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

Markus Schnare^a Martin Röllinghoff^a Salman Qureshi^b

^aInstitute for Clinical Microbiology, Immunology and Hygiene, University of Erlangen-Nuremberg, Erlangen, Germany; ^bCentre for the Study of Host Resistance, McGill University, Montreal, Canada

Key Words

Toll-like receptor · Myeloid differentiation factor 88 · Mouse infection model, innate/adaptive immune response · Human bacterial infections

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.

Copyright © 2006 S. Karger AG, Basel

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-

KARGER

Fax +41 61 306 12 34
E-Mail karger@karger.ch
www.karger.com

© 2006 S. Karger AG, Basel
1018–2438/06/1391–0075\$23.50/0

Accessible online at:
www.karger.com/iaa

Correspondence to: Dr. Markus Schnare
Institute for Clinical Microbiology, Immunology and Hygiene
University of Erlangen-Nuremberg, Wasserturmstr. 3–5, DE–91054 Erlangen (Germany)
Tel. +49 9131 85 22577, Fax +49 9131 85 1001
E-Mail markus.schnare@mikrobio.med.uni-erlangen.de

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 of structurally 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.

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

TLR deficiency/ adaptor deficiency	Causative agent for infectious disease model	Resulting Th response ¹
TLR2	<i>Mycobacterium tuberculosis</i>	Th1 (stronger) [64]
TLR2	<i>Listeria monocytogenes</i>	Unclear, but a lower IFN- γ production [58]
TLR2	<i>Streptococcus pneumoniae</i>	Presumably Th2 [83]
TLR4	<i>Brucella abortus</i>	Unclear, but a reduced IFN- γ production [73]
TLR4	<i>Salmonella typhimurium</i>	Unclear, but strong reduction of IFN- γ producing CD4+ T cells [68]
MyD88	<i>Borrelia burgdorferi</i>	Th2 [89]
MyD88	<i>Streptococcus pneumoniae</i>	Presumably Th2 [83]
MyD88	<i>Mycobacterium tuberculosis</i>	Reduced Th1 (after BCG immunization) [93]

¹ Different from the response in wild-type control mice.

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].

TLR2 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

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 or-

ganism 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 lipoarabinomannan 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 ($LD_{50} > 2 \times 10^3$) [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 im-

mune 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-1 α and diminished activation of NF- κ B 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

may be due to the decreased pulmonary levels of TNF, IL-12p40 and MCP-1 in the lungs of the mutant mice during the infection [76]. It should be noted, however, that for unclear reasons the role of TLR4 in *M. tuberculosis* remains controversial, with two negative studies in the literature [78, 79].

Toll-Like Receptor 11

TLR11 has a unique expression pattern in the epithelial cells of the kidney and the liver, suggesting that it might play an important role in host defence of these organs. Indeed TLR11-deficient mice are highly susceptible to intra-urethral infection with uropathogenic *E. coli*, developing a bacterial burden in the kidney that was >10,000 times higher relative to wild-type controls [80]. A diminished 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-dependent 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* ligand 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 activation of more than one TLR [87] and most of these signals would be abrogated by MyD88 deficiency. In addition to these studies, there are some new and unique findings 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 aeruginosa* MyD88 regulates several early host responses beyond those of TLR2 or TLR4 deficiency alone, resulting in nearly absent cytokine and chemokine production 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 predominantly 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 experimental polymicrobial septic peritonitis. Despite the finding 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-independent 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 peptidoglycan or muramyl dipeptide, respectively, do not use MyD88 in their signalling pathway [91] and it is well established that TLR4 (as well as TLR3) can activate a variety of defence genes in the absence of MyD88 [8]. The requirement for MyD88 in antigen-presenting cells of different 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-1 β , IFN- γ and TNF) as well as chemokines (MIP-1 α , 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^{392STOP}) 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^{A89G} and TLR4^{C1196T}, 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

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 TLR4^{D299G} 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-deficient 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.

Acknowledgements

S.Q. is a recipient of a Canada Research Chair; his research is supported by a Career Award in the Biomedical Sciences from the Burroughs Wellcome Fund. The research of M.S. is supported by the German Research Foundation (DFG) and the Mukoviszidose e.V.

References

- Hooper L-V, Gordon JI: Commensal host-bacterial relationships in the gut. *Science* 2001; 292:1115–1118.
- Berg RD: The indigenous gastrointestinal microflora. *Trends Microbiol* 1996;4:430–435.
- Rakoff-Nahoum S, Paglino J, Eslami-Varzaneh F, Edberg S, Medzhitov R: Recognition of commensal microflora by Toll-like receptors is required for intestinal homeostasis. *Cell* 2004;118:229–241.
- Reid G, Howard J, Gan B-S: Can bacterial interference prevent infection? *Trends Microbiol* 2001;9:424–428.
- Guarner F, Malagelada JR: Gut flora in health and disease. *Lancet* 2003;361:512–519.
- Takeda K, Kaisho T, Akira S: Toll-like receptors. *Annu Rev Immunol* 2003;21:335–376.
- Medzhitov R, Janeway CA Jr: Decoding the patterns of self and nonself by the innate immune system. *Science* 2002;296:298–300.
- Akira S, Takeda K: Toll-like receptor signalling. *Nat Rev Immunol* 2004;4:499–511.
- Tabeta K, Georgel P, Janssen E, Du X, Hoebe K, Crozat K, Mudd S, Shamel L, Sovath S, Goode J, et al: Toll-like receptors 9 and 3 as essential components of innate immune defense against mouse cytomegalovirus infection. *Proc Natl Acad Sci USA* 2004;101:3516–3521.
- Bowie A-G, Haga I-R: The role of Toll-like receptors in the host response to viruses. *Mol Immunol* 2005;42:859–867.
- Chuang T-H, Ulevitch RJ: Cloning and characterization of a sub-family of human toll-like receptors: hTLR7, hTLR8 and hTLR9. *Eur Cytokine Netw* 2000;11:372–378.
- Schroder M, Bowie A-G: TLR3 in antiviral immunity: key player or bystander? *Trends Immunol* 2005;26:462–468.
- Krieg A-M: CpG motifs in bacterial DNA and their immune effects. *Annu Rev Immunol* 2002;20:709–760.
- Deng J-C, Moore TA, Newstead MW, Zeng X, Krieg AM, Standiford TJ: CpG oligodeoxynucleotides stimulate protective innate immunity against pulmonary *Klebsiella* infection. *J Immunol* 2004;173:5148–5155.
- Ito S, Ishii KJ, Shirota H, Klinman DM: CpG oligodeoxynucleotides improve the survival of pregnant and fetal mice following *Listeria monocytogenes* infection. *Infect Immun* 2004; 72:3543–3548.
- Ito S, Ishii KJ, Gursel M, Shirota H, Ihata A, Klinman DM: CpG oligodeoxynucleotides enhance neonatal resistance to *Listeria* infection. *J Immunol* 2005;174:777–782.
- Edwards L, Williams AE, Krieg AJ, Rae AJ, Snelgrove RJ, Hsuell T: Stimulation via Toll-like receptor 9 reduces *Cryptococcus neoformans*-induced pulmonary inflammation in an IL-12-dependent manner. *Eur J Immunol* 2005;35:273–281.
- Lund J, Sato A, Akira S, Medzhitov R, Iwasaki A: Toll-like receptor 9-mediated recognition of Herpes simplex virus-2 by plasmacytoid dendritic cells. *J Exp Med* 2003;198:513–520.
- Krug A, French AR, Barchet W, Fischer JA, Dzionek A, Pingel JT, Orihuela MM, Akira S, Yokoyama WM, Colonna M: TLR9-dependent recognition of MCMV by IPC and DC generates coordinated cytokine responses that activate antiviral NK cell function. *Immunity* 2004;21:107–119.
- Krug A, Luker GD, Barchet W, Leib DA, Akira S, Colonna M: Herpes simplex virus type 1 activates murine natural interferon-producing cells through Toll-like receptor 9. *Blood* 2004; 103:1433–1437.
- Janeway CA Jr, Medzhitov R: Innate immune recognition. *Annu Rev Immunol* 2002;20: 197–216.
- Hoffmann JA: The immune response of *Drosophila*. *Nature* 2003;426:33–38.
- Janssens S, Beyaert R: Role of Toll-like receptors in pathogen recognition. *Clin Microbiol Rev* 2003;16:637–646.

- 24 Fitzgerald K-A, Rowe DC, Golenbock DT: Endotoxin recognition and signal transduction by the TLR4/MD2-complex. *Microbes Infect* 2004;6:1361-1367.
- 25 da Silva Correia J, Soldau K, Christen U, Tobias PS, Ulevitch RJ: Lipopolysaccharide is in close proximity to each of the proteins in its membrane receptor complex: transfer from CD14 to TLR4 and MD-2. *J Biol Chem* 2001;276:21129-21135.
- 26 Choe J, Kelker MS, Wilson I-A: Crystal structure of human toll-like receptor 3 (TLR3) ectodomain. *Science* 2005;309:581-585.
- 27 Tsan M-F, Gao B: Endogenous ligands of Toll-like receptors. *J Leukoc Biol* 2004;76:514-519.
- 28 Schnare M, Barton GM, Holt AC, Takeda K, Akira S, Medzhitov R: Toll-like receptors control activation of adaptive immune responses. *Nat Immunol* 2001;2:947-950.
- 29 Hoebe K, Janssen E, Beutler B: The interface between innate and adaptive immunity. *Nat Immunol* 2004;5:971-974.
- 30 Liew F-Y, Xu D, Brint EK, O'Neill LA: Negative regulation of Toll-like receptor-mediated immune responses. *Nat Rev Immunol* 2005;5:446-458.
- 31 Lord KA, Hoffman-Liebermann B, Liebermann DA: Nucleotide sequence and expression of a cDNA encoding MyD88, a novel myeloid differentiation primary response gene induced by IL6. *Oncogene* 1990;5:1095-1097.
- 32 Wesche H, Henzel WJ, Shillinglaw W, Li S, Cao Z: MyD88: an adapter that recruits IRAK to the IL-1 receptor complex. *Immunity* 1997;7:837-847.
- 33 Medzhitov R, Preston-Hurlburt P, Kopp E, Stadlen A, Chen C, Ghosh S, Janeway CA Jr: MyD88 is an adaptor protein in the hToll/IL-1 receptor family signaling pathways. *Mol Cell* 1998;2:253-258.
- 34 McGettrick A-F, O'Neill LA: The expanding family of MyD88-like adaptors in Toll-like receptor signal transduction. *Mol Immunol* 2004;41:577-582.
- 35 Yamamoto M, Takeda K, Akira S: TIR domain-containing adaptors define the specificity of TLR signaling. *Mol Immunol* 2004;40:861-868.
- 36 Kawai T, Takeuchi O, Fujita T, Inoue J, Muhlradt PF, Sato S, Hoshino K, Akira S: Lipopolysaccharide stimulates the MyD88-independent pathway and results in activation of IFN-regulatory factor 3 and the expression of a subset of lipopolysaccharide-inducible genes. *J Immunol* 2001;167:5887-5894.
- 37 O'Neill L-A, Fitzgerald KA, Bowie AG: The Toll-IL-1 receptor adaptor family grows to five members. *Trends Immunol* 2003;24:286-290.
- 38 Mink M, Fogelgren B, Olszewski K, Maroy P, Csizsar K: A novel human gene (SARM) at chromosome 17q11 encodes a protein with a SAM motif and structural similarity to Armadillo/ β -catenin that is conserved in mouse, *Drosophila*, and *Caenorhabditis elegans*. *Genomics* 2001;74:234-244.
- 39 Bogdan C: Nitric oxide and the immune response. *Nat Immunol* 2001;2:907-916.
- 40 Iwasaki A, Medzhitov R: Toll-like receptor control of the adaptive immune responses. *Nat Immunol* 2004;5:987-995.
- 41 Remer KA, Brcic M, Jungi TW: Toll-like receptor-4 is involved in eliciting an LPS-induced oxidative burst in neutrophils. *Immunol Lett* 2003;85:75-80.
- 42 Becker M-N, Diamond G, Verghese MW, Randell SH: CD14-dependent lipopolysaccharide-induced beta-defensin-2 expression in human tracheobronchial epithelium. *J Biol Chem* 2000;275:29731-29736.
- 43 Hertz C-J, Wu Q, Porter EM, Zhang YJ, Weismuller KH, Godowski PJ, Ganz T, Randell SH, Modlin RL: Activation of Toll-like receptor 2 on human tracheobronchial epithelial cells induces the antimicrobial peptide human beta defensin-2. *J Immunol* 2003;171:6820-6826.
- 44 Eisenbarth S-C, Piggott DA, Huleatt JW, Visintin I, Herrick CA, Bottomly K: Lipopolysaccharide-enhanced, Toll-like receptor 4-dependent T helper cell type 2 responses to inhaled antigen. *J Exp Med* 2002;196:1645-1651.
- 45 Takeuchi O, Hoshino K, Kawai T, Sanjo H, Takada H, Ogawa T, Takeda K, Akira S: Differential roles of TLR2 and TLR4 in recognition of gram-negative and gram-positive bacterial cell wall components. *Immunity* 1999;11:443-451.
- 46 Alexopoulou L, Thomas V, Schnare M, Lobet Y, Anguita J, Schoen RT, Medzhitov R, Fikrig E, Flavell RA: Hyporesponsiveness to vaccination with *Borrelia burgdorferi* OspA in humans and in TLR1- and TLR2-deficient mice. *Nat Med* 2002;8:878-884.
- 47 Takeuchi O, Kawai T, Muhlradt PF, Morr M, Radolf JD, Zychlinsky A, Takeda K, Akira S: Discrimination of bacterial lipoproteins by Toll-like receptor 6. *Int Immunol* 2001;13:933-940.
- 48 Brightbill H-D, Libraty DH, Krutzik SR, Yang RB, Belisle JT, Bleharski JR, Maitland M, Norgard MV, Plevy SE, Smale ST, et al: Host defense mechanisms triggered by microbial lipoproteins through Toll-like receptors. *Science* 1999;285:732-736.
- 49 Brown G-D, Herre J, Williams DL, Willment JA, Marshall AS, Gordon S: Dectin-1 mediates the biological effects of beta-glucans. *J Exp Med* 2003;197:1119-1124.
- 50 Gantner B-N, Simmons RM, Canavera SJ, Akira S, Underhill DM: Collaborative induction of inflammatory responses by dectin-1 and Toll-like receptor 2. *J Exp Med* 2003;197:1107-1117.
- 51 Hoebe K, Georgel P, Rutschmann S, Du X, Mudd S, Crozat K, Sovath S, Shamel L, Hartung T, Zahringer U, et al: CD36 is a sensor of diacylglycerides. *Nature* 2005;433:523-527.
- 52 Takeuchi O, Hoshino K, Akira S: Cutting edge: TLR2-deficient and MyD88-deficient mice are highly susceptible to *Staphylococcus aureus* infection. *J Immunol* 2000;165:5392-5396.
- 53 Echchannaoui H, Frei K, Schnell C, Leib SL, Zimmerli W, Landmann R: Toll-like receptor 2-deficient mice are highly susceptible to *Streptococcus pneumoniae* meningitis because of reduced bacterial clearing and enhanced inflammation. *J Infect Dis* 2002;186:798-806.
- 54 Koedel U, Angele B, Rupprecht T, Wagner H, Roggenkamp A, Pfister HW, Kirschning CJ: Toll-like receptor 2 participates in mediation of immune response in experimental pneumococcal meningitis. *J Immunol* 2003;170:438-444.
- 55 Koedel U, Rupprecht T, Angele B, Heesemann J, Wagner H, Pfister HW, Kirschning CJ: MyD88 is required for mounting a robust host immune response to *Streptococcus pneumoniae* in the CNS. *Brain* 2004;127:1437-1445.
- 56 Mancuso G, Midiri A, Beninati C, Biondo C, Galbo R, Akira S, Henneke P, Golenbock D, Teti G: Dual role of TLR2 and myeloid differentiation factor 88 in a mouse model of invasive group B streptococcal disease. *J Immunol* 2004;172:6324-6329.
- 57 Meng G, Rutz M, Schiemann M, Metzger J, Grabiec A, Schwandner R, Lippa PB, Ebel F, Busch DH, Bauer S, et al: Antagonistic antibody prevents toll-like receptor 2-driven lethal shock-like syndromes. *J Clin Invest* 2004;113:1473-1481.
- 58 Torres D, Barrier M, Bihl F, Quesniaux VJ, Maillet I, Akira S, Ryffel B, Erard F: Toll-like receptor 2 is required for optimal control of *Listeria monocytogenes* infection. *Infect Immun* 2004;72:2131-2139.
- 59 Underhill DM, Ozinsky A: Phagocytosis of microbes: complexity in action. *Annu Rev Immunol* 2002;20:825-852.
- 60 Darville T, O'Neill JM, Andrews CW Jr, Nagarajan UM, Stahl L, Ojcius DM: Toll-like receptor-2, but not Toll-like receptor-4, is essential for development of oviduct pathology in chlamydial genital tract infection. *J Immunol* 2003;171:6187-6197.
- 61 Wooten R-M, Ma Y, Yoder RA, Brown J-H, Weis JH, Zachary JF, Kirschning CJ, Weis JJ: Toll-like receptor 2 is required for innate, but not acquired, host defense to *Borrelia burgdorferi*. *J Immunol* 2002;168:348-355.
- 62 Means TK, Wang S, Lien E, Yoshimura A, Golenbock DT, Fenton MJ: Human Toll-like receptors mediate cellular activation by *Mycobacterium tuberculosis*. *J Immunol* 1999;163:3920-3927.
- 63 Thoma-Uszynski S, Stenger S, Takeuchi O, Ochoa M, Engele M, Sieling P-A, Barnes F, Rollinghoff M, Beleskei PL, Wagner M, et al: Induction of direct antimicrobial activity through mammalian Toll-like receptors. *Science* 2001;291:1544-1547.
- 64 Drennan M-B, Nicolle D, Quesniaux VJ, Jacobs M, Allie N, Mpagi J, Fremont C, Wagner H, Kirschning CJ, Ryffel B: Toll-like receptor 2-deficient mice succumb to *Mycobacterium tuberculosis* infection. *Am J Pathol* 2004;164:49-57.
- 65 Sing A, Rost D, Tvardovskaia N, Roggenkamp A, Wiedemann A, Kirschning CJ, Aepfelbacher M, Heesemann J: Yersinia V-antigen exploits Toll-like receptor 2 and CD14 for interleukin 10-mediated immunosuppression. *J Exp Med* 2002;196:1017-1024.
- 66 Poltorak A, He X, Smirnova I, Liu MY, Van Huffel C, Du X, Birdwell D, Alejos E, Silva M, Galanos C, et al: Defective LPS signaling in C3H/HeJ and C57BL/10ScCr mice: mutations in Tlr4 gene. *Science* 1998;282:2085-2088.

- 67 O'Brien AD, Rosenstreich DL, Scher I, Campbell GH, MacDermott RP, Formal SB: Genetic control of susceptibility to *Salmonella typhimurium* in mice: role of the LPS gene. *J Immunol* 1980;124:20–24.
- 68 Vazquez-Torres A, Vallance BA, Bergman MA, Finlay B, Cookson BT, Jones-Carson J, Fang FC: Toll-like receptor 4 dependence of innate and adaptive immunity to *Salmonella*: importance of the Kupffer cell network. *J Immunol* 2004;172:6202–6208.
- 69 Woods J-P, Frelinger JA, Warrack G, Cannon JG: Mouse genetic locus Lps influences susceptibility to *Neisseria meningitidis* infection. *Infect Immun* 1988;56:1950–1955.
- 70 Hopkins W-J, Gendron-Fitzpatrick E, Balish E, Uehling DT: Time course and host responses to *Escherichia coli* urinary tract infection in genetically distinct mouse strains. *Infect Immun* 1998;66:2798–2802.
- 71 Wang X, Moser C, Louboutin JP, Lysenko ES, Weiner DJ, Weiser JN, Wilson JM: Toll-like receptor 4 mediates innate immune responses to *Haemophilus influenzae* infection in mouse lung. *J Immunol* 2002;168:810–815.
- 72 Branger J, Knapp S, Weijer S, Leemans JC, Pater JM, Speelman P, Florquin S, van der Poll T: Role of Toll-like receptor 4 in gram-positive and gram-negative pneumonia in mice. *Infect Immun* 2004;72:788–794.
- 73 Campos M-A, Rosinha GM, Almeida IC, Salgueiro XS, Jarvis BW, Splitter GA, Qureshi N, Bruna-Romero O, Gazzinelli RT, Oliveira SC: Role of Toll-like receptor 4 in induction of cell-mediated immunity and resistance to *Brucella abortus* infection in mice. *Infect Immun* 2004;72:176–186.
- 74 Saint Andre A, Blackwell NM, Hall LR, Hoerauf A, Brattig NW, Volkmann L, Taylor MJ, Ford L, Hise AG, Lass JH, et al: The role of endosymbiotic *Wolbachia* bacteria in the pathogenesis of river blindness. *Science* 2002;295:1892–1895.
- 75 Malley R, Henneke P, Morse SC, Cieslewicz MJ, Lipsitch M, Thompson CM, Kurt-Jones E, Paton JC, Wessels MR, Golenbock DT: Recognition of pneumolysin by Toll-like receptor 4 confers resistance to pneumococcal infection. *Proc Natl Acad Sci USA* 2003;100:1966–1971.
- 76 Abel B, Thieblemont N, Quesniaux VJ, Brown N, Mpagi J, Miyake K, Bihl F, Ryffel B: Toll-like receptor 4 expression is required to control chronic *Mycobacterium tuberculosis* infection in mice. *J Immunol* 2002;169:3155–3162.
- 77 Branger J, Leemans JC, Florquin S, Weijer S, Speelman P, van der Poll T: Toll-like receptor 4 plays a protective role in pulmonary tuberculosis in mice. *Int Immunol* 2004;16:509–516.
- 78 Reiling N, Holscher C, Fehrenbach A, Kroger S, Kirschning CJ, Goyert S, Ehlers S: Cutting edge: Toll-like receptor (TLR)2- and TLR4-mediated pathogen recognition in resistance to airborne infection with *Mycobacterium tuberculosis*. *J Immunol* 2002;169:3480–3484.
- 79 Shim T-S, Turner OC, Orme I-M: Toll-like receptor 4 plays no role in susceptibility of mice to *Mycobacterium tuberculosis* infection. *Tuberculosis (Edinb)* 2003;83:367–371.
- 80 Zhang D, Zhang G, Hayden MS, Greenblatt MB, Bussey C, Flavell RA, Ghosh S: A Toll-like receptor that prevents infection by uropathogenic bacteria. *Science* 2004;303:1522–1526.
- 81 Klaffenbach D, Rascher W, Rollinghoff M, Dotsch J, Meissner U, Schnare M: Regulation and signal transduction of Toll-like receptors in human chorioncarcinoma cell lines. *Am J Reprod Immunol* 2005;53:77–84.
- 82 Yarovinsky F, Zhang D, Andersen JF, Bannenberg GL, Serhan CN, Hayden MS, Hieny S, Sutterwala FS, Flavell RA, Ghosh S, et al: TLR11 activation of dendritic cells by a protozoan profilin-like protein. *Science* 2005;308:1626–1629.
- 83 Khan A-Q, Chen Q, Wu Z-Q, Paton JC, Snapper CM: Both innate immunity and type 1 humoral immunity to *Streptococcus pneumoniae* are mediated by MyD88 but differ in their relative levels of dependence on Toll-like receptor 2. *Infect Immun* 2005;73:298–307.
- 84 Edelson B-T, Unanue ER: MyD88-dependent but Toll-like receptor 2-independent innate immunity to *Listeria*: no role for either in macrophage listericidal activity. *J Immunol* 2002;169:3869–3875.
- 85 Weiss D-S, Raupach B, Takeda K, Akira S, Zychlinsky A: Toll-like receptors are temporally involved in host defense. *J Immunol* 2004;172:4463–4469.
- 86 Adachi O, Kawai T, Takeda K, Matsumoto M, Tsutsui H, Sakagami M, Nakanishi K, Akira S: Targeted disruption of the MyD88 gene results in loss of IL-1- and IL-18-mediated function. *Immunity* 1998;9:143–150.
- 87 Nau G-J, Schlesinger A, Richmond JF, Young RA: Cumulative Toll-like receptor activation in human macrophages treated with whole bacteria. *J Immunol* 2003;170:5203–5209.
- 88 Power M-R, Peng Y, Maydanski E, Marshall JS, Lin TJ: The development of early host response to *Pseudomonas aeruginosa* lung infection is critically dependent on myeloid differentiation factor 88 in mice. *J Biol Chem* 2004;279:49315–49322.
- 89 Bolz D-D, Sundsbak RS, Ma Y, Akira S, Kirschning CJ, Zachary JF, Weis JH, Weis JJ: MyD88 plays a unique role in host defense but not arthritis development in Lyme disease. *J Immunol* 2004;173:2003–2010.
- 90 Weighardt H, Kaiser-Moore S, Vabulas RM, Kirschning CJ, Wagner H, Holzmann B: Cutting edge: myeloid differentiation factor 88 deficiency improves resistance against sepsis caused by polymicrobial infection. *J Immunol* 2002;169:2823–2827.
- 91 Inohara N, Chamillard M, McDonald C, Nunez G: NOD-LRR proteins: role in host-microbial interactions and inflammatory disease. *Annu Rev Biochem* 2005;74:355–383.
- 92 Piggott D-A, Eisenbarth SC, Xu L, Constant SL, Huleatt JW, Herrick CA, Bottomly K: MyD88-dependent induction of allergic Th2 responses to intranasal antigen. *J Clin Invest* 2005;115:459–467.
- 93 Fremont C-M, Yermeev V, Nicolle DM, Jacobs M, Quesniaux VF, Ryffel B: Fatal *Mycobacterium tuberculosis* infection despite adaptive immune response in the absence of MyD88. *J Clin Invest* 2004;114:1790–1799.
- 94 Casanova JL, Abel L: Inborn errors of immunity to infection: the rule rather than the exception. *J Exp Med* 2005;202:197–201.
- 95 Medvedev AE, Lentsch A, Kuhns DB, Blanco JC, Salkowski C, Zhang S, Arditi M, Gallin JI, Vogel SN: Distinct mutations in IRAK-4 confer hyporesponsiveness to lipopolysaccharide and interleukin-1 in a patient with recurrent bacterial infections. *J Exp Med* 2003;198:521–531.
- 96 Picard C, Puel A, Bonnet M, Ku CL, Bustamante J, Yang K, Soudais C, Dupuis S, Feinberg J, Fieschi C, et al: Pyogenic bacterial infections in humans with IRAK-4 deficiency. *Science* 2003;299:2076–2079.
- 97 Hawn TR, Verbon A, Lettinga KD, Zhao LP, Li SS, Laws RJ, Skerrett SJ, Beutler B, Schroeder L, Nachman A, et al: A common dominant TLR5 stop codon polymorphism abolishes flagellin signaling and is associated with susceptibility to Legionnaires' disease. *J Exp Med* 2003;198:1563–1572.
- 98 Hawn T-R, Verbon A, Janer M, Zhao LP, Beutler B, Aderem A: Toll-like receptor 4 polymorphisms are associated with resistance to Legionnaires' disease. *Proc Natl Acad Sci USA* 2005;102:2487–2489.
- 99 Agnese D-M, Calvano E, Hahn SJ, Coyle SM, Corbett SA, Calvano SE, Lowry SF: Human Toll-like receptor 4 mutations but not CD14 polymorphisms are associated with an increased risk of gram-negative infections. *J Infect Dis* 2002;186:1522–1525.
- 100 Lorenz E, Mira JP, Frees KL, Schwartz DA: Relevance of mutations in the TLR4 receptor in patients with gram-negative septic shock. *Arch Intern Med* 2002;162:1028–1032.
- 101 Smirnova I, Mann N, Dols A, Derks HH, Hibberd ML, Levin M, Beutler B: Assay of locus-specific genetic load implicates rare Toll-like receptor 4 mutations in meningococcal susceptibility. *Proc Natl Acad Sci USA* 2003;100:6075–6080.
- 102 Lorenz E, Mira JP, Cornish KL, Arbour NC, Schwartz DA: A novel polymorphism in the Toll-like receptor 2 gene and its potential association with staphylococcal infection. *Infect Immun* 2000;68:6398–6401.
- 103 Ogun A-C, Yoldas B, Ozdemir T, Uguz A, Oicun S, Keser L, Coskun M, Cilli A, Yegin O: The Arg753Gln polymorphism of the human toll-like receptor 2 gene in tuberculosis disease. *Eur Respir J* 2004;23:219–223.
- 104 Schroder N-W, Diterich I, Zinke A, Eckert J, Draing C, Baehr VV, Hassler D, Priem S, Hahn K, Michelsen KS, et al: Heterozygous Arg753Gln polymorphism of human TLR-2 impairs immune activation by *Borrelia burgdorferi* and protects from late stage Lyme disease. *J Immunol* 2005;175:2534–2540.