Toll-Like Receptor 4 Protects against Lethal Leptospira interrogans Serovar Icterohaemorrhagiae Infection and Contributes to In Vivo Control of Leptospiral Burden

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Received 13 September 2005/Returned for modification 20 October 2005/Accepted 10 November 2005

Leptospirosis is a zoonotic disease of global importance caused by more than 11 species and 200 serovars of spirochetes of the genus Leptospira (6). In recent years, a grave form of leptospirosis, pulmonary hemorrhage, has emerged worldwide as an important clinical syndrome (25, 29, 30). Mechanisms of pathogenesis, host defense, and protective immunity in leptospirosis remain poorly understood (6). Given that case fatality rates in the disease can be as high as 25% (31), an improved understanding of host defense mechanisms in leptospirosis may help to more effectively treat and prevent this disease.

Leptospirosis is known reasons (7). Some isolates freshly isolated from dis-

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The relationship of single genes to susceptibility to bacterial and parasitic diseases has been established in mouse models of infections due to organisms such as Leishmania major, Myco-

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bacterium tuberculosis, Salmonella enterica serovar Typhi-
murium, and Escherichia coli (15). While the NRAMP-1 iron
transporter gene has been shown to be important for host
resistance to intracellular infections, TLR4 has been shown to
be critical in susceptibility to gram-negative LPS. Although the
innate immune response to Leptospira has been explored in
vitro (37), whether protection from or susceptibility to severe
leptospirosis is related to a single Mendelian trait has not been
explored in vivo. In a report of in vitro L. interrogans stimula-
tion of the innate immune system, leptospiral LPS was shown
paradoxically to stimulate the TLR2-dependent pathway but
not the TLR4 pathway (37). The potential for TLR2 to be
stimulated by Porphyromonas gingivalis LPS, an LPS with a
structure distinctly different from those of E. coli and Salmo-
nella spp., has also been proposed (10). Whether TLR2 has an
in vivo role in the host response to Leptospira has not been
shown in challenge models.

In this study, we report results of experiments from an in
vitro murine model of severe leptospirosis in which a single
gene, TLR4, determined the clinical outcome of infection as
well as tissue burden of leptospires in sublethal infection. A
lack of antibody and B and T lymphocytes did not prevent
jaundice or pulmonary hemorrhage, suggesting that these arms
of the immune system are not involved in Leptospira-induced
immunopathology, at least in mice. In in vitro assays, Lepto-
spira-stimulated proinflammatory cytokines and chemokines
are dependent on TLR4 and MD-2 present in the TLR4 sig-
ning complex but independent of CD14. These observations
indicate that a CD14-independent, TLR4-dependent pathway
of the innate immune response contributes an unexpectedly
important component of protection against death in severe
murine leptospirosis and in controlling leptospirosis prolifera-
tion during chronic infection.

MATERIALS AND METHODS

Reagents. Salmonella minnesota Re595 LPS was purchased from List Biologi-
cal Laboratories. Leptospira interrogans LPS was kindly provided by Catherine
Wertz (Institute Pasteur, Paris, France). Heat-killed Sphingococcus aurous was
obtained from Calbiochem. An anti-CD14 monoclonal antibody (MAb), 28C5,
as a generous gift from P. Tobias (Research Institute of the Scripps Clinic, La
Jolla, CA). MAb anti-mouse TLR4/MD-2 clone MTS510 was obtained from
Ebioscience. MAb anti-TLR4/MD-2 clone MTS510 was obtained from
Calbiochem. An anti-CD14 monoclonal antibody (MAb), 28C5,
(PE Bio
tek), and MyD88
(BD Bioscience), counted using a Petroff-Hauser counting chamber under dark-
field microscopy, and heat killed at 56°C for 30 min. All preparations were tested
for endotoxin contamination using a QCL-1000 chromogenic LAL assay (Bio-
Whittaker).

Challenge infections. For lethal infection, 3-week-old mice were infected intraperitoneally (i.p.) with 6 × 10^8 Leptospira cells in 1.0 ml of PLM-5. For
sublethal infection, 6-week-old mice were infected i.p. with 10^9 Leptospira cells
(1.0 ml of PLM-5). Control, uninfected animals received the same volume of sterile
culture medium. Infected animals were clinically observed twice daily.

Activation of elicited mouse peritoneal macrophages. Mice (6 to 8 weeks old)
were injected i.p. with 1 ml of 5 mM sodium periodate in phosphate-buffered
saline per mouse 4 days prior to harvesting of macrophages. The peritoneal
macrophages were harvested in phosphate-buffered saline; washed with a solu-
tion containing Dulbecco’s modified Eagle’s medium high-glucose medium, 10%
fetal calf serum, 10 mM HEPES, and 2 mM glutamine; and seeded at 2 × 10^5
cells per well of 96-well plates. The plate was incubated for 1 to 2 h at 37°C to
allow cells to adhere. After washing once with medium, cells were stimulated
with various reagents. After 16 h of incubation, the supernatant was harvested and
assayed for mouse cytokines using enzyme-linked immunosorbent assay
(ELISA) kits for tumor necrosis factor alpha (TNF-α), interleukin-6 (IL-6)
(OptiEIA set; Pharmingen), and macrophage inflammatory protein 2 (MIP-2)
(Duo set; R&D Systems) according to the manufacturers’ protocols.

Real-time PCR for quantification of Leptospira cells in tissues of mice. Mice
were euthanized with halothane. Organs were harvested using aseptic techniques
and stored in 70% ethanol at −20°C until used for extraction. Three sections of
approximately 25 mg were accurately weighed (for normalization of bacterial
concentrations) and used for genomic DNA extraction. DNA extraction was
done using the DNeasy tissue DNA extraction kit according to the manufactur-
er’s directions (QIAGEN). Five microliters of DNA was added to a PCR reac-
tion mixture containing 25 μl Platinum Quantitative PCR Supermix-UDG
(In-
vitrogen), 0.6 μM each forward and reverse 16S rRNA gene primer, and a FamTam probe (5’-CTC ACC AAC GCA CAC ATC GCT AGA C-3’) as
described previously by Smythe et al. (32). The concentration of the final PCR
product of 87 bp was determined using a standard curve generated with serial
dilutions (10^6 to 10^9) of genomic DNA extracted from in vitro-cultivated bacte-
ria. Tissue sections from an uninfected control animal were spiked with appro-
priate number of bacteria and then extracted using a standard protocol using the
DNeasy tissue DNA extraction kit for use as a template in the standard curve.

Statistical analysis. Data were analyzed in a one-way analysis of variance with
post hoc Tukey tests for pairwise comparisons to avoid type I error inflation using
Prysm software (GraphPad). Probabilities (P values) less than 0.05 were consid-
ered significant.

RESULTS

High-dose challenge infection of TLR4 mutant but not
TLR4 WT mice with Leptospira interrogans serovar Icterohaem-
morrhagiae strain HAI188 recapitulates severe human lepto-
spirosis. To investigate the role of innate immune responses in
leptospirosis, we infected 3-week-old C3H/HeJ mice
(TLR4 deficient), C3H/HeJ-SCID mice (TLR4 deficient and lacking T and B cells), and C3H/OuJ mice (WT) with a high
dose (6 × 10^8 cells) of Leptospira interrogans serovar Icterohaem-
morrhagiae strain HAI188. The challenge inoculum used a
low-passage isolate (passage 2) obtained originally from a fatal
case of human leptospirosis in the Peruvian Amazon which
clinically manifested as jaundice, severe hemoptysis, and re-
fractory shock (4). Infected sick mice developed piloerection,
progressing to hunching and listlessness before becoming se-
verely moribund, which necessitated euthanasia at 4 to 5 days
for C3H/HeJ mice and 3 to 4 days for C3H/HeJ-SCID mice
and at day 8 for one C3H/OuJ mouse. The C3H/HeJ and
C3H/HeJ-SCID mice all had obvious jaundice (Fig. 1A), pul-
monary hemorrhage (Fig. 1B), blood in nares (not shown),
mediastinal hemorrhage (not shown), and massive splenic
enlargement. C3H/HeJ SCID mice appeared to die at the same
rate as or only slightly more rapidly than the C3H/HeJ mice
(Table 1). In contrast, eight out of nine C3H/OuJ (wild-type
TLR4) mice survived infection without external evidence of
clinical illness. The one C3H/OuJ mouse that died was not jaundiced, nor did it have pulmonary hemorrhage; the cause of death was not able to be determined. The surviving eight C3H/OuJ mice were sacrificed at day 14 after infection. At necropsy, all organs appeared grossly normal. Cultures of kidneys for *Leptospira* were positive in all groups, including those of kidneys from the wild-type mice at 14 days, confirming infection.

**Sublethal infection resulted in higher leptospiral density in kidneys of TLR4 mutant mice than in those of WT mice.** When 6-week-old C3H/HeJ and C3H/OuJ mice were infected with a lower dose (1 × 10^8^ cells) of *Leptospira interrogans* serovar Icterohaemorrhagiae strain HAI188, all mice survived. Organs were removed 21 days after infection, and the presence of *Leptospira* was determined quantitatively by real-time PCR. *Leptospira* was presented in heart, lung, liver, spleen, and kidneys in both C3H/HeJ and C3H/OuJ mice. However, a significantly higher organism burden was observed in the kidneys and lungs of C3H/HeJ mice than in C3H/OuJ mice (Fig. 2).

FIG. 1. Clinical manifestations of *Leptospira interrogans* serovar Icterohaemorrhagiae strain HAI188 infection in mice. Fourteen days after challenge infection, C3H/OuJ (TLR4 wild-type) mice appeared grossly identical to the uninfected C3H/HeJ SCID (TLR4-deficient) mice, while the infected C3H/HeJ (TLR4-deficient) mice died within 3 to 4 days after challenge infection with jaundice (A) and pulmonary hemorrhage (B). (A) Gross examination of thorax and abdomen. (B) Gross examination of perfused lungs showing pulmonary hemorrhage in C3H/HeJ mice (right panel) but not in the other mice.
Leptospira
to
3B). As a further control, MIP-2, IL-6, and TNF-
with leptospiral LPS in the presence and absence of PMB (Fig.
produced cytokines in response to the LPS component of heat-
TLR activation (37). To investigate whether the macrophages
were inhibited by PMB. In contrast, the cytokine responses to
heat-killed Leptospira. As a positive control, 28C5 did inhibit
Salmonella minnesota Re595 LPS stimulation of MIP-2 produc-
tion. This result suggests that Leptospira stimulates MIP-2 produc-
tion in a CD14-independent manner (Fig. 4). As expected,
anti-TLR4/MD2, anti-TLR4, and anti-CD14 antibod-
ies significantly inhibited MIP-2 production induced by Salmo-
nella minnesota Re595 LPS (Fig. 4).

As independent confirmation that CD14 does not play a role
in leptosporal stimulation of proinflammatory cytokines, peri-
toneal macrophages from C57BL/6 (WT) and CD14-/- mice were
incubated with heat-killed Leptospira. Salmonella minnesota
Re595 LPS was used as a control. While Re595 LPS stimulated much higher levels of MIP-2 and IL-6 from macro-
phages of the WT mice than from macrophages of CD14-/-
mice, the responses of both MIP-2 and IL-6 production to
Leptospira stimulation were similar in both the WT and CD14-/-
macrophages (Fig. 5).

The role of TLR2 in macrophage responses to Leptospira."

Leptospira stimulates periodate-elicited mouse peritoneal
macrophages to produce proinflammatory cytokines. To deter-
de whether Leptospira stimulated production of proinflam-
matorcytokines by mouse macrophages, heat-killed L.
interrogans serovar Icterohaemorrhagiae strain HAI188 at
doses varying from 10^4 to 10^6 cells/ml was incubated with
peritoneal macrophages overnight. Leptospira stimulated pro-
duction of MIP-2, IL-6, and TNF-α in a dose-dependent man-
er (Fig. 3A). In the presence of PMB, which is known to bind
to and inhibit LPS activation, no significant difference was
found in the cytokine production stimulated by Leptospira (Fig.
3A). As a positive control, PMB inhibited cytokine production
induced by Salmonella minnesota Re595 LPS (Fig. 3A). It was
reported that PMB also bound to leptosporal LPS and inhibited
TLR activation (37). To investigate whether the macrophages
produced cytokines in response to the LPS component of heat-
killed Leptospira, we activated mouse peritoneal macrophages
with leptosporal LPS in the presence and absence of PMB (Fig.
3B). As a further control, MIP-2, IL-6, and TNF-α responses to
Leptospira LPS as well as Salmonella minnesota Re595 LPS
were inhibited by PMB. In contrast, the cytokine responses to
heat-killed whole Leptospira cells were not inhibited by PMB.
This result is consistent with a non-LPS leptosporal component
being involved in most of the cytokine induction of mouse macrophages by heat-killed Leptospira.

Leptospira stimulation of MIP-2 production is TLR4 depen-
dent but CD14 independent. Using MIP-2 production as a
sensitive measure of proinflammatory cytokine secretion, we
determined whether Leptospira stimulation of macrophages from wild-type C57BL/6 mice was dependent on normal TLR4 and
CD14 function. Anti-TLR4/MD-2 complex MAb (MTS510) and anti-TLR4 MAb (14H4E12) both significantly
decreased MIP-2 production from macrophages stimulated by
Leptospira (Fig. 4A). As negative controls, neither tissue cul-
ture medium alone nor isotype control antibody had a signif-
icent effect on MIP-2 production. An anti-CD14 monoclonal antibody, 28C5, did not reduce MIP-2 production in response
to heat-killed Leptospira. As a positive control, 28C5 did inhibit
Salmonella minnesota Re595 LPS stimulation of MIP-2 produc-
tion. This result suggests that Leptospira stimulates MIP-2 produc-
tion in a CD14-independent manner (Fig. 4). As expected,
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Re595 LPS was used as a control. While Re595 LPS stimulated much higher levels of MIP-2 and IL-6 from macro-
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Leptospira stimulation were similar in both the WT and CD14-/-
macrophages (Fig. 5).

The role of TLR2 in macrophage responses to Leptospira."

Previously, Werts et al. showed that Leptospira LPS, unlike
Salmonella or E. coli LPS, activates cells through human TLR2
(37). We determined whether intact Leptospira isolates behave
similarly by using peritoneal macrophages from TLR2-/-
and C56BL/6 (WT) mice. Macrophages from both mice secreted
similar levels of IL-6 and TNF-α upon activation with heat-
killed Leptospira and Re595 LPS (Fig. 6). Leptospiral LPS,
however, induced a much higher level of cytokine responses
from WT murine macrophages than from macrophages from
TLR2-/- mice. The cytokine levels were similar to cytokine
responses induced by heat-killed Staphylococcus aureus, a li-
gand known to stimulate TLR2-dependent responses. We also
observed that 1 μg/ml of leptosporal LPS induced levels of
TNF-α similar to those induced by 1 ng/ml Re595 LPS in the
WT mice. This observation suggests that leptosporal LPS is at
least 1,000-fold less active than Re595 LPS in stimulating cy-
tokine responses.

Macrophage cytokine responses to Leptospira are dependent
on TLR4. To assess directly whether leptosporal stimulation of
peritoneal macrophages was dependent on intact TLR4, we
compared cytokine production of peritoneal macrophages

These data suggest that intact TLR4 is involved in the control
of leptosporal burden in tissues of subletally infected mice,
which more closely mimics the natural state of infection.

Leptospira stimulation periodate-elicited mouse peritoneal
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de whether Leptospira stimulated production of proinflam-
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3B). As a further control, MIP-2, IL-6, and TNF-α responses to
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were inhibited by PMB. In contrast, the cytokine responses to
heat-killed whole Leptospira cells were not inhibited by PMB.
This result is consistent with a non-LPS leptosporal component
being involved in most of the cytokine induction of mouse macrophages by heat-killed Leptospira.
from C3H/HeJ and control C3H/OuJ mice. Leptospira-induced MIP-2 and IL-6 responses in macrophages were abrogated in C3H/HeJ mice, while high levels of these cytokines were observed in C3H/OuJ mice. Heat-killed Staphylococcus and Leptospira LPS induced high levels of the cytokines independent of TLR4 (Fig. 7A). When macrophages from transgenic TLR4−/− mice were compared with those from WT mice, ex vivo-obtained TLR4−/− macrophages failed to produce MIP-2, IL-6, and TNF-α after stimulation with all doses of Leptospira (Fig. 7B). The responses to other control stimuli, Re595 LPS, heat-killed Staphylococcus LPS, and leptospiral LPS, were similar to the results shown in Fig. 7A (data not shown).

Leptospira activates macrophages predominantly through the MyD88 pathway. TLRs signal through MyD88-dependent and -independent pathways. To determine whether the cellular signaling pathway induced by Leptospira is mediated through MyD88, peritoneal macrophages from MyD88−/− mice were stimulated with Leptospira. As with heat-killed Staphylococcus and Re595 LPS, heat-killed Leptospira stimulated MIP-2, IL-6, and TNF-α in WT mice but induced very low levels of cytokines in macrophages obtained from MyD88−/− mice (Fig. 8). This result suggests that Leptospira-stimulated signal transduction pathways leading to cytokine secretion are predominantly mediated through the signaling molecule MyD88.
DISCUSSION

The key finding in this study was that intact TLR4 function in a murine model of severe leptospirosis was critical for mouse survival and for preventing jaundice, pulmonary hemorrhage, and death. This effect was not related to leptospiral LPS since polymyxin B had no effect on *Leptospira*-induced cytokine production. Leptospiral stimulation of murine peritoneal macrophages was not dependent on CD14, consistent with the lack of LPS stimulation of the TLR4 pathway. In contrast to findings in the guinea pig model of leptospiral pulmonary hemorrhage (22), immunopathology related to either T or B cells in this model system did not occur.

Jaundice and pulmonary hemorrhage are unique clinical syndromes associated with leptospirosis and differ from other bacterial diseases caused by intra- and extracellular pathogens such as *E. coli*, *Salmonella* spp., and *Listeria*. Leptospirosis is therefore a unique system of bacterial pathogenesis. This murine model, which directly recapitulates severe human leptospirosis, suggests a critical role of the innate immune system, particularly the TLR4 gene or its associated components, in early infection by *Leptospira*.

Our results also demonstrate that intact TLR4 signaling contributes to the control of the tissue burden of *Leptospira* in nonlethal infection. Natural mammalian reservoir hosts of leptospires generally do not develop severe pathology in leptospiral infection. It has been well documented that leptospires can persist for prolonged periods of time in renal tubules of a wide variety of mammals (cattle, dogs, pigs, rats, mice, and humans) as well as in other tissues, such as brain, even in the absence of antileptospiral antibodies (6, 17, 35). Therefore, our finding that TLR4-deficient mice have significantly higher numbers of leptospires, particularly in the target organs mediating both leptospiral disease (liver, lung, and kidney) and transmission (kidney), is novel and important.

Our studies are consistent with the observations of Werts et al. (37), who previously observed that leptospiral LPS activates mouse macrophages through TLR2 and that there are no differences between mouse and human TLR2 (37) in the ability to recognize leptospiral LPS. Although a high quantity of purified leptospiral LPS can cause death in mice (37), our studies here clearly demonstrate that leptospiral ligands other than LPS play crucial roles in the pathogenesis of intact *Leptospira*. In contrast to our results, Werts et al. found that intact, heat-killed leptospires activate cells in a CD14-dependent manner using a human THP.1 cell line transfected with a human CD14 construct. We were unable to confirm this finding with murine macrophages when we used intact, heat-killed leptospires either to stimulate ex vivo-obtained CD14-deficient CD14 wild-type murine macrophages in the presence of an anti-CD14 monoclonal antibody or to stimulate macrophages from wild-type and CD14−/− mice. A similar discrepancy in the CD14 dependence of cytokine production between ex vivo-obtained murine macrophages and CD14-transfected THP.1 cell lines has previously been observed with *Aspergillus fumigatus* (18). The most likely

FIG. 5. Production of cytokines by periodate-elicited peritoneal macrophages of CD14-deficient mice. Mouse peritoneal macrophages of C57BL/6 wild-type or C57BL/6 CD14−/− mice either were left unstimulated (Uns) or were stimulated with 10^6 *Leptospira* (Lepto) cells/ml or 1 ng/ml of Re595 LPS. MIP-2 (left panel) and IL-6 (right panel) concentrations were measured by ELISA after 18 h of stimulation. Bars represent means ± SE of duplicate experiments. *, P < 0.05.

FIG. 6. Production of cytokines by peritoneal macrophages of TLR2-deficient mice. Mouse peritoneal macrophages of C57BL/6 wild-type or C57BL/6 TLR2−/− mice either were left unstimulated (Uns) or were stimulated with *Leptospira* (Lepto) at 10^6 bacteria/ml, 1 ng/ml Re595 LPS (LPS), 1 μg/ml *Leptospira* LPS (Lepto LPS), or 10^6 cells/ml of heat-killed *Staphylococcus aureus* (Staph). IL-6 and TNF-α concentrations were measured by ELISA after 18 h of stimulation. Bars represent means ± SE of assay performed in duplicate. *, P < 0.05.
explanation for this discrepancy is that CD14-transfected cell lines may produce biased cytokine responses compared to those of wild-type cells. It is also possible that human and mouse CD14 differ in their ability to interact with intact leptospires.

It is also known that there are differences between human and mouse cells in their abilities to respond to gram-negative LPS and taxol in a TLR4-dependent manner and that the species specificity of the recognition lies in the TLR4 adaptor molecule MD-2, which directly binds to the ligand (36). The requirement of MD-2 in leptospiral recognition needs to be investigated in more detail. Our preliminary studies using mouse and human TLR4-transfected cell lines indicate that there are no differences between mouse and human recognition of intact Leptospira (S. Viriyakosol, unpublished observation). Our finding that the TLR4 pathway protects mice from severe leptospirosis has direct implications for understanding leptospirosis in humans. Most cases of leptospiral infection are asymptomatic; the vast majority of symptomatic infections result in self-resolving undifferentiated fever (1). Relatively few,
perhaps only 5 to 10% of symptomatic cases, manifest as severe disease (Weil's syndrome [fever, jaundice, and renal failure]) (4), and of these cases, only a minority result in severe pulmonary hemorrhage (12, 29). An understanding of the mechanisms underlying the predisposition of humans to develop severe leptospirosis would be a major advance in the development of new approaches to treat and prevent this disease, a major problem in the developing world. The present study provides a strong rationale for carrying out a population-based analysis of TLR4 gene polymorphisms and their relationship to the clinical expression of leptospiral infection.

Of substantial significance is that the data presented here suggest that the magnitude of TLR4 stimulation by Leptospira in vivo likely far outweighs the leptomospial LPS stimulation of TLR2 after challenge infection, given the need for pharmacological levels of LPS to induce cytokine production but relatively low levels of heat-killed intact leptospires (similar to the blood or tissue burden that would be present in natural infection) that stimulate TLR4.

Unlike experimental infections such as E. coli pneumonia (16) or Pneumocystis carinii (34), our experiments also demonstrated the correlation of macrophage cytokine responses in vitro and in vivo disease outcomes. Macrophages from TLR4-defective mice showed less inflammatory cytokine responses than those from the wild-type mice. The reduced inflammatory response is associated with impaired bacterial elimination in the kidneys in the sublethal infection experiments as well as death in the lethal infection in the TLR4-deficient mice. The role of inflammatory cytokines in the disease process in vivo is being investigated. Although macrophages of TLR2−/− mice showed no defect in responses to intact Leptospira, the role of TLR2 in experimental infection still remained to be determined.

Mechanisms by which Leptospira activates the TLR4 pathway remain to be determined. In other microbiological systems, several ligands of TLR4 have been identified. The best characterized of these ligands is gram-negative bacterial LPS (28), which, depending upon whether it is the smooth or the rough type, stimulates TLR4 in either a CD14-dependent or -independent manner (11). CD14-independent TLR4 stimulation of cytokines is mediated solely by MyD88-dependent responses (11). We have shown that Leptospira activates the murine TLR4 pathway in a predominantly MyD88-dependent, CD14-independent manner. It is possible that the TLR4 responses to Leptospira that we observed were due to a hitherto-uncharacterized leptomospial LPS that stimulates TLR4 but not TLR2. Bacteria such as Porphyromonas gingivalis have been shown to produce different, structurally distinct lipid A components of LPS (23) that may stimulate different TLRs. How- ever, the TLR4-dependent responses to intact Leptospira were not inhibited by polymyxin B, which was known to inhibit LPS responses (37). Therefore, our data suggest that it is unlikely that the leptomospial TLR4 ligand(s) in our studies includes a CD14-independent manner (20). Identifying the Leptospira component(s) that stimulates TLR4 will be essential for understanding the role of innate immunity in human leptospirosis as well as TLR-dependent cellular responses in general.

The experiments presented here establish a clear basis for pursuing several distinct lines of investigation that will provide additional mechanistic insight into the pathogenesis of leptospirosis and in vivo dynamics during asymptomatic leptosporial infection. Important avenues of further research include elucidating the effector cell type of the TLR4-containing cell, the relationship of human TLR4 polymorphisms to severity of human leptospirosis, the biochemical components of Leptospira that interact with TLR4 and TLR4-containing protein complexes, and whether differences in these leptomospial components between strains explains virulence potential and human predisposition to severe rather than mild clinical outcomes of leptosporial infection.

ACKNOWLEDGMENTS

We thank Frances Multer and Lucia Hall for their excellent technical assistance. We are grateful to Eduardo Gotuzzo, Humberto Guerra, Robert H. Gilman, and James Olson and the U.S. Naval Medical Research Center Detachment, Lima, Peru, for their continued support of our work, which represents ongoing efforts of the Peru-United States Leptospirosis Consortium. We are particularly grateful to Gary Klimpel for his continued collaboration and support. This work was supported by NIH grants R01TW005860 and R01AI053422 to G. Klimpel and J.M.V. and R01AI19149 to T.N.K.

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