Host Response to *Leptospira* Infection

Richard L. Zuerner

Abstract Pathogenic *Leptospira* has the capacity to infect a broad range of mammalian hosts. Leptospirosis may appear as an acute, potentially fatal infection in accidental hosts, or progress into a chronic, largely asymptomatic infection in natural maintenance hosts. The course that *Leptospira* infection follows is dependent upon poorly understood factors, but is heavily influenced by both the host species and bacterial serovar involved in infection. Recognition of pathogen-associated molecular patterns (PAMPs) by a variety of host pattern recognition receptors (PRRs) activates the host immune system. The outcome of this response may result in bacterial clearance, limited bacterial colonization of a few target organs, principally the kidney, or induction of sepsis as the host succumbs to infection and dies. This chapter describes current knowledge of how the host recognizes *Leptospira* and responds to infection using innate and acquired immune responses. Aspects of immune-mediated pathology and pathogen strategies to evade the host immune response are also addressed.

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R.L. Zuerner (✉)
Department of Biomedical Sciences and Veterinary Public Health, Swedish University for Agricultural Sciences, 75007 Uppsala, Sweden
e-mail: Richard.Zuerner@gmail.com

1 Introduction

Leptospira can cause two distinctly different disease manifestations depending on the mammalian host and infecting serovar. Infection leads to either a chronic, nearly asymptomatic infection or an acute, potentially life-threatening disease. The most severe form, classic Weil's disease, or acute leptospirosis, occurs most commonly in accidental hosts, including humans, with a wide range of disease manifestations (Faine et al. 1999). In contrast, infection of a normal maintenance host will typically result in a chronic infection with little outward sign of infection (Faine et al. 1999). Maintenance hosts most commonly show evidence of infection during pregnancy, manifested by the appearance of reproductive failure (infertility, abortions, still-births, or birth of weak offspring). It is important to note that the same bacterial strain can often cause both acute and chronic infections, depending largely on the mammalian species that is infected. Development of chronic or acute infection is dependent upon poorly understood factors that pair specific mammalian host species with selected *Leptospira* serovars. Presumably, the interplay between the host immune system and infecting strain of bacteria is critical in directing the outcome of *Leptospira* infection.

Keeping in mind the dual nature of *Leptospira* is key to understanding the disease and how the immune system responds to infection. Early work by Adler, Faine, and coworkers clearly established the importance of antibody in providing immune protection in leptospirosis, at least for some host species (Adler and Faine 1977). However, these studies do not tell the full story. Components of both innate and acquired immune systems have been identified that respond to *Leptospira* infection. Both humoral and cell-mediated immunity (CMI) are needed for immune protection against *Leptospira,* yet at the same time immunological processes may also contribute to tissue damage during infection. Infected hosts are challenged by a number of bacterial properties that alter the host response or that contribute to immune evasion and persistent infection.

Although *Leptospira* can infect a wide range of mammalian species, most experimental infection studies have been conducted in golden Syrian hamsters, a species particularly susceptible to acute infection (Morton 1942). However, lack of well characterized immunological reagents for use with hamster tissues has limited our understanding of many aspects of the immune response to *Leptospira* infection. Although a wide variety of reagents are available for characterization of the mouse immune response, most mouse strains are refractory to infection by pathogenic strains of *Leptospira* after about 1 month of age (Packchanian 1940). This situation has slowed analysis of many aspects of the host immune response during

leptospirosis. Studies involving experimental infection of other mammalian hosts, notably cattle, have helped to establish a prominent role for components of the cellular immune response in development of protective immunity, especially in maintenance hosts. Thus, the overall view of the immune response during leptospirosis draws on knowledge gained from the host responses in widely divergent mammalian genera infected with diverse species of *Leptospira*.

From the perspective of the host, pathogenic spirochetes, including *Leptospira,* are difficult adversaries to remove. Pathogenic spirochetes express few proteins on the outer membrane surface (Haake et al. 1991, see the chapter by D.A. Haake and W.R. Zückert, this volume), and may vary expression of surface proteins in response to environmental factors such as iron, temperature, and osmolarity (Lo et al. 2006; Matsunaga et al. 2005, 2007). Limited expression of antigenic surface proteins presents few targets for pathogen recognition and development of a protective immune response by the host. Additionally, spirochete outer membranes are loosely attached to the peptidoglycan layer and are easily removed by mild detergent (Haake et al. 1991; Zuerner et al. 1991). Lateral movement of outer membrane antigens through the lipid bilayer with little or no impediment enables the bacteria freedom of movement when bound by antibody (Charon et al. 1981). Thus, attachment of antibody to surface proteins does not impede motility, often a key factor in tissue penetration. Although leptospiral LPS is less pyrogenic than typical Gram-negative LPS, it stimulates a strong immune response that may, or may not, be important for immunoprotection, depending on the nature of the host–serovar interaction.

This chapter reviews historic and recent findings related to the immune response of the host to *Leptospira* infection, the bacterial targets of the immune response, and possible role of the immune response in contributing to disease manifestations. Complicating our ability to develop an understanding of how hosts resist *Leptospira* infection is the variability associated with past experimentation, which has often used different *Leptospira* species, serovars, and strains, and different mammalian host species. Additionally, a critical flaw in some studies has been the use of strains that had undergone many in vitro passages in bacteriological media without first assessing infectivity (ID_{50}) or lethality (LD_{50}) before animal experimentation. Consequently, findings from different laboratories may appear contradictory, yet could simply represent differences between mammalian hosts or the species, serovar, or strain used in experimentation. Recent development of genetic tools has facilitated construction of defined leptospiral mutants, and this is allowing us to discern the importance of specific bacterial genes in virulence (see the chapter by M. Picardeau, this volume). Likewise, recent development of highly inbred or genetic knockout strains of mice with known genetic deficiencies is now leading to a limited, but growing, understanding of the genetic basis of host susceptibility to infection. It is therefore important to bear in mind that most of the studies described in this chapter are drawn from experiments conducted with widely variable use of hosts and pathogens. Continued development and use of highly infectious (i.e., strains with low ID_{50}) knockout mutants should help to resolve the relative importance of specific genes in host selection and virulence.

2 Animal Models

A wide variety of animal species have been used as hosts for experimental leptospirosis. Early studies used guinea pigs as a preferred animal model host to study acute infection (Noguchi 1918). In the mid-twentieth century, it was discovered that hamsters were particularly susceptible to *Leptospira* infection (Morton 1942). Owing to their general good health, rapid growth, and lower cost, hamsters are now routinely used as the primary model for acute leptospirosis (Haake 2006). Hamsters retain susceptibility to acute infection with increasing age more than many other animal species and mimic acute infections that share some similarities to clinical disease in humans. Hamsters have been used extensively to test bacterial strain infectivity and virulence, and as a model for testing vaccine efficacy. Indeed, the hamster model of infection is so predictable that many government and international organizations use the hamster model for testing vaccine efficacy.

Common laboratory mice (*Mus musculus*) and rats (*Rattus novegicus*) are generally unsuitable as hosts for acute leptospirosis; these species are only susceptible to developing acute leptospirosis within a very short window of time after birth (Packchanian 1940), unless they have specific genetic deficiencies. Infection of mice or rats older than a few weeks of age will more likely lead to development of chronic infections limited to colonization of kidney. However, as will be described in more detail below, infection of cyclophosphamide-treated mice (Adler and Faine 1976) or mice with toll-like receptor (TLR) deficiencies (Pereira et al. 1998) can result in lethal infection. Use of well-defined genetic knockout (KO) mouse strains in leptospirosis research is becoming more common. Rats are also being used as experimental hosts to study chronic leptospirosis (Athanazio et al. 2008; Monahan et al. 2008; Tucunduva de Faria et al. 2007). Use of rats and mice allows access to well-defined tools and the availability of genetically defined strains is leading to new knowledge on components of the immune system that are important for protective immunity.

Although experimental studies using livestock are quite expensive, cattle, goats, and pigs have been used as experimental hosts for infection and vaccination studies for leptospirosis. This is due in large part on the concern for zoonotic transmission between livestock and humans, the impact of leptospirosis on livestock production costs, and the need for effective vaccines in production animals. Research on leptospirosis in cattle has provided new information on the role of CMI in controlling leptospirosis.

Experimental small animals are most often inoculated with *Leptospira* by intraperitoneal (IP) injection (Haake 2006). Although an unnatural method of disease transmission, IP injection is an efficient, reproducible method for inoculation of small animals such as hamsters and leads to rapid dissemination in the animal. Two alternative methods of experimental inoculation of *Leptospira* thought to mimic natural routes of infection include conjunctival instillation (Thiermann and Handsaker 1985) and subcutaneous injection (Truccolo et al. 2002).

Virulent leptospires injected into the peritoneum establish a transient peritonitis and can be detected in and around blood vessels within 2 days (Zuerner et al. 2012). In acute leptospirosis, the bacteria may first migrate to the pancreas and potentially reduce insulin production, as seen in some human infections (Spichler et al. 2007). Within 3–4 days enough bacteria can be detected in the kidney to be visualized by microscopic examination of stained sections. If the infection does not progress to fulminant leptospirosis, the bacteria often remain in the kidney with occasional migration to other tissue (including brain and pancreas) (Zuerner et al. 2012). However, in hamsters, many *Leptospira* strains produce acute infections and the bacteria can be detected in nearly all tissue in the body, including freely swimming in blood. The interval between injection of virulent *Leptospira* and onset of clinical signs of infection varies, and is dependent upon the strain used, number of in vitro passages, and infectious dose. Standardized tests for vaccine potency or virulence checks for strains are limited to 28 days, by which time most animals should have shown clinical signs of infection.

Many *Leptospira* infection studies have used death as an endpoint, a practice that has been replaced with use of alternative criteria such as onset of clinical signs of infection, at which point animals are euthanized to avoid pain and distress. This approach leads to calculation of a modified LD_{50}. Determination of ID_{50} is often more difficult, due to the requirement to detect *Leptospira* by culture or through direct examination of tissue. There is considerable variation in LD_{50} values for different strains. Critical points for consideration when planning experimental infections are the age and number of in vitro passes of the culture. Older cultures, or cultures that have been propagated in vitro for several passages, tend to lose virulence. Using hamster virulence as a guide, LD_{50} values for *Leptospira* strains may vary from <10 to >10^8 (Haake 2006).

3 Host Detection of Pathogens

3.1 Pattern Recognition Receptors

How does the immune system detect the presence of microbial pathogens? Mammalian cells display a variety of receptors on the cell surface with the design and purpose to recognize molecular signatures that are characteristic for microbial pathogens; these signatures are referred to as pathogen-associated molecular patterns (PAMPs) (Akira et al. 2006; Iwasaki and Medzhitov 2010). PAMPs include a wide variety of molecules such as bacterial LPS, lipoproteins, peptidoglycan, and flagella proteins. The host receptors, referred to as pattern recognition receptors (PRRs), interact with PAMPs, initiating a series of intracellular signals that trigger the host response to infection. Host receptors that recognize PAMPs include toll-like receptors (TLRs) and C-type lectin receptors (CLRs) (Akira et al. 2006; Iwasaki and Medzhitov 2010). A related group of receptors that recognize damage associated

molecular patterns (DAMPs), which develop during infection, include receptors for advanced glycosylation end products (RAGE) (Williams et al. 2010). Nucleotide-binding oligomerization domain (NOD)-like receptors (NLRs) recognize both PAMPs and DAMPs. Interaction of PAMPs and DAMPs with appropriate receptors leads to intracellular signaling cascades that trigger the innate immune response and help direct acquired immune responses (Iwasaki and Medzhitov 2010; Williams et al. 2010). Therefore, understanding these receptors and the responses they initiate are essential in developing an understanding of how the host responds to infection.

3.1.1 Toll-like Receptors

Toll-like receptors are the most thoroughly studied PRRs and in many cases initiate the first part of the host response to infection (Aderem and Ulevitch 2000). Most of the published research on recognition of *Leptospira* by PRRs has focused on TLRs, and therefore this section will focus on TLR detection of PAMPs and subsequent cellular responses. TLRs are a group of related transmembrane proteins with an extracellular pattern recognition domain and a cytoplasmic domain responsible for transmitting the signal generated from the extracellular domain to the host response network (Napetschnig and Wu 2013). The cytoplasmic portions of TLRs share a protein domain with the interleukin 1 receptor (IL-1R). This intracellular toll/inter-leukin 1 receptor (TIR) domain interacts with cytoplasmic proteins to initiate a signaling cascade that triggers the host cell to respond to the threat of infection.

LPS has been used extensively to study the intricacies of TLR signaling and the host response to LPS will be used here to provide an overview of the TLR signaling pathway (Fig. 1 summarizes this process) and to highlight some unusual attributes of the response to *Leptospira*. Detection of LPS from most Gram-negative bacterial genera involves TLR4, myeloid differentiation protein 2 (MD-2), and CD14. CD14 binds LPS and transfers the molecule to MD-2 (Kawai and Akira 2010). When MD-2 binds the lipid A portion of a LPS molecule, it undergoes a structural change and forms a protein pocket that interacts with TLR4 (Park et al. 2009). This interaction initiates the intracellular TIR domains to come together and form a site where adaptor proteins assemble into oligomeric structures and initiate the intra-cellular signaling cascade. The TIR domains of most TLRs interact with MyD88, a protein that forms an oligomeric structure called a Myddosome with IRAK4, IRAK1, and IRAK2 (Napetschnig and Wu 2013). MyD88 forms the top of the structure with IRAK4 forming a layer between MyD88 and IRAK1/IRAK2. MyD88, IRAK1, IRAK2, and IRAK4 are phosphokinases, and one proposed model suggests MyD88 promotes phosphorylation of IRAK4, with subsequent down-stream phosphorylation from IRAK4 to IRAK1 and IRAK2 (Napetschnig and Wu 2013). Phosphorylation by the myddosome complex to additional kinases (including p38 MAP kinase) ensues, thereby activating transcriptional regulatory proteins, including nuclear factor kB (NFkB), AP1, and Sp-1 (Napetschnig and Wu 2013), described in more detail below.
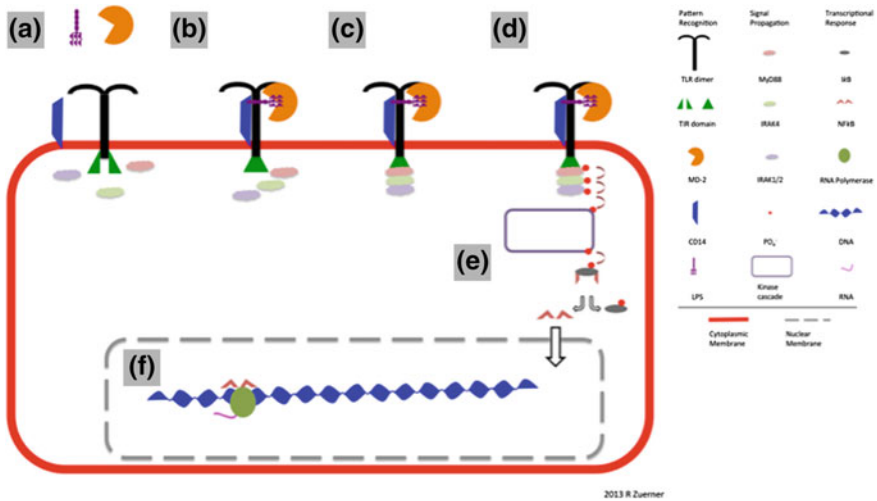
Fig. 1 Pattern recognition and host response. **a** Before PAMP (shown here as LPS) interaction with MD-2, the two TIR domains of the TLR1/TLR2 heterodimer or TLR2/TLR2 homodimer are disassociated from each other, and CD14 is not tightly associated with the TLRs. **b** The MD-2/LPS complex binds the TLR dimer, and CD14 joins the complex; this process leads to structural changes in the TLRs that bring the TIR domains together. **c** MyD88 binds the TIR domain and forms the myddosome consisting of MyD88 (*top*), IRAK4 (*middle*), and IRAK1/2 (*bottom*). **d** Phosphorylation from MyD88 through the myddosome is passed to one of several cytoplasmic kinases. **e** The phosphorylation cycle is propagated through a new cascade, ultimately leading to phosphorylation of IκB. Phosphorylated IκB undergoes ubiquitination, releasing NFκB. **f** NFκB migrates to the nucleus and activates transcription of immune response genes resulting in induction of the immune response

Although most of the TLR research on *Leptospira* has focused on TLR2 and 4 (see below), studies on other spirochetes, particularly *Borrelia burgdorferi*, have shown that TLR5 (Bernardino et al. 2008; Cabral et al. 2006), TLR8 (Cervantes et al. 2011), and TLR7 and TLR9 (Petzke et al. 2009) also play a role in recognizing spirochetes. TLR1, TLR2 and TLR6 are structurally similar and can form heterodimers that are involved in lipoprotein detection, TLR5 detects the presence of flagellin, TLR7 and TLR8 recognize single stranded RNA and imidazoquinoline compounds, and TLR9 recognizes unmethylated CpG motifs in DNA (Akira et al. 2006).

TLRs use a variety of accessory proteins during PAMP recognition and some of these proteins, e.g., MD-2, appear to have multiple functions. For example, LPS-bound MD-2 is also phosphorylated, possibly during endocytosis of the LPS-TLR4-MD-2 complex (Gray et al. 2011). In addition, TLR complexes can also function from within intracellular compartments. TLR4-MD-2 appears to be capable of sensing the presence of LPS; LPS binding through this pathway induces a distinct set of LPS inducible genes (Shibata et al. 2011). While the Myddosomes connect most TLRs to the intracellular signaling network, TIR-domain-containing adapter-inducing interferon-β (TRIF), a protein recognized by TLR3 for signal propagation, is also used for this purpose (Napetschnig and Wu 2013; Petnicki-Ocwieja et al. 2013).

Myddosome-mediated signals primarily result in induction of inflammatory cytokine and chemokine expression, whereas TRIF-mediated signals induce type I interferons (Kawai and Akira 2010). TLR2-TRIF mediated signaling was recently demonstrated in response to *B. burgdorferi* infection in mice (Miller et al. 2010), and may have a role in detecting other spirochetes, including *Leptospira*. These studies highlight the complexity of signal transduction in recognition of microbial pathogens and rapid growth in our understanding of how host cells sense the presence of microbial pathogens.

3.1.2 Other Pattern Recognition Receptors

NLRs are large oligomeric cytoplasmic proteins that contain the NOD domain, which in NLRs is referred to as NACHT (Leemans et al. 2011). The NOD domain is centrally located, and NLRs also have a carboxy-terminal leucine-rich repeat (LRR) motif. Interaction of NLRs with the appropriate PAMP or DAMP leads to formation of multiprotein structures called inflammasomes. Once formed, inflammasomes activate caspase-1, which in turn activates proinflammatory cytokines IL-1β and IL-8 (Leemans et al. 2011). If the inflammasome is not properly regulated, then a pro-inflammatory cycle ensues leading to host directed tissue damage (Leemans et al. 2011), for example, during acute lung injury (ALI). Currently, the NLRP3 inflammasome is the best characterized, and is often associated with a variety of inflammatory disorders including chronic kidney disease (Anders and Muruve 2011).

Other PRRs include the mannose receptor, a member of the CLR family. The mannose receptor has been associated with binding of *B. burgdorferi* to monocyte/macrophages (Cinco et al. 2002). *B. burgdorferi* interaction with $\alpha_3\beta_1$ integrin also induces proinflammatory cytokine production, independent of TLRs or MyD88 (Behera et al. 2006). The binding to the $\alpha_3\beta_1$ integrin sends a signal to the c-Jun NH(2)-terminal kinase (JNK) pathway and leading to induction of a proinflammatory response (Behera et al. 2006). The roles of these other PRRs in *Leptospira* infection have not been well defined.

3.1.3 PRR-Mediated Transcription Response

The signaling cascade triggered by interaction of PAMPs with the appropriate receptor results in activation of several transcription factors, most notably NFkB (Napetschnig and Wu 2013). Nonactivated NFkB is located in the cytosol and bound by the inhibitor IkB. A portion of the signaling cascade leads to phosphorylation of IkB kinase (IKK), which in turn phosphorylates IkB. Phosphorylated IkB is recruited to proteosomes and degraded, thereby releasing NFkB (Napetschnig and Wu 2013). IkB-free NFkB migrates to the nucleus where it binds chromosomal DNA at specific sites with the assistance of accessory transcription factors, e.g., activator protein-1 (AP-1), that are also activated by phosphorylation (Napetschnig and Wu 2013). These events lead to transcriptional activation of

response genes enabling the activated cells to respond to infection. NFkB can be activated by a variety of molecular signals and pathways, including TNF-α, and IL-1 via TRAF6. This process initiates the innate response to infection, induces the expression of inflammatory cytokines and chemokines, and helps direct development of the acquired immune response.

4 Innate Immunity

4.1 TLR Recognition and Response

The vast majority of research on pattern recognition of *Leptospira* has focused on the role of TLRs. Although leptospiral LPS has low endotoxicity, it stimulates a strong antibody response during infection (Chapman et al. 1988), or as a result of vaccination with whole-killed cell bacterins (Bolin et al. 1989). As noted above, LPS from most bacteria is predominantly detected using TLR4. However, LPS from *Leptospira* spp. is primarily recognized in humans by TLR2/TLR1 (Werts et al. 2001). Leptospiral LPS is recognized primarily by TLR2, but TLR4 also contributes to activation of murine cells (Nahori et al. 2005). Information regarding TLR recognition of leptospiral LPS is not available for other mammalian species.

The C3H/HeJ mouse strain lacks functional TLR4. Infection of C3H/HeJ mice with *Leptospira interrogans* serovar Icterohaemorrhagiae leads to acute leptospirosis and death (Pereira et al. 1998). Subsequent studies have shown that C3H/HeJ mice are also susceptible to infection with *L. interrogans* serovars Copenhageni and Manilae (Viriyakosol et al. 2006; Koizumi 2003; Nally et al. 2005) indicating that this is not a serovar-specific property. These studies suggest that TLR4 is of critical importance in controlling *Leptospira* infection in mice. The respective roles of TLR2 and TLR4 were studied using mice with genetically defined mutations in TLR2 and TLR4 (Chassin et al. 2009), and results from this and related studies have helped to define links between innate and adaptive immunity and control of *Leptospira* infection. In these studies, C57BL/6 J wild type (WT) mice were used as a control group and bred with $TLR2^{-/-}$, $TLR4^{-/-}$, and double TLR2/TLR4 knockout (DKO) mice, followed by backcrossing to insure that the TLR mutations were studied in a uniform C57BL/6 J genetic background. The resulting mutant strains and WT were inoculated with *L. interrogans* serovar Icterohaemorrhagiae and the outcome of infection assessed. Consistent with previous results using TLR4 deficient C3H/HeJ mice as hosts for *Leptospira*, infection of C57BL/6 J $TLR4^{-/-}$ mice also resulted in lethal infections, as did infections in DKO mice (Chassin et al. 2009). $TLR4^{-/-}$ mice survived longer than DKO mice, a finding that suggests loss of TLR2 increases susceptibility to lethal infection over the TLR4 deficiency alone. The bacterial loads in liver, lung, and kidney were assessed, and compared to WT C57BL/6 J mice in which bacteria were cleared. DKO strains had high bacterial loads in all three organs, but $TLR2^{-/-}$ or $TLR4^{-/-}$ knockout mice had significantly

lower bacterial loads in kidney and lung, as compared to DKO mice, and in these two organs resembled WT mice. Infected TLR4$^{-/-}$ mice had high bacterial loads in the liver, whereas TLR2$^{-/-}$ mice resembled WT mice with little or no bacteria detected (Chassin et al. 2009). These studies led to a model where TLR2 and TLR4 have overlapping functions in kidney, but functional TLR4 receptors are needed for bacterial clearance in liver (Chassin et al. 2009). Removal of leptospires from liver in TLR2$^{-/-}$ mice is consistent with the finding that cytokine expression in mouse macrophages involves LPS interaction with TLR4 (Chassin et al. 2009; Viriyakosol et al. 2006). In addition, there is also evidence for TLR-independent induced inflammation (Chassin et al. 2009).

4.1.1 NLR Mediated Response

Detection of PAMP and DAMP signals by NLRs also occurs during leptospirosis. The NLRP3 inflammasome is primed by TLR2/4 interaction with LPS and activated by a depression of the Na/K-ATPase pump by leptospiral glycolipoprotein (Burth et al. 1997; Lacroix-Lamande et al. 2012). Leptospiral glycoliporotein is a suspected cytotoxic component of the *Leptospira* outer membrane (Vinh et al. 1986), and has previously been shown to activate peripheral blood mononuclear cells (Diament et al. 2002). Chronic inflammasome activation may be one pathway leading to the development of tissue lesions, especially in kidney; the NLRP3 inflammasome can be triggered by either sterile or infectious stimuli (Anders and Muruve 2011; Vilaysane et al. 2010). Inflammasome activation has been implicated in a variety of chronic kidney diseases (Anders and Muruve 2011), and specific activation of the NLRP3 inflammasome and induction of IL-1β and IL-18 secretion has been associated with the development of chronic kidney disease in a noninfectious mouse model (Vilaysane et al. 2010).

4.1.2 CLR Mediated Response

DC-SIGN and mannose-binding protein are two CLRs that have been identified as having possible roles in *Leptospira* recognition. The mannose-binding lectin (MBL) is elevated in serum during leptospirosis, with higher serum levels being detected in human patients during an outbreak of more classical Weil's disease than in an outbreak of more moderate disease manifestations (Miranda et al. 2009). This finding suggests that MBL may be useful as a marker for severe leptospirosis (Miranda et al. 2009). *Leptospira* detection by human monocyte-derived dendritic cells via DC-SIGN induced secretion of the proinflammatory cytokine TNF-α and IL-12 (which enhances the cytotoxicity of NK and CD8$^+$ T cells) (Gaudart et al. 2008). However, DC-SIGN induced limited secretion of the anti-inflammatory cytokine IL-10 (Gaudart et al. 2008). Low passage, virulent serovar Pyrogenes strain 2317 induced greater TNF-α and IL-12 secretion than a high passage avirulent derivative of strain 2317 (Gaudart et al. 2008). These results are consistent

with studies that show high serum TNF-α correlates with a poor prognosis in human leptospirosis patients (Tajiki and Salomão 1996; Kyriakidis et al. 2011; Wagenaar et al. 2009a). Serum IL-10 levels are also elevated during acute leptospirosis, but this may be in response to high levels of inflammatory cytokines; IL-10 functions to restrain the inflammatory response. The inability of high serum levels of IL-10 to control the inflammation response may lead to immune pathology (see Sect. 6). IL-10 is important for limiting *Borrelia turicatae* growth in mice, showing a commonality in the need for a balanced immune response to survive infections caused by these spirochetes (Londoño et al. 2008).

4.1.3 Cytokine Induction

The role of inflammatory cytokines in C57BL/6 J mice and assorted mutants was also examined (Chassin et al. 2009). Levels of inflammatory cytokines were highest in DKO mice in kidney and liver as compared to WT, TLR2$^{-/-}$, or TLR4$^{-/-}$ mice. This result may be due in part to the higher bacterial load in liver and kidney because DKO mutants were unable to clear bacteria during infection, and the observed induction of inflammatory cytokines was by a non-TLR based pathway (Chassin et al. 2009). MyD88$^{-/-}$ mice have approximately the same levels of inflammatory cytokines as TLR2-TLR4 DKO mice, leading to the conclusion that TLRs other than TLR2 and TLR4 do not play a significant role in detecting *Leptospira,* or for inducing production of IL-1β, IL-6, and chemokines There is no evidence that TLR3, which uses a MyD88-independent signaling pathway, has a significant role in recognition of *Leptospira* antigens. TNF-α-induced CCL5 (also known as RANTES), CXCL1 (also known as KC) and CXCL2 (also known as MIP-2) (Chassin et al. 2009). This conclusion is inconsistent with another report concluding that TNF-α production is largely TLR5-dependent in human leukocytes (Goris et al. 2011). These differences may indicate either a difference in the relative importance of TLRs in different tissues or the ability of leukocytes from different host species to use different TLRs for pathogen detection. However, a general program of robust production of inflammatory cytokines is consistent across diverse mammalian hosts; the findings cited above are similar to studies conducted in hamsters that also have high inflammatory cytokine production during the acute phase of infection (Lowanitchapat et al. 2010; Marinho et al. 2009; Matsui et al. 2011; Vernel-Pauillac and Merien 2006; Vernel-Pauillac and Goarant 2010).

The array of circulating cytokines and chemokines induced at significant levels in both humans and mice during leptospirosis includes IL1-β, IL-6, IL-10, MCP-1, and TNF-α (Wang et al. 2012). In humans, GM-CSF (granulocyte macrophage colony-stimulating factor) and CCL2 (also known as macrophage chemotactic protein 1, MCP-1) were also found at increased levels in sera (Wang et al. 2012); these two compounds promote granulocyte and monocyte production and recruit leukocytes to sites of inflammation, respectively. Human patients also had elevated IL-11; IL-11 contributes to platelet replenishment due to thrombocytopenia (a frequent complication of leptospirosis) and induction of acute phase proteins (Wang

et al. 2012). There is also in vitro evidence of locally produced cytokine and chemokine production; CXCL1/KC, iNOS, and CCL2/MCP-1, are produced by cultured renal cells using a TLR1/2 driven pathway via MyD88 and a MAP38 kinase pathway (Hung et al. 2006a, b; Yang et al. 2006). The result of CXCL1/KC synthesis in the kidney may help recruit neutrophils to the site of infection. However, another consequence of chemokine/cytokine synthesis in the kidney may be accumulation of fibrous tissue and decreased kidney function (see Sect. 6).

4.2 Cellular Response

Monocytes/macrophages utilize PRR-mediated activation to provide innate immune protection of the host, especially during the early stages of infection. Leptospiral LPS and hemolysins stimulate macrophages to produce IL-1β, IL-6, IFN, and TNF-α (Isogai et al. 1990; Wang et al. 2012). In addition, macrophages treated with leptospiral LPS have enhanced phagocytic activity (Isogai et al. 1990). The role of macrophages in protection against *Leptospira* infection was suggested in a study where mice were treated with silica to inhibit macrophage function, and it was found that treated mice were impaired in bacterial clearance and had increased susceptibility to infection (Isogai et al. 1986). The role of antibody appears to be important for macrophage-mediated killing of *Leptospira*; several independent studies have shown that phagocytosis leading to decreased bacterial viability requires opsonization with homologous antibody (Banfi et al. 1982; Cinco et al. 1981; Vinh et al. 1982). Opsonization may overcome an inherent immune evasion mechanism of *Leptospira* that impairs macrophage function; freshly isolated virulent strains can induce macrophage apoptosis (Merien et al. 1997; Jin et al. 2009) or limit lysosomal fusion with bacteria-laden phagosomes (Toma et al. 2011).

Polymorphonuclear leukocytes (PMNs) include granulocytes, eosinophils, and neutrophils, and these cells comprise important components of the innate immune response, but their role in protection against *Leptospira* infection is unclear. Two antibacterial peptides produced by bovine neutrophils, Bac5 (also known as cathelicidin-2) and Bac7 (cathelicidin-7), can kill *Leptospira* (Scocchi et al. 1993). Intact *Leptospira,* leptospiral peptidoglycan or LPS induce PMN adherence to endothelial cells (Isogai et al. 1989a; Dobrina et al. 1995). However, resistance to PMN phagocytosis has been suggested as a potential virulence factor for *Leptospira* (Wang et al. 1984). Acute infection with a hamster lethal strain of *Leptospira borgpetersenii* serovar Hardjo resulted in the in vivo formation in blood of large bacterial–neutrophil aggregates without bacterial clearance (Zuerner et al. 2012). Much like the findings described above noting that macrophages depend on antibody for phagocytosis, PMNs also require assistance from the humoral response to provide immune sera for phagocytosis of *Leptospira* (McGrath et al. 1984; Wang et al. 1984). These results are similar to those reported for *B. burgdorferi*, which appears to be more susceptible to PMN attack in the presence, rather than absence, of antibody (Lusitani et al. 2002).

   Although platelets have traditionally been associated with hemostasis, recent studies have shown these cells are important components of the innate immune response (Yeaman 2009). Platelets utilize TLRs to detect PAMPs, and produce antimicrobial peptides and cytokines (Cognasse et al. 2008). Activated platelets contribute to the activation of neutrophils and neutrophil extracellular formation (Yeaman 2009). Leptospiral LPS induces platelet aggregation (Isogai et al. 1989b), and thrombocytopenia often occurs in about half of human leptospirosis patients (Edwards et al. 1986). By contributing to platelet removal, *Leptospira* may be able to subvert part of the innate immune system during the early stages of infection.

5 Acquired Immunity

5.1 *Humoral Response*

Antibodies against *Leptospira* have a key role in providing immune protection against lethal infection in many potential host species. Adler and Faine concluded that antibody to *Leptospira* is essential for protective immunity with the discovery that mice treated with cyclophosphamide, which preferentially kills B cells, were susceptible to lethal *Leptospira* infection (Adler and Faine 1976, 1977). Nude mice, which lack a thymus, and therefore cannot produce T cells, were resistant to infection (Adler and Faine 1977). Mice lacking a functional Rag gene are unable to produce functional B and T cells due to an inability to undergo V(D)J recombination in immunoglobulin and T cell receptor genes. Likewise, SCID mice lack an enzyme responsible for DNA recombination needed for maturation of antigen specific B and T cells. μMT mice lack functional B cells. Infection with *L. interrogans* of Rag$^{-/-}$, SCID mice, μMT, or nude mice treated with cyclophosphamide results in lethal infection (Adler and Faine 1977; Bandeira et al. 2011; Chassin et al. 2009; Nally et al. 2005). Adler and Faine (1977) clearly demonstrated the importance antibody (and therefore B cells) by injecting cyclophosphamide-treated nude mice with antibody to *Leptospira* resulting in protection against lethal *Leptospira* infection. Chassin et al. (2009) reported similar results: μMT mice were protected from lethal challenge using passive transfer of immune sera collected from infected WT mice at 20-days PI. These studies are consistent with an earlier study that showed transfer of maternal antibodies protected mice from becoming chronic carriers of *Leptospira* (Birnbaum et al. 1972). In contrast, T cells do not appear to have an important role in providing protection from lethal challenge in the mouse model; CD3$^{-/-}$ (T cell deficient) mice are resistant to lethal challenge (Chassin et al. 2009).

   A key *Leptospira* antigen that is important for the development of immune protection in many host species is LPS. Immunization with *Leptospira* LPS protects hamsters against homologous challenge (Jost et al. 1989). Passive transfer of antibody to LPS has also been used successfully to protect mice, guinea pigs, monkeys, and dogs before lethal infectious challenge (Jost et al. 1986; Challa et al.

2011; Schoone et al. 1989). Indeed, development of antibody to the LPS component of whole cell bacterins is thought to be key for immune protection against lethal infection with several *Leptospira* serovars in many animal species. Development of agglutinating antibodies, predominantly IgM (Adler and Faine 1978), is important for serological diagnosis of exposure to *Leptospira* using the microscopic agglutination test (MAT). Antibodies to LPS develop early in infection (Chapman et al. 1991). However, LPS is the serovar-specific antigen in *Leptospira* and therein lies one of the problems with relying on the LPS component in vaccines for developing protective immunity against a broad spectrum of serovars; antibodies against LPS provide limited cross-protection against other serovars and may provide short duration of immunity. Development of antibody to leptospiral proteins has greater likelihood of cross-protection (Sonrier et al. 2000; Srikram et al. 2011; see the chapters by D.A. Haake and W.R. Zückert and by B. Adler, this volume). The major outer membrane protein of pathogenic *Leptospira,* LipL32, stimulates a significant early and sustained antibody response during infection (Haake et al. 1991, 2000; Zuerner et al. 1991). LipL32 is proteolytically cleaved in vitro (Haake et al. 2000; Zuerner et al. 1991; Cullen et al. 2002) and the protein is post-translationally modified (Witchell et al. 2014), processes that may limit exposure of antigenic epitopes of this protein on the cell surface (Pinne and Haake 2013). While antibodies to LipL32 are good indicators of infection, development of antibody to this protein does not appear to be protective (Lucas et al. 2011), and LipL32 is not needed for successful infection (Murray et al. 2009). Differential methylation of OmpL32 glutamic residues is another method by which Leptospira may alter exposure of antigenic epitopes during infection (Eshghi et al. 2012b). A more complete review of vaccine development is presented in the chapter by B. Adler, this volume, but, on the whole, the most successful *Leptospira* vaccines produced to date are composed of whole, killed bacteria (Bey and Johnson 1982), suggesting that a complex mixture of antigens may be required for protection.

5.2 Cell-Mediated Immunity

Although the data described above show the critical importance of a Th2, or B cell-mediated protective immunity, both B and T lymphocytes have important roles in promoting an immune response to *Leptospira* infection. Rag$^{-/-}$ mice do not produce significant levels of IFN-γ in liver or kidney, indicating either B or T cells, or both classes of lymphocytes, are required for IFN-γ production during infection (Chassin et al. 2009). B cells appear to be primarily responsible for IFN-γ production and bacterial clearance in the liver, whereas T cells are responsible for these roles in the kidney (Chassin et al. 2009). Furthermore, histological evidence of kidney tissue damage is greater in CD3$^{-/-}$ animals lacking functional T cells, as compared to WT or μMT (B cell deficient) mice. Histological examination of infected kidneys showed evidence of interstitial inflammation and development of nodular infiltrates in T cell deficient mice that were absent from kidneys of infected

WT or B cell deficient mice (Chassin et al. 2009). Finally, serological markers of renal damage were elevated in infected T cell deficient mice, but not infected WT or B cell deficient mice. Combined, these data provide a compelling argument that the Th1, or CMI, response involving T cells is an important component of the immune response as it relates to *Leptospira* infection.

The studies above focused on protection against lethal *Leptospira* challenge. There is less information on what components of the immune response are critical to prevent against development of chronic infection by *Leptospira*. Elimination of chronic leptospirosis, especially in maintenance hosts, is important to reduce the likelihood of disease transmission to accidental hosts. Vaccination and challenge studies using serovar Hardjo infections of cattle have led to a better understanding of the immune response in regard to *Leptospira* infection of its natural maintenance host. In contrast to the animal studies described above that show antibody to LPS to be protective against lethal leptospirosis, similar studies in cattle have shown that high titers of antibody to LPS fail to protect cattle from infection with serovar Hardjo, the most common serovar associated with chronic bovine leptospirosis (Bolin et al. 1989). Vaccines in this class fail to induce a Th1, or CMI, response in cattle (Naiman et al. 2001b, 2002; Zuerner et al. 2011). Refined whole cell monovalent serovar Hardjo vaccines that protect against significant renal colonization stimulate antibody production, but also induce CMI (Bolin et al. 2000; Bolin and Alt 2001; Ellis et al. 2000). These vaccines stimulate CD4$^+$ and γδ T cells to proliferate and produce IFN-γ in a recall response when exposed to serovar Hardjo antigens (Blumerman et al. 2007a; Naiman et al. 2001b, 2002). In short-term vaccine efficacy studies, where cattle were challenged approximately 4 months after vaccination, these vaccines either prevented renal colonization and urinary shedding of bacteria (Bolin et al. 2000; Bolin and Alt 2001; Ellis et al. 2000) or eliminated urinary shedding within a few weeks after challenge (Zuerner et al. 2011). However, duration of protective immunity remains a problem. Some animals may develop renal colonization with lesion formation if infected 1 year after vaccination (Zuerner et al. 2011). Nevertheless, the general success of these vaccines in limiting renal colonization provides a model to understand the role of CMI in controlling *Leptospira* infection in cattle, and perhaps other species.

Analysis of how bovine peripheral blood mononuclear cells (PBMCs) respond to vaccination and subsequent infectious challenge has revealed several characteristics of a protective Th1 response to *Leptospira* infection. Analysis of how cattle respond to *L. borgpetersenii* serovar Hardjo has played a key role in characterizing bovine γδ T cells because this class of lymphocytes replicate in response to vaccination with a monovalent serovar Hardjo and demonstrate a strong recall response to leptospiral antigens (Baldwin et al. 2002; Naiman et al. 2001a, b; Zuerner et al. 2011). γδ T cells are a unique class of CD4$^-$ CD8$^-$ T cells that comprise approximately 30 % of the normal adult ruminant PBMC population (Hein and Mackay 1991). This unique class of T cells comprises a smaller percentage of PBMCs in nonruminant species, and is not as well characterized as CD4$^+$ and CD8$^+$ αβ T cells. Most bovine γδ T cells possess the WC1 scavenger receptor on the cell surface (Baldwin et al. 2000). WC1 is one of several members of the scavenger receptor cysteine-rich (SRCR) family (Wang et al. 2011), a group of surface

proteins that bind a variety of PAMPs including lipoproteins, lipoteichoic acid, and leucine-rich repeat proteins (Loimaranta et al. 2009). A protective monovalent serovar Hardjo vaccine induces a positive recall response to leptospiral antigens in CD4$^+$ αβ- and γδ-T cells, but the response of CD8$^+$ cells has varied between studies (Baldwin et al. 2002; Naiman et al. 2001b, 2002; Zuerner et al. 2011). Several studies have shown that the phenotype of *Leptospira* antigen-stimulated WC1$^+$ γδ T cells is consistent with Th1 polarized cells: (1) they express the transcription factors T-bet and GATA-3, as well as IL-12Rβ2 which encodes the high affinity IL-12 receptor (Rogers et al. 2005a, b); (2) they are activated by treatment with IL-12; (3) γδ T cells have elevated transcription of genes associated with cytotoxic activity (Blumerman et al. 2007b); and (4) cells have elevated expression of B-cell activating factor (BAFF, also referred to as BLysS, for B lymphocyte stimulator), and NDFIP2 (Blumerman et al. 2007b), a gene that promotes IFN-γ production in Th1 polarized lymphocytes (Lund et al. 2007). One of the characteristics of CD4$^+$ T cells stimulated with *Leptospira* antigens is increased transcription of genes encoding cytotoxic functions and CXCL6 (granulocyte chemotactic protein), a chemokine that attracts neutrophils (Blumerman et al. 2007b).

Several studies on human patients have shown that exposure to *Leptospira* antigens induces a proliferative response in PBMCs. In particular, there is preferential expansion of γδ T cells in leptospirosis patient blood samples stimulated with *Leptospira* antigens (Barry et al. 2006; Klimpel et al. 2003). However, Tuero et al. (2010) did not detect the presence of a T cell memory response in patient blood following infection. The presence of inflammatory cytokines (de Fost et al. 2003) showed that *Leptospira* induced significant increases in human PBMC expression of IFN-γ, TNF-α, and IL-12 receptor, consistent with a strong Th1 response to infection. In addition, patients with, or suspected of having, leptospirosis had elevated concentrations of T- and NK-cell derived cytotoxic compounds or chemokines (De Fost et al. 2007).

NK cells are a group of cytotoxic lymphocytes often considered part of the innate immune response. However, recent reports have shown that NK cells have the capacity to mount a recall response to antigens (Cooper et al. 2009; Sun et al. 2009). NK cells, defined as CD335$^+$ (i.e., containing the natural cytotoxicity receptor, NKp46) from cattle vaccinated with a monovalent serovar Hardjo vaccine demonstrated a recall response by expressing IFN-γ when exposed to *Leptospira* antigens (Zuerner et al. 2011). Surprisingly, NK cells from sham vaccinated cattle also demonstrated an antigen recall response, that by 6 weeks post challenge, was indistinguishable from cells obtained from vaccinated animals (Zuerner et al. 2011). A key difference in the response of lymphocytes from vaccinates versus sham vaccinates may be the ability of the vaccine to induce sustained lymphocyte proliferation with IFN-γ expression following infectious challenge (Naiman et al. 2001b, 2002; Zuerner et al. 2011). The presence of immune cells in vaccinated animals at initiation of infection may limit the initial tissue colonization by *Leptospira*, and allow the host to eventually resolve the infection. In contrast, infection of nonimmune animals likely allows substantial colonization of host tissue that cannot be removed by the host without additional forms of intervention, e.g., antibiotic treatment.

6 Immune Pathology

Some of the manifestations of acute leptospirosis may be the result of unrestrained activation of the host immune response. Several lines of evidence suggest that robust activation of inflammasomes may contribute to tissue damage, particularly lung and kidney. The two triggers (e.g., LPS and potassium efflux) needed to obtain high-level activation of inflammasomes resulting in robust production of IL-1β (Mariathasan and Monack 2007) are present during acute leptospirosis. LPS is an outer membrane component and potassium efflux is induced by the leptospiral glycolipoprotein (Lacroix-Lamande et al. 2012). Overstimulation of the inflammasome can contribute to tissue damage to lung (Xiang et al. 2011) and kidney (Vilaysane et al. 2010; Anders and Muruve 2011). Consistent with this hypothesis, evidence for "massive overexpression" of proinflammatory cytokines in hamsters experiencing severe leptospirosis has been presented (Matsui et al. 2011). Other studies have found high inflammatory cytokine levels associated with lethal infection of hamsters (Marinho et al. 2009; Vernel-Pauillac and Goarant 2010). Human patients suffering from acute leptospirosis also have evidence of overexpression of inflammatory cytokines, and soluble ST2 and long pentraxin PTX3 have been indicated as possible markers for acute infection (Wagenaar et al. 2009a, b). Elevated expression of neutrophil chemokines CXCL1 (Hung et al. 2006a, b) and CXCL6 (Blumerman et al. 2007b) may promote PMN migration, cell activation, and inflammation, all of which are associated with acute lung injury (Li et al. 2009). The presence of neutrophil–bacterial aggregates during acute infection (Zuerner et al. 2011), coupled with extracellular net formation can result in release of neutrophil granules and tissue damage (Abraham 2003; Lee and Downey 2001). Several immune receptors were upregulated in lung tissue from human patients that had died from acute leptospirosis and experienced pulmonary involvement (Del Carlo Bernardi et al. 2012).

Pathogenic *Leptospira* binds to chondroitin sulfate B, a proteoglycan of host cells (Breiner et al. 2009). Interaction of pathogenic *Leptospira* with endothelial cells induces cellular changes that are consistent with disruption of endothelial barriers, thereby aiding bacterial invasion (Martinez-Lopez et al. 2010). Interestingly, this process, at least in vitro, can be blocked using the ACE inhibitor lysinopril, suggesting that pharmaceutical treatments that supplement antibiotic therapy may be useful in limiting tissue damage (Martinez-Lopez et al. 2010). Tissue from infected animals often shows evidence of tubulointerstitial nephritis, an accumulation of extracellular matrix (ECM) proteins in the kidney (Araujo et al. 2010), a process that can be replicated with mouse proximal tubule cells treated with *Leptospira* OMP preparation, which may well contain LPS (Yang et al. 2000). Evidence for a TLR2-driven signaling cascade stimulating ECM production in response to exposure to *Leptospira* outer membrane proteins, especially LipL32, was recently shown using immortalized human kidney cells (Hsu et al. 2010; Tian et al. 2011). Production of CXCL1/KC attracts neutrophils and monocytes (Hsu et al. 2010), and production TGF-B1 likely drives synthesis of type I and type IV collagen (Tian 2006), leading to fibrosis. In situ expression of inflammatory

cytokines has been suggested as contributing to tissue damage in acute lung injury and pulmonary fibrosis (Kolb et al. 2001), and a similar mechanism of tissue damage and errant repair may also occur in renal tissue following infection with *Leptospira* and contribute to the development of tubulointerstitial nephritis.

iNOS expression may present a double-edged sword with respect to protecting the host and damaging tissue. Production of iNOS is important for host survival (Prêtre et al. 2011), but two studies have suggested that iNOS activity may contribute to damage observed during *Leptospira*-induced pulmonary hemorrhage (Chen et al. 2007; Yang and Hsu 2005).

Leptospirosis induced autoimmunity may contribute to the development of uveitis and loss of eyesight in horses (Faber et al. 2000; Verma and Stevenson 2012) and humans (Chu et al. 1998; Mancel et al. 1999). Antibodies from uveitis horses recognize two *Leptospira* proteins, LruA and LruB that share antigenic cross reactivity with eye proteins in horses (Verma et al. 2005). Sera from human uveitis patients also recognize LruA and LruB (Verma et al. 2008). Development of antibodies to other host proteins in leptospirosis patients has been reported, for example antibodies to host cardiolipin following infection (Rugman et al. 1991), but this has not been associated with development of autoimmune disease.

Autoimmunity, in the form of Goodpasture's syndrome, has also been suggested as contributing to the development of pulmonary hemorrhage in a guinea pig model (Nally et al. 2004). In that model it was proposed that autoantibodies to connective tissue triggered by *Leptospira* infection were the cause of pulmonary damage. This hypothesis has since been disproven (Craig et al. 2009).

7 Immune Evasion

Leptospira has several proteins that have the capacity to bind components of the complement system, including factor H (fH) and factor H-like (fHl) proteins. By binding components of the complement system, *Leptospira* avoids complement activation and cell damage. Among the leptospiral proteins capable of binding components of the complement system are two endostatin-like proteins LenA (LfhA) and LenB (Stevenson et al. 2007; Verma et al. 2010), LigA and LigB (Castiblanco-Valencia et al. 2012), and LIC11834, and LIC12253 (Domingos et al. 2012). Some of these proteins, as well as many other *Leptospira* proteins including other members of the endostatin-like proteins (Stevenson et al. 2007) and LipL32 (Choy et al. 2011), bind a variety of host proteins, thereby masking bacterial antigens and contributing to evasion from the host immune system.

The major outer membrane protein of pathogenic *Leptospira,* LipL32, induces a strong antibody response and is a TLR2 agonist (Hsu et al. 2010). However, mutants in LipL32 are still infectious, and LipL32 monovalent vaccines are not protective (Lucas et al. 2011), findings consistent with a recent report that challenges the long-held belief that LipL32 is exposed on the bacterial surface (Pinne and Haake 2013). If the subsurface localization of this protein is confirmed, then perhaps the robust

antigenic activity of LipL32 serves to divert the immune response away from effective targets for immune clearance and instead direct the immune response to antigens for which antibodies do not impair in vivo bacterial survival.

Leptospira may also mask antigenic sites on outer membrane proteins. Amino acids in OmpL32 are methylated, and the pattern of methylation changes depending on various environmental factors (Eshghi et al. 2012b). This process likely alters antibody access to this protein, thereby interfering with opsonization. As noted above, LipL32 is also post-translationally modified in a way that may alter antibody binding to antigenic domains on the major outer membrane protein (Witchell et al. 2014).

Leptospira is also able to penetrate host cells, including macrophages, and induce apoptosis (Merien et al. 1997, 1998; Jin et al. 2009). This function likely abrogates the normal role of macrophages in innate immunity and bacterial clearance. Expression of catalase may contribute to intracellular survival of *Leptospira* (Eshghi et al. 2012a).

8 Conclusions

We are gaining new insight into the complexities involved as mammalian hosts respond to *Leptospira* infection. The infection is a perpetual battle between host and bacterium, each with its own arsenal of weapons to attack the other. On the host side, an array of inflammatory cytokines and chemokines is used to stimulate cells to resist infection and kill the bacteria. The bacteria present an array of molecules to the host immune system that may initiate a destructive immune response leading to sepsis and death, or in a more limited setting, chronic tissue damage as the bacteria establishes a persistent infection. Information is needed on how to avoid over induction of a proinflammatory response and instead develop a measured response that limits host tissue damage while contributing to bacterial death. Identification of bacterial evasion strategies and key bacterial antigens is needed to enable development of safe, effective vaccines that promote clearance of bacteria from the host. These findings provide a basis for future study on the immune response to *Leptospira* infection. Future work may involve genetic determinants of susceptibility; several genetic polymorphisms that may predispose humans to severe infection have been identified (Fialho et al. 2009), and may be important for anticipating treatment strategies, especially in areas where humans are chronic carriers of endemic strains of *Leptospira*.

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