Important Role for Toll-Like Receptor 9 in Host Defense against Meningococcal Sepsis

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Neisseria meningitidis is a leading cause of meningitis and sepsis (29). This gram-negative diplococ, which is commonly carried asymptomatically by humans through colonization of the nasopharynx, may occasionally switch to invasive infection and rapid disease progression through a process characteristic of meningococcal disease (29). This gram-negative diplococ, which is responsible for some of the severe symptoms characteristic of meningococcal infection, both as a potent stimulator of proinflammatory signaling pathways and bactericidal activity and as a player in the eventual resolution of the inflammatory response.

We have previously demonstrated that nonlipooligosaccharide meningococcal pathogen-associated molecular patterns also contribute to disease progression in a MyD88-dependent manner (25), indicating a role for other TLRs. However, the roles of TLR2 and TLR9 in meningococcal disease have not been described.

Here we have investigated the roles of TLR2 and TLR9 in a murine model for meningococcal sepsis, as well as invasive disease in children (10, 31). We have previously demonstrated that nontipooligosaccharide meningococcal pathogen-associated molecular patterns also contribute to disease progression in a MyD88-dependent manner (25), indicating a role for other TLRs. However, the roles of TLR2 and TLR9 in meningococcal disease have not been described.

Here we have investigated the roles of TLR2 and TLR9 in a murine model for meningococcal sepsis. We report that TLR9 is essential for bacterial clearance whereas TLR2 plays only a minor role in the model used in this study. Our data further suggest a dual role for TLR9 in the host response to meningococcal infection, both as a potent stimulator of proinflammatory signaling pathways and bactericidal activity and as a player in the eventual resolution of the inflammatory response.
Bacteria and TLR ligands. We used the N. meningitidis strain FAM20, which belongs to serogroup C, multilocus sequence type 11. The bacteria were grown overnight in brain heart infusion broth with 10% L-levulin broth, reaching a concentration of (18.0 ± 2.2) × 10^8 bacteria per ml as determined in a Thoma counting chamber. For stimulations with pure TLR ligands, we used Pam2CSK4, lipopolysaccharide (LPS) (Ultrapure LPS; E. coli 0111:B4), and ODN1826 (all from InvivoGen).

Mouse model of infection. The mice used were 8- to 10-week-old female C57BL/6J, TLR2−/−, TLR9−/−, or TLR2/9−/− mice. The TLR single-knockout mice were obtained from Oriental Yeast Co Ltd. TLR2/9−/− animals were generated by mating TLR2−/− and TLR9−/− animals. The F2 animals were then intercrossed to get homozygous TLR2/9−/− mice, which were identified by PCR of tail snips. All mice were bred at Taconic M&B (Laven, Denmark). The animals had been backcrossed onto the background of the wild-type C57BL/6J strain for eight generations prior to use in experiments. The animal experiments were approved by the Swedish Ethical Committee. Mice were challenged intraperitoneally (i.p.) with 2 × 10^9 bacteria in 100 µl phosphate-buffered saline (PBS). Control mice were injected with 100 µl PBS. In survival studies, mice were monitored for 7 days. Bacteremia was defined by recovery of bacteria from blood samples taken from the tail vein at different time points post-i.p. challenge. Briefly, 5 µl of blood was diluted in GC liquid and serial dilutions were plated on GCB plates; the CFU were enumerated the next day. Blood smears were made for analysis of neutrophil/macrophage recruitment by light microscopy after Wright’s staining (Sigma, St. Louis, MO). Mice (n = 6 per group) were anesthetized by isoflurane (Forene, Abbott) before blood was collected by retro-orbital bleeding. Blood was allowed to clot at 4°C, and sera were collected after centrifugation.

Isolation and culture of mouse peritoneal macrophages. Peritoneal cells were isolated as described previously by lavage of the peritoneal cavity (21). The cells were seeded onto tissue culture plates and allowed to settle for 4 h. Nonadherent cells were removed by washing of the wells three times with ice-cold PBS. For determination of evivo cytokine production, peritoneal cells from infected or vehicle-treated mice were cultured for 20 h after isolation, at which point supernatants were harvested and cytokine concentrations determined.

Generation of mouse bone marrow-derived dendritic cells (BM-DCs). BM-DCs were prepared as described previously (4). Briefly, mice were sacrificed by cervical dislocation and femur and tibia were removed. Bone marrow cells were flushed from the bone shafts and cultured at 2 × 10^5 cells/ml in medium containing 40 ng/ml recombinant murine granulocyte-macrophage colony-stimulating factor (R&D Systems). Fresh medium containing 40 ng/ml granulocyte-macrophage colony-stimulating factor was added after days 3 and 6. On days 7, nonadherent cells were harvested, washed, and resuspended in fresh medium, and used for experiments. The cells were 70% CD11c positive as determined by flow cytometry.

Isolation of pDCs from mouse spleens. Spleens were surgically removed and transferred to RPMI with 5% fetal calf serum (FCS). The spleens were then transferred to a 1-mg/ml suspension of collagenase D (Roche). The enzyme was injected into the organ, which was subsequently cut into small pieces, followed by incubation in the collagenase D suspension for 30 min at 37°C. The suspension was filtered over a 70-µm-pore-size cell strainer (BD Falcon), spun down, and suspended in RPMI-1% FCS. Erythrocytes were removed using Lympholyte-M (Cedarlane Laboratories), following the instructions of the manufacturer. After centrifugation, the cells were resuspended in PBS with 2 mM EDTA and 0.5% bovine serum albumin (MACS running buffer) in concentrations according to the manufacturer’s instructions (MACS; Miltenyi Biotech). Anti-mPDCA-1 microbeads were added, and after incubation for 15 min at 4°C, the suspension was spun down and suspended in running buffer. Plasmacytoid DCs (pDCs) were then isolated in an Auto-MACS separator by positive selection factor (R&D Systems). Fresh medium containing 40 ng/ml granulocyte-macrophage colony-stimulating factor (R&D Systems) was added to the pDCs, and the culture supernatants were harvested after the appropriate amount of time for measurement of cytokines. For measurement of intracellular signaling proteins, the cells were lysed using a cell lysis kit from Bio-Rad.

Determination of cytokines and chemokines. Cytokines were measured by enzyme-linked immunosorbent assay and Luminox technology assay as described earlier (19, 21, 25, 26). Tumor necrosis factor alpha (TNF-α) was measured using a kit (Ultrasensitive; sensitively, 0.01 pg/ml) from BioSource. Type I interferon (IFN) bioactivity was measured using an L929-cell-based bioassay (28).

Measurement of signal transduction pathways. To measure activation of signal transduction pathways, cells receiving the appropriate treatment were lysed using a Bio-Rad cell lysis kit. The levels of phosphorylated IkBα was measured using a Bio-Rad Lumix kit, following the instructions of the manufacturer, similar to the Luminox protocol described above.

Nitrile determination. Nitrile is generated by the rapid oxidation of nitric oxide (NO). It is stable, and its accumulation in the culture medium reflects the amount of NO produced. To assay nitrile, we used the Griess assay. Aliquots of 100 µl culture supernatants were mixed with equal volumes of the Griess reagent (equal volumes of 0.1% N-(1-naphthyl)-ethylenediamine dihydrochloride and 1% p-aminoazobenzene sulfanilamide diluted in 2.5% phosphoric acid) in a 96-well microtitrater plate (Maxisorb Immunoplate; Nunc). After a 10-min incubation at room temperature, the absorbance at a wavelength of 540 nm was measured in a microplate reader. A range of twofold dilutions of sodium nitrite (0.05 to 100 µM) in RPMI medium was run in each assay to generate a standard curve.

Phagocytosis of N. meningitidis by macrophages. For fluorescein labeling, organisms (0.3 ml in RPMI 1640 medium without phenol red) at an A490 value of 1 were mixed with an equal volume of fluorescein isothiocyanate (FITC) solution (1 mg/ml in RPMI medium without phenol red) and incubated at 37°C for 30 min. The organisms were spun at 9,500 × g for 1 min and washed three times with 0.5 ml of RPMI medium. Organisms were fixed by the addition of 0.5% (wt/vol) (final concentration) paraformaldehyde and resuspended in RPMI medium. FITC-labeled organisms (50 µl) were incubated with peritoneal macrophages (five bacteria per macrophage) in a total volume of 1 ml, and the suspension was incubated at 37°C for 60 min. The cells were washed three times in 1% RPMI and fixed, and phagocytosis was determined by flow cytometry.

Growth of meningococci in mouse whole blood. Whole blood was collected from B6, TLR2−/−, TLR9−/−, and TLR2/9−/− mice (n = 3), pooled, and mixed 1:1 with bacteria suspended in Dulbecco’s modified Eagle cell culture medium (10 × 10^6 CFU of FAM20/ml). The mixtures were incubated for 1 h at 37°C, and the number of surviving bacteria was determined by spreading 10-fold serially diluted supernatants on GCB plates incubated overnight in air plus 5% CO2 at 37°C. The number of CFU was determined the next day. All data were performed twice in triplicate, and data show the percentage recovery of bacteria compared to the initial inoculum.

Statistical analysis. Survival rates were assessed using Fisher’s exact test. Cytokine concentrations, phagocytosis, and the serum bacterialidal assay were evaluated with a two-tailed Student t test. P values of <0.05 were considered significant.

RESULTS

TLR9 deficiency is associated with impaired survival and increased bacteremia during infection with N. meningitidis. To examine the role of TLR2 and TLR9 in host defense against N. meningitidis infection, we used a murine model for meningococcal sepsis (25, 30). In a first series of experiments, we infected C57BL/6, TLR2−/−, TLR9−/−, and TLR2/9−/− mice i.p. with the FAM20 strain and monitored survival of the animals. As expected, the majority of the wild-type mice (10 out of 12) survived the infection with the dose of bacteria used (Fig. 1). TLR2 deficiency did not affect the survival rate of the mice, but mice lacking TLR9 displayed a significantly lower survival rate than wild-type mice. The TLR2/TLR9 double-deficient mice responded to the infection with a phenotype intermediate between those of the TLR2−/− and TLR9−/− mice.

To examine if the differential survival rate of TLR9−/− mice versus wild-type and TLR2−/− mice was reflected in differences in bacterial loads in the bloodstream, we harvested blood samples from the mice at the indicated time points postinfection (p.i.) and measured bacteria in the samples. At 6 h p.i., a high bacterial load was observed for all mouse strains with no significant differences between the strains (Fig. 2A). However, at later time points (24, 48, and 72 h p.i.), the level of bac-

5422 SJÖLINDER ET AL. INFECT. IMMUN.
teremia for TLR9\(^{-/-}\) mice was significantly higher than that for C57BL/6 mice (Fig. 2B and D). TLR2\(^{-/-}\) and TLR2\(^{-/-}/9^{-/-}\) were statistically indistinguishable from wild-type mice at all time points. Thus, TLR9 deficiency is associated with impaired survival and increased levels of bacteremia during infection with *N. meningitidis*.

**TLR9 is required for optimal activation of bactericidal activity.**

Given the elevated mortality and bacterial load of TLR9\(^{-/-}\) mice, we were interested in knowing how TLR9\(^{-/-}\) cells controlled meningococcal infection. We first examined recruitment of macrophages to the peritoneal cavity after infection. By 2 h postchallenge, significantly elevated levels of macrophages were found in the peritoneal cavity of all mouse strains compared to results for vehicle-treated mice (Fig. 3A). However, the peritoneal washes from infected TLR2\(^{-/-}\) mice contained significantly fewer macrophages than the washes from the C57BL/6 mice. When looking in the bloodstream, we found that cell types of this lineage did migrate to the bloodstream during sepsis, particularly in TLR9\(^{-/-}\) mice, from which a significantly higher number of blood monocytes were recovered than from C57BL/6 mice (Fig. 3B). In contrast, in TLR2\(^{-/-}\) mice, the infection did not lead to significantly higher levels of blood monocytes. Next, we examined the ability of meningococci to grow in mouse whole blood. Interestingly, significantly more bacteria were recovered from the blood of TLR9\(^{-/-}\) mice than from that of C57BL/6 mice (Fig. 3C). The bacterial growth in blood from TLR2\(^{-/-}\) and TLR2/9\(^{-/-}\) mice was comparable to what was observed in blood from C57BL/6 mice. The observed augmented bacterial growth in a TLR9-deficient environment was not due to an impaired ability of TLR9\(^{-/-}\) macrophages to perform phagocytosis, since all four genotypes displayed similar abilities to take up *N. meningitidis* (Fig. 3D).

One way through which macrophages kill meningococci is through production of reactive nitrogen and oxygen radicals (24, 33). To test if the production of NO in response to meningococcal infection was dependent on either TLR2 or TLR9, we harvested and cultured peritoneal macrophages and spleen cells from C57BL/6, TLR2\(^{-/-}\), TLR9\(^{-/-}\), and TLR2/9\(^{-/-}\) mice and treated them as indicated in Fig. 3E and F. Nitrite, which is a stable oxidation product of NO, was measured 24 h postinfection. TLR2\(^{-/-}\) peritoneal macrophages and spleen cells produced levels of nitrite comparable to those of wild-type cells. However, cells lacking TLR9 produced significantly less nitrite than wild-type cells. Thus, *N. meningitidis* displays augmented growth in blood from TLR9\(^{-/-}\) mice, and leukocytes from these mice produce reduced levels of NO.

**Prolonged cytokine production in TLR9-deficient mice.**

TLR9 agonists are well described as potent inducers of a number of cytokines and chemokines (11, 17), but important roles in negative control of the inflammatory response in vivo also...
have been ascribed to them (16). Therefore, we were interested in examining the production of cytokines in mice during meningococcal infection. First we infected mice with *N. meningitidis*. Two hours later, peritoneal cells were isolated and cultured for 20 h to allow ongoing cytokine production to continue. Supernatants were harvested, and cytokines were measured. The cells from infected mice produced large amounts of TNF-α, interleukin 6 (IL-6), and KC (Fig. 4A to C), and for IL-6 the response was partly dependent on TLR2, whereas the two other cytokines were produced independently of TLR2 and TLR9. Next, we harvested serum from uninfected mice and mice challenged for 1, 6, and 24 h with FAM20. For TNF-α, peak levels were observed 1 h postchallenge (Fig. 4D), while the other cytokines measured (IL-6, KC, IL-10, IL-12, and gamma IFN [IFN-γ]) reached the highest levels about 6 h postinfection (Fig. 4E and F; also data not shown). The level of the complement cleavage product C5a was also elevated, with peak levels at 6 h post-bacterial challenge (data not shown). For all parameters, no significant differences between the mouse strains were observed. However, after 24 h, the cytokine levels were close to background levels for C57BL/6 and TLR2−/− mice but remained elevated for TLR9−/− and TLR2/9−/− mice (Fig. 4G to I). For the former mouse strain, the difference was significant for all cytokines and C5a (relative to results for C57BL/6 mice), while for the latter strain, only the levels of IL-10 and IFN-γ were significantly higher. Thus, infection with *N. meningitidis* induced a potent cytokine response, which was prolonged in TLR9-deficient mice.

*N. meningitidis* is recognized by pDCs through TLR9. The data shown above suggest a role for TLR9 in protection against meningococcal sepsis. Therefore, we wanted to investigate the specific nature of the cells recognizing *N. meningitidis* in a TLR9-dependent manner. We focused on macrophages, BM-DCs, and pDCs, of which pDCs are known to be the major TLR9-expressing cell type (2). All three cell types responded to stimulation with the TLR9 agonist ODN1826 with expression of IFN-α/β (data not shown), suggesting that the receptor was expressed on these cell types. When the macrophages and
BM-DCs were stimulated with *N. meningitidis*, expression of TNF-α and IL-6 was strongly induced (Fig. 5A and B and D and E). In addition, type I IFN (Fig. 5C and F), which has been suggested to be a negative modulator of the inflammatory response in certain settings (16), was detected in the culture supernatants. The IL-6 response was partly dependent on TLR2. The pDCs also responded to meningococcal treatment with expression of TNF-α and IL-6, although to a more modest extent than the two other cell types (Fig. 5G and H). Interestingly, the pDCs produced large amounts of IFN-α/β after bacterial stimulation, and this was dependent on TLR9 (Fig. 5I).

To look further into the differential requirement for TLR9 in recognition of meningococci by macrophages, BM-DCs, and pDCs, we examined the ability of *N. meningitidis* to activate intracellular signaling in these cell types. Cell lysates were prepared from cells incubated with the bacteria for 2 h, and phosphorylation of IκBα was determined as a measure of activation of the NF-κB signaling pathway. In macrophages and BM-DCs, *N. meningitidis* strongly activated the NF-κB pathway in a TLR9-independent manner (Fig. 6A and B). In macrophages, the NF-κB response displayed a partial dependence on TLR2. The pDCs also mediated activation of the NF-κB pathway in response to bacteria, and in this cell type the response was dependent on TLR9 (Fig. 6C). Thus, *N. meningitidis* is recognized by pDCs in vitro in a TLR9-dependent manner, leading to activation of the NF-κB pathway and expression of a number of cytokines, including IL-6, TNF-α, and type I IFN.

**DISCUSSION**

TLRs are responsible for innate recognition of microbes, and TLR2, -4, and -9 have previously been reported to be involved in recognition of *N. meningitidis* (18, 20, 35). While the importance of TLR4 in the host response to meningococcal infection is well described (25, 31), the functions of TLR2 and TLR9 remain unknown. Here we have investigated the roles of TLR2 and TLR9 in a murine model for meningococcal sepsis.

It has been reported that preinjection of iron compounds enhances meningococcal infection in mouse models (13, 34). However, the main disadvantage of this experimental model is the unknown effects of iron on the host immune response, especially on the innate immune defense system. Therefore, in this study we challenged mice i.p. without iron pretreatment. We report that TLR9−/− mice displayed elevated levels of *N. meningitidis* bacteremia and reduced survival compared to wild-type mice but also responded to the infection with enhanced recruitment of macrophages and elevated expression of cytokines at later stages of infection. In contrast, TLR2−/− mice controlled the infection in a manner comparable to that of wild-type mice.

TLR2 recognizes meningococcal porins and is a potent inducer of inflammatory cytokines (18). Accordingly, we found
reduced macrophage recruitment to the peritoneal cavity in TLR2−/− mice after infection. In addition, bacterium-induced expression of IL-6 in vitro and ex vivo was reduced in the absence of TLR2. However, in the model used in this study, we did not observe any effect of TLR2 deficiency on bacteremia levels or survival. Thus, our data suggest that TLR2 plays only a minor role in triggering the inflammatory response during N. meningitidis sepsis. These data are in line with our previous observation that TLR2−/− mice displayed unaltered sensitivity to meningococcal infection and that production of inflammatory cytokines and chemokines was not impaired in TLR2−/− mice (26).

A much more pronounced phenotype was observed in the TLR9−/− mice, displaying both reduced bactericidal activity and prolonged production of cytokines. This prolonged expression of cytokines in TLR9−/− mice could indicate that this PRR plays a role in negative regulation of the host response or might alternatively be due to the higher bacterial load in the TLR9−/− mice. However, the elevated cytokine expression 24 h postchallenge was also observed in the TLR2/9−/− mice, which did not exhibit an elevated bacterial load, thus suggesting that TLR9 may play a role in the eventual resolution of the inflammatory response during meningococcal infection.

TLR9 is expressed on a number of cell types, with the highest abundance on pDCs, which are potent inducers of type I IFN (2). By in vitro stimulation, we found that pDCs produced type I IFN and other cytokines in response to live meningococci, and this was dependent on TLR9. The signaling pathways activated by TLR9 include the NF-kB and mitogen-activated protein kinase pathways, which stimulate potent proinflammatory activities (2), but also the IFN regulatory factor pathway, which together with the DNA-dependent protein kinase induces type I IFN and anti-inflammatory activities (2). For instance, stimulation through TLR9 protects mice from experimental colitis through induction of type I IFN (16). Also, stimulation of allergen-loaded macrophages with TLR9 agonists suppressed

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**FIG. 5.** Cytokine production by macrophages, BM-DCs, and pDCs after infection in vitro. Macrophages (A to C), BM-DCs (D to F), and pDCs (G to I) from wild-type and KO mice were treated with 2 × 10⁷ CFU of FAM20 per ml or vehicle control, and supernatants were harvested 16 h later for measurement of TNF-α, IL-6, and IFN-α/β. The data are shown as means for triplicate cultures ± standard errors. *, P < 0.05 (Student’s t test).

**FIG. 6.** Activation of intracellular signaling in macrophages, BM-DCs, and pDCs after infection in vitro. Macrophages (A), BM-DCs (B), and pDCs (C) from wild-type and KO mice were treated with 2 × 10⁷ CFU of FAM20 per ml or vehicle control, and whole-cell lysates were prepared 2 h later. The levels of phospho-IκBα (P-IκBα) were determined by using Luminex. The data are shown as means for triplicate cultures ± standard errors.
allergic airway inflammation in mice by a mechanism dependent on IL-10 (32). In contrast, type I IFN is essential for mounting the inflammatory response during Francisella infection through activation of the inflammasome, which triggers production of bioactive IL-1β and IL-18 (12). It remains unknown whether type I IFN is responsible for the prolonged cytokine production observed in our experimental model and more globally how IFN may either promote or inhibit the inflammatory response dependent on the conditions.

One of the key findings in this work is that TLR9 is essential for optimal host defense against *N. meningitidis*. This is the first report to demonstrate an essential role for this PRR in meningococcal disease. TLR9 recognizes DNA from meningococci and other bacteria (11, 20) and has previously been reported to play a role in defense against infections with *Streptococcus pneumoniae* and *Klebsiella pneumoniae* in vivo (3, 8). In the case of pneumococcal infection, TLR9 was essential for uptake and intracellular killing of the bacteria (3), whereas during infection with *K. pneumoniae*, the role of TLR9 was to recruit and activate leukocytes, including dendritic cells and macrophages, and to promote development of a Th1 response (8). Similar results have been obtained in other studies, where TLR9 has been ascribed important functions in establishment of an IL-12/IFN-γ response (15, 22). In our studies, TLR9 was essential for restriction of bacterial growth but not for phagocytosis. We observed that TLR9+/− and TLR2/9−/− macrophages, as well as total spleen cells, produced reduced levels of NO after meningococcal infection, suggesting that this may contribute to the impaired bacterial activity of the TLR9−/− mice. In agreement with these data, TLR9-deficient macrophages have been reported to produce reduced levels of NO synthase in a model for mycobacterial antigen-elicited pulmonary granuloma (14). Collectively, TLR9 seems to play an important role in host defense against a range of bacteria and to utilize a whole array of mechanisms to shape the immune response and control bacterial growth.

It has recently been demonstrated that TLR9 contributes to the pathogenesis of polymicrobial sepsis following experimental peritonitis induced by cecal ligation and puncture (27). It was reported that TLR9−/− mice exhibited lower levels of both bacteremia and serum inflammatory cytokines than wild-type mice and had improved survival. These findings are somewhat in contrast to our observation that TLR9−/− mice displayed higher levels of bacteremia and prolonged expression of serum cytokines. However, similar to findings of Plitas et al. (27), we also observed that TLR9 deficiency led to enhanced migration of certain types of leukocytes. The microbial flora giving rise to polymicrobial sepsis in the model used by Plitas and colleagues is very different from the well-defined meningococcal strain used in our study, and the observed differences are most likely explained by this fact. Therefore, these studies suggest that induction as well as control of bacterial sepsis may rely on different immunological mechanisms, including the possible involvement of several different TLRs, depending on the invading pathogen.

TLR2 and TLR9 were previously reported to synergistically mount protective host responses against pathogens. TLR2/9−/− mice were severely impaired in defense against *Mycobacterium tuberculosis* and *Trypanosoma cruzi* (5, 6), and this involved reduced production of IL-12 and IFN-γ. However, in our model, no synergy between TLR2 and TLR9 was observed. In fact, the TLR2/9−/− mice displayed a phenotype which in many respects was intermediate between those of the two single knockout mouse strains. The apparent beneficial effect of TLR2 deficiency on the TLR9-deficient background may at least in part be explained by the proinflammatory activity of TLR2 in the model, as discussed above. TLR2 deletion may thus lead to reduced inflammation and hence less immunopathology.

In conclusion, we report that *N. meningitidis* is recognized by TLR9 in vivo and contributes to production of cytokines and NO by pDCs and macrophages, respectively. Our work further demonstrates that TLR9 is essential for bacterial clearance in vivo, and hence mice deficient in this PRR are more susceptible to meningococcal sepsis.

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**REFERENCES**


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