes and dendritic cells predominantly by stimulation of TLR4 [Eckert et al., in press].

**TLRs Control Bacterial Infection**

TLR2 and TLR4 have both been clearly shown to play a functional role in the control of bacterial infection, and both of these TLRs signal innate immune responses through a common adaptor protein, MyD88. Interestingly, MyD88 deficiency in mice is not compensated by preserved TLR2- or TLR4-dependent, MyD88-independent signalling that presumably still occurs during experimental infection. It is clear that TLRs are strong inducers of Th1-mediated immune responses; this was first demonstrated using MyD88-deficient animals that were immunized with complete Freund’s adjuvant [28]. MyD88-deficient mice failed to mount a Th1-adaptive immune response but had preserved, if not enhanced, Th2 responses. Precisely how a Th2 response is instructed is not known, but it has been shown that, under some conditions, certain TLR ligands may promote this pattern of adaptive immunity [44]. Furthermore, the site of infection or the repertoire of TLR expression on different antigen-presenting cells may also be involved in this decision [40]. Analysis of MyD88/TRIF-double-deficient mice that should not exhibit any signalling capacity after TLR stimulation may clarify this question. In this section we will review the current knowledge and understanding of the role TLRs play during an immune response against live bacteria. A summary of the resulting phenotypes in TLR- or MyD88-deficient mice in different infection models is presented in table 1.
Toll-Like Receptor 2

TLR2 was first described to recognize peptidoglycan, a major structural component of the cell wall of gram-positive bacteria [45] as well as microbial lipopeptides found in both gram-positive and gram-negative bacteria [46–48]. Subsequent studies have shown that TLR2 has to form heterodimers with either TLR1 [46] or TLR6 [47] to exhibit its function and thereby recognize the widest spectrum of microbial ligands. TLR1 and TLR6 recognize bacterial ligands only in combination with TLR2. Recently, TLR2 was also shown to cooperate with a C-type lectin, dectin 1, to recognize fungal β-glucans [49, 50], as well as with CD36 to recognize diacylglycerides [51].

Toll-Like Receptor 2 and Gram-Positive Bacteria

The potential function of TLR2 in host defence was first described by infecting TLR2-deficient mice with various gram-positive bacteria. Compared to their wild-type littermates, TLR2-deficient mice rapidly succumb to a high-dose *Staphylococcus aureus* infection [52]. Interestingly, no difference in survival was observed when the mice were challenged with a low dose of the same organism, suggesting that additional gram-positive microbial recognition pathways are active and compensate for the lack of TLR2 activity during infection in this model. Two studies have specifically investigated the role of TLR2 during *Streptococcus pneumoniae* meningitis. In the first, TLR2-deficient mice exhibited greater severity of clinical disease and earlier mortality compared to controls following either an intracerebral or intracisternal route of infection. TLR2-deficient animals developed a higher bacterial load compared to controls, as well as a greater degree of meningeal inflammation and enhanced permeability of the blood-brain barrier based on comparative analysis of CSF TNF levels and extravasation of Evans blue dye, respectively. In contrast to the localized differences in microbial replication, similar bacterial counts and IL-6 production were observed in the blood, suggesting that following a local infection, TLR2-deficient mice were not predisposed to developing generalized bacterial sepsis [53]. Another group also reported similar findings, specifically that, compared to wild-type controls, TLR2-deficient mice had significant increases in clinical disease score, permeability of the blood-brain barrier, bacterial growth and intracranial pressure after direct intracisternal injection of *S. pneumoniae*. Somewhat surprisingly, this was associated with insignificant differences in the expression of several inflammatory parameters in the brain, suggesting the presence of additional host recognition mechanisms for *Pneumococcus*. A conflicting observation between the two studies was that in the latter, higher bacterial titres were also detected in the blood, indicative of enhanced systemic sepsis in TLR2-deficient mice [54]. A follow-up report by the same group demonstrated reduced cerebral complications and local inflammation among MyD88-deficient mice (with impaired IL-1, IL-18 and TLR signalling) compared to wild-type mice, yet a worse overall clinical course associated with severe pneumonia and bacteraemia [55].

TLR2 plays also an important role in the control of the group B streptococcus (GBS), an important cause of neonatal sepsis and meningitis. Following subcutaneous administration of a low dose (3 CFU) of this gram-positive organism, neonatal TLR2-deficient mice showed decreased survival and a higher bacterial burden at various sites including the blood, spleen and kidney, whereas no organisms were detectable in wild-type control animals at the

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**Table 1.** Adaptive immune responses in the absence of individual TLRs or MyD88

<table>
<thead>
<tr>
<th>TLR deficiency/adaptor deficiency</th>
<th>Causative agent for infectious disease model</th>
<th>Resulting Th response¹</th>
</tr>
</thead>
<tbody>
<tr>
<td>TLR2</td>
<td><em>Mycobacterium tuberculosis</em></td>
<td>Th1 (stronger) [64]</td>
</tr>
<tr>
<td>TLR2</td>
<td><em>Listeria monocytogenes</em></td>
<td>Unclear, but a lower IFN-γ production [58]</td>
</tr>
<tr>
<td>TLR2</td>
<td><em>Streptococcus pneumoniae</em></td>
<td>Presumably Th2 [83]</td>
</tr>
<tr>
<td>TLR4</td>
<td><em>Brucella abortus</em></td>
<td>Unclear, but a reduced IFN-γ production [73]</td>
</tr>
<tr>
<td>TLR4</td>
<td><em>Salmonella typhimurium</em></td>
<td>Unclear, but strong reduction of IFN-γ producing CD4+ T cells [68]</td>
</tr>
<tr>
<td>MyD88</td>
<td><em>Borrelia burgdorferi</em></td>
<td>Th2 [89]</td>
</tr>
<tr>
<td>MyD88</td>
<td><em>Streptococcus pneumoniae</em></td>
<td>Presumably Th2 [83]</td>
</tr>
<tr>
<td>MyD88</td>
<td><em>Mycobacterium tuberculosis</em></td>
<td>Reduced Th1 (after BCG immunization) [93]</td>
</tr>
</tbody>
</table>

¹ Different from the response in wild-type control mice.
same time point after infection. Bacteraemia in the mutant neonatal mice was associated with elevated serum levels of TNF and IL-6. Strikingly different results were obtained with a larger inoculum (60 CFU) of GBS in neonatal mice; in this case, both groups had a similar bacterial burden; however, wild-type mice were actually more susceptible to lethal infection compared to TLR2-deficient mice and had higher levels of TNF and IL-6 in the blood, suggesting a greater inflammatory response. A similar dose-dependent pattern of host defence was obtained in an invasive GBS model in adult animals; TLR2-deficient mice were relatively susceptible to an intermediate dose challenge but relatively resistant to a high-dose infection [56]. Parallel studies using MyD88 knockout mice showed more profound effects relative to TLR2 deficiency, once again suggesting that other TLRs play an important role in the host defence against gram-positive bacteria.

An interesting study supporting the observation that TLR activation during an immune response might not always be beneficial for the host is the model of lethal shock induced by the gram-positive bacteria [57]. Based on the fact that TLR2-deficient mice were protected against death due to infection by viable Bacillus subtilis, administration of monoclonal antibodies directed against the extracellular domain of TLR2 also protected wild-type mice against lethal shock syndromes due to triacylated bacterial lipoproteins as well as heat-inactivated B. subtilis. In contrast, TLR2 plays a beneficial role in the immune response to intravenously administered gram-positive Listeria monocytogenes [58]. TLR2-deficient mice infected with Listeria display an increased susceptibility characterized by a higher bacterial load in the liver and an increased number of hepatic micro-abscesses in comparison to wild-type mice. TLR2 is expressed mainly on the cell surface and mediates protection despite the fact that Listeria is a facultative intracellular pathogen that enters the cytosol of the host cells. The exact mechanism by which Listeria or its ligands and TLR2 come into contact is unknown, but may occur either at the cell surface prior to bacterial internalization or at an intracellular location such as the phagosome following TLR2 recruitment [59]. Another explanation for this susceptibility phenotype may be a lower serum IFN-γ level in the TLR2 knockout mice.

**TLR2 and Obligate Intracellular Bacteria**

In a genital tract infection model of Chlamydia trachomatis, an obligate intracellular pathogen, less severe disease as judged by histology was observed in TLR2-deficient mice; this was associated with an equivalent organism burden yet decreased production of both pro-inflammatory cytokines (TNF, IL-6 and IFN-γ) as well as chemokines (MIP-2) in genital tract secretions. The TLR2 ligand of C. trachomatis is still unclear; however, activation of other TLRs by this organism is also likely since this organism is known to express LPS on its surface [60].

**TLR2 and Spirochaetes**

TLR2 deficiency did not significantly influence histological parameters of arthritis compared to wild-type mice despite an average 40-fold increase in the spirochaetal burden in the infected joints of the mutant mice 2 weeks following infection [61]. Four weeks after infection, the organism burden was 100-fold higher in ear tissue of mutant mice and 10-fold higher in the heart. Remarkably, no significant differences were detected in the character or kinetics of the humoral immune response, suggesting that clonal selection and activation of B cells in this model are TLR2 independent.

**TLR2 and Mycobacteria**

A variety of mycobacterial products such as lipo-arabinomannan and lipoproteins were previously identified as TLR2 ligands [62, 63]. Mice deficient in TLR2 were able to survive a standard low-dose aerosol challenge of Mycobacterium tuberculosis (100 CFU) but exhibited impaired control of mycobacterial growth in the lung with dissemination to the liver and spleen [64]. In response to a high-dose aerosol infection (500 CFU), these mice developed a uniformly fatal chronic pneumonia with increased neutrophil inflammation and an impaired granulomatous response. TLR2 deficiency in this setting was associated with a marked reduction of lung inducible nitric oxide synthase activity and an increased pro-inflammatory cytokine response. Surprisingly, TLR2-deficient mice develop an even stronger antigen-specific Th1 response in comparison to wild-type mice, although this was not effective in restricting microbial growth or promoting disease resolution. The mechanism for this altered adaptive immune response is unclear; however, the current view is that all TLRs are able to induce a Th1 response, and therefore other TLR family members might be responsible for the enhanced antigen-specific T cell activation. Alternatively, M. tuberculosis may attenuate host inflammatory responses in a TLR2-dependent manner. Overall, the data from this study suggest that TLR2 appears to have a more important or primary role when the mice are infected with a high dose of mycobacteria and other TLRs may activate host defence during low-dose infection.
**TLR2 and Gram-Negative Bacteria**

TLR2-deficient mice are more resistant to an oral infection of the gram-negative bacteria *Yersinia enterocolitica* as illustrated by higher CFUs in the spleen as well as in the liver 5 days after the infection. In contrast to most other experimental models where TLR2 functions as a PRR to activate the immune response, in this case TLR2 is a target of a bacterial virulence protein referred to as LcrV [also known as the virulence-(V)-protein]. TLR2-LcrV interactions are responsible for host IL-10 production that mediates immunosuppression required for the virulence of *Yersinia* [65].

**Toll-Like Receptor 4**

**TLR4 and Gram-Negative Bacteria**

TLR4 achieved prominence with the discovery that it is the primary cellular sensor of bacterial LPS, a major constituent of the cell wall of gram-negative bacteria [66]. In order to efficiently recognize LPS, TLR4 forms a receptor complex together with CD14, MD-2 and possibly LPS-binding protein. Long before TLR4 was identified as the *Lps* gene, it was clear that C3H/HeJ mice were highly susceptible to progressive overwhelming infection with *Salmonella typhimurium* (LD$_{50}$ < 2) as compared to the closely related C3H/HeN strain (LD$_{50}$ > 2 × 10$^3$) [67]. Indeed genetic analysis revealed that a point mutation resulting in non-conservative amino acid substitution in the cytoplasmic signalling domain of TLR4 underlies this susceptibility phenotype of the C3H/HeJ strain. Recent studies using TLR4-deficient mice subjected to an *S. typhimurium* infection administered by the oral route has confirmed the initial findings [68]. The role of TLR4 has been studied in numerous other bacterial infection models. For example, C3H/HeJ mice were shown to display a prolonged bacteraemia to infection with another gram-negative pathogen, *Neisseria meningitidis*. The bacterial load of *N. meningitidis* was several hundred times higher in TLR4 mutant mice compared to control mice with the same genetic background, and the clearance of the bacteraemia was significantly delayed relative to control mice [69]. In a uropathogenic *Escherichia coli* infection model, TLR4 mutant mice were also more susceptible to the infection in comparison to control animals, exhibiting a prolonged and elevated bacterial burden in the bladder as well as in the kidney during a 6-day observation period [70]. Pneumonia due to *Haemophilus influenzae*, another gram-negative bacterium, was prolonged in TLR4 mutant mice and associated with an impaired innate immune response characterized by reduced recruitment of neutrophils to the site of infection, impaired pro-inflammatory cytokine production in the broncho-alveolar lavage, reduced production of the chemokine MIP-1α and diminished activation of NF-κB in the lung [71]. TLR4 also plays a central role in the control of *Klebsiella pneumoniae* infection; mutant mice have shortened survival associated with enhanced bacterial growth and progressive pneumonia [72]. TLR4 is also important in the immune defence against *Brucella abortus*, a gram-negative organism that causes chronic zoonotic infection in humans. Mice deficient for TLR4 exhibit impaired clearance of the bacteria from the spleen associated with diminished IFN-γ production during the infection [73]. The role of TLR4 in the induction of river blindness was elucidated in a mouse infection model where the corneal cells were directly infected with an extract of filarial nematodes carrying the live endosymbiotic gram-negative bacterium *Wolbachia*, the causative agent of the river blindness. Inflammation caused by *Wolbachia* was dependent on the expression of TLR4 and CD14 by corneal cells [74].

**TLR4 and Gram-Positive Bacteria**

Given such overwhelming evidence for innate immune defence against gram-negative organisms, it may appear surprising that TLR4 also plays a role in the control of gram-positive bacteria. For example, it was shown that TLR4 mutant mice are highly susceptible in a sepsis model due to pneumolysin-producing *S. pneumoniae*. Pneumolysin is a cytolytic toxin and bacterial virulence factor present in almost all clinical isolates that was shown to activate macrophages in a TLR4 and MyD88-dependent manner and to function synergistically in the presence of TLR2 agonists. Therefore, in addition to LPS, TLR4 has multiple ligands including pneumolysin and plays a non-redundant role in the recognition and inflammatory response against gram-positive bacteria producing this virulence factor [75].

**TLR4 and Mycobacteria**

Two publications support the observation that TLR4 mutant mice have a reduced capacity to eliminate bacteria from the lung in an infection model of *M. tuberculosis* [76, 77]. In one of these [76], mycobacterial infection quickly spread to the spleen and the liver, resulting in a 10- to 100-fold higher CFU count relative to controls and resulted in death within 5–7 months. A clinical picture of chronic pneumonia with increased neutrophil infiltration but reduced macrophage recruitment was observed and...
TLR11-deficient mice. The attributes of uropathogenic infections renal inflammatory response was observed in the to more common, virulent E. coli times higher relative to wild-type controls [80]. A dimin-
ished renal inflammatory response was observed in the TLR11-deficient mice. The attributes of uropathogenic E. coli that facilitate its recognition by TLR11 in comparison to more common, virulent E. coli strains are at present unknown. An evolutionarily interesting feature is the finding that the human TLR11 sequence bears premature stop codons. This suggests that human TLR11 is either not expressed or that any translated protein does not participate in the recognition of uropathogenic E. coli [81]. Very recently, a profilin-like protein derived from the protozoan Toxoplasma gondii was identified as a TLR11 ligand that activates dendritic cells to produce IL-12 in a MyD88-de-
pendent manner [82]. TLR11-deficient mice were more susceptible to chronic infection with T. gondii, suggesting a potential role for this receptor in rodent host resistance against protozoal infection. These observations also raise the possibility that the putative uropathogenic E. coli li-
gand for mouse TLR11 is a protein.

Myeloid Differentiation Factor 88

As outlined previously, MyD88 is a central adaptor molecule that plays an important role in the transmission of signals from all known TLRs with the exception of TLR3. Therefore, integrity of MyD88-dependent responses is important for the control of a wide variety of infections.

MyD88 and Bacterial Infection

As a general rule, the altered host resistance phenotype of MyD88-deficient mice is even more pronounced than that of mice with individual TLR deficiencies and is ex-
emplified by a heightened susceptibility to infection with S. aureus, GBS, S. pneumoniae, Listeria and Salmonella [52, 56, 83–85]. There are at least two factors that explain this differential host susceptibility. First, MyD88 is not an exclusive adaptor protein for TLR signalling and also mediates activation via the IL-1R and IL-18R, two pro-
inflammatory cytokines that also play an important role in host defence [86]. Second, many bacteria trigger acti-
vation of more than one TLR [87] and most of these sig-
als would be abrogated by MyD88 deficiency. In addi-
tion to these studies, there are some new and unique find-
ings in MyD88-deficient mice that were not necessarily predictable from the in vitro data. For example, in an acute model of lung infection with Pseudomonas aerugi-
osa MyD88 regulates several early host responses be-

ding the bacterial burden and neutrophil infiltration that is necessary for recruitment of neutrophils to the lung [88]. Following infection with Borrelia burgdorferi, MyD88-deficient mice developed significantly higher numbers of spirochaetes in ankle joints, ears and heart compared to susceptible TLR2-deficient mice, as detected by quantitative PCR. Although MyD88 knockout mice were able to mount an antigen-specific humoral immune response, in this case the isotypes were predomi-
nantly Th2 induced [89]. This observation is consistent with previous data showing that in the absence of MyD88, a Th2-dominated adaptive immune response still occurs [28].

MyD88 and Polymicrobial Infection

A further unexpected finding is that, in contrast to TLR2, TLR4 or combined TLR2/TLR4 deficiencies, MyD88 deficiency improved resistance against exper-
imental polymicrobial septic peritonitis. Despite the find-
ing that the bacterial burden and neutrophil infiltration of the peritoneal cavity was unaltered, the serum levels of pro-inflammatory cytokines were dramatically reduced in comparison to wild-type animals [90]. In this case, it is conceivable that additional TLR- or MyD88-inde-
pendent mechanisms of innate immune responsiveness might be engaged by the diverse microbial stimulus associated with intestinal perforation. For example NOD-1/2, which are expressed in the intestine and are activated by pepti-
doglycan or muramyl dipeptide, respectively, do not use MyD88 in their signalling pathway [91] and it is well es-

ed that TLR4 (as well as TLR3) can activate a va-

riety of defence genes in the absence of MyD88 [8]. The requirement for MyD88 in antigen-presenting cells of dif-

ferent tissues might also vary, reflecting a site-specific in-
nate mechanism for the control of adaptive immune responses [92]. Finally, in addition to TLRs the innate immune system is equipped with other PRRs (for example, C-type lectin receptor family, mannose receptor, pentraxin family, complement receptor, Fc receptor and others) and their contribution to the immune response has not been clearly established [21]. Therefore, during a particular infection, a beneficial or detrimental role for TLRs/MyD88 may depend on the relative contribution of an increasing bacterial burden or a progressive inflammatory shock syndrome to disease and/or death. Since these two conditions occur together during the severest infections, this issue may prove difficult to resolve. Further studies, including the possibility to generate MyD88/TRIF-double-deficient mice, in which no TLR signalling should occur, may provide an additional experimental tool to address this question.

**MyD88 and Mycobacterial Infection**

In an aerosol infection model with *M. tuberculosis*, mice deficient in MyD88 succumb rapidly with a 100-fold higher microbial burden in the lung and dissemination to the liver and spleen [93]. A vigorous lung inflammatory response was observed, characterized by an increased recruitment of macrophages and neutrophils in the absence of granuloma formation. This was associated with elevated levels of pro-inflammatory cytokines (IL-1β, IFN-γ and TNF) as well as chemokines (MIP-1α, MCP-1 and RANTES) in the lungs 5 weeks after infection. In sharp contrast to the in vivo findings, a dramatically decreased secretion of TNF, IL-12p40, nitric oxide and IL-6 was observed after in vitro infection of MyD88-deficient macrophages and dendritic cells with *M. tuberculosis* or *M. bovis* BCG. Although BCG-immunized MyD88-deficient mice were able to mount an antigen-specific immune response and control the acute phase of *M. tuberculosis* infection, this did not provide durable protection and the mice later succumbed, suggesting that a competent innate immune response is required for long-term disease containment. The reason for the seemingly discordant in vitro and in vivo observations may be similar to what has been described for polymicrobial infection, specifically that additional PRRs (especially DC-SIGN) and/or alternative TLR signalling pathways may participate in the immune response to *M. tuberculosis*. Another obvious consideration is that the in vivo infection model is much more complex compared to in vitro infection of individual cell types. For example, aerosolized mycobacteria may initially come into contact and be phagocytosed by lung alveolar macrophages, or may cross the epithelium and interact with interdigitating dendritic cells. Such dynamic interactions not only elicit an immune response from the lung mucosa, but also trigger complex changes in the gene and protein expression programmes of the microbe. A simultaneous analysis of both of these processes has not yet been realized, owing to limitations of the current experimental systems.

**The Functions of TLRs in the Pathogenesis of Bacterial Infections in Humans**

Heritable predisposition to infectious disease among humans is now considered to be extremely common [94]. Notwithstanding, a hallmark of innate immune signalling mechanisms is the evolutionary conservation of both structure and function. Therefore, identification of premature stop codon polymorphisms of human IRAK-4 that abolish signal transmission of TLRs and render patients highly susceptible to pyogenic infections was received with great interest [95, 96]. Interestingly, IRAK-deficient mice are highly susceptible to both viral and bacterial infection, while humans with IRAK mutations appear to have a more selective defect in antibacterial host defence. This difference suggests that humans possess redundant mechanisms for immune defence against non-bacterial pathogens. Various TLR polymorphisms have been associated with altered susceptibility to Legionnaires’ disease. In 1 case, a premature dominant stop codon polymorphism was identified in the extracellular domain of TLR5 (TLR5<sup>392STOP</sup>) with a prevalence of 10% of apparently healthy individuals. A case-control study demonstrated a significant association of this polymorphism with pneumonia due to *Legionella pneumophila*, a flagellated bacterium [97]. However, another report by the same group showed that TLR4<sup>A896G</sup> and TLR4<sup>C1196T</sup>, two polymorphisms that were previously associated with increased susceptibility to gram-negative bacteria, actually conferred resistance to Legionnaires’ disease [98]. The underlying reasons for these discrepant findings remain to be elucidated and attest to the complex nature of human genetic predisposition to infection as well as the specificity of the immune response for individual pathogens.

TLR1 has not been formally linked to infectious disease susceptibility in humans; however, patients that were low responders to a vaccine containing the outer surface protein A, a lipoprotein from *B. burgdorferi*, displayed a defect in the cell surface expression of TLR1 protein without any identifiable polymorphism of the
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coding sequence of the gene [46]. Subsequent in vitro studies demonstrated that TLR2 recognizes outer surface protein A in cooperation with TLR1 and initiates an adaptive immune response. Based on these findings, an underlying defect among these individuals is suspected either in the TLR1 promoter or in another protein necessary for intracellular trafficking yet its precise identification remains elusive.

As described previously, TLR4 is a crucial mediator of the immune response to gram-negative bacteria. Therefore, several groups have studied the effect of human TLR4 polymorphisms on the risk of developing sepsis. A polymorphism TLR4D299G was found to increase the susceptibility to gram-negative infections [99] and gram-negative septic shock [100], and other rare polymorphisms in TLR4 have been linked to meningococcal infections [101]. The TLR2 polymorphism R753Q may predispose to infection with S. aureus [102] or M. tuberculosis [103]. Finally in a very recent publication, it was demonstrated that the same R753Q polymorphism in TLR2 protects from late-stage Lyme disease [104]. Collectively, these human studies have confirmed the important role that TLRs play in a protective immune response and are generally consistent with the information obtained from gene-decient mice.

Future Directions

The ﬁeld of innate immunity and genetic susceptibility to infection has greatly advanced during the past decade since the discovery of mammalian TLRs. The innate immune response to many important human pathogens has been well deﬁned using experimental mouse models and will continue to advance with the increasing application of genomics to human infection. Future approaches may include the use of multiply gene-deﬁcient mice to better deﬁne TLR-independent immune responses and signalling pathways, as well as novel experimental models that are more relevant to human infections. Ultimately the hope is to translate the rapidly expanding knowledge of innate immunity into more effective management strategies that will reduce the burden of human infectious disease and death.

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