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Toll-Like Receptor 2 Participates in Mediation of Immune Response in Experimental Pneumococcal Meningitis

Uwe Koedel,* Barbara Angele,* Tobias Rupprecht,* Hermann Wagner,† Andreas Roggenkamp,† Hans-Walter Pfister,‡ and Carsten J. Kirschning‡

Heterologous expression of Toll-like receptor (TLR)2 and CD14 in Chinese hamster ovary fibroblasts was reported to confer responsiveness to pneumococcal peptidoglycan. The present study characterized the role of TLR2 in the host immune response and clinical course of pneumococcal meningitis. Pneumococcal infection of mice caused a significant increase in brain TLR2 mRNA expression at both 4 and 24 h postchallenge. Mice with a targeted disruption of the TLR2 gene (TLR2−/−) showed a moderate increase in disease severity, as evidenced by an aggravation of meningitis-induced intracranial complications, a more pronounced reduction in body weight and temperature, and a deterioration of motor impairment. These symptoms were associated with significantly higher cerebellar and blood bacterial titers. Brain expression of the complement inhibitor complement receptor-related protein y was significantly higher in infected TLR2−/− mice, while the expression of the meningitis-relevant inflammatory mediators IL-1β, TNF-α, IL-6, macrophage-inflammatory protein (MIP)-2, inducible NO synthase, and C3 was similar in both genotypes. We first ectopically expressed single candidate receptors in HEK293 cells and then applied peritoneal macrophages from mice lacking TLR2 and/or functional TLR4 for further analysis. Overexpression of TLR2 and TLR4/MD-2 conferred activation of NF-κB in response to pneumococcal exposure. However, pneumococci-induced TNF-α release from peritoneal macrophages of wild-type and TLR2/functional TLR4/double-deficient mice did not differ. Thus, while TLR2 plays a significant role in vivo, yet undefined pattern recognition receptors contribute to the recognition of and initiation of the host immune defense toward Streptococcus pneumoniae infection. The Journal of Immunology, 2003, 170: 438–444.

Bacterial meningitis caused by Streptococcus pneumoniae, the principal etiologic agent in adults (1), is still a serious disease with death rates up to 20%. Moreover, neurologic morbidity affects up to one-third of survivors. Clinical and neuropathological studies have clearly demonstrated that a fatal outcome of the disease is often caused by intracranial complications including brain edema formation, increased intracranial pressure, and cerebrovascular insults (2, 3). Therefore, during the past 15 years, investigations have focused on the pathogenesis of meningitis-associated CNS complications (4). It has become clear that the development of these complications is not simply dependent on the presence of viable bacteria, but rather occurs as a consequence of the host inflammatory reaction to the pathogen (5). Although the exact mechanisms of immune activation in pneumococcal meningitis are unknown, recent in vitro experiments provide a clue. The first step in immune activation is thought to be the binding of the pathogen associated molecular pattern (6) peptidoglycan and/or lipoteichoic acid to the pattern recognition receptor membrane CD14 (mCD14) (7–9). However, mCD14 by itself cannot transmit the activating signal into the cell due to the lack of a transmembrane domain connecting the extracellular domain to the cytoplasm (10). Transmission of the activating signal through the cellular membrane into the cell potentially occurs through the Toll-like receptor (TLR)2 (for review, see Refs. 11 and 12). Accordingly, heterologous expression of TLR2 in CD14-expressing Chinese hamster ovary (CHO/CD14) fibroblasts was reported to confer responsiveness to pneumococcal peptidoglycan (as evidenced by inducible translocation of the transcription factor NF-κB) (13). When CHO fibroblasts were exposed to heat-treated S. pneumoniae, a partial immune response was observed in CHO/CD14 cells in the absence of TLR2 overexpression (13). Taken together, this study indicates the existence of TLR2-dependent and TLR2-independent immune stimulation by infection with S. pneumoniae.

Constitutive expression of TLR2 was found in the mouse brain by in situ hybridization, particularly in the choroid plexus lining the lateral ventricle (14). Because meningeal pathogens were reported to accumulate in large numbers in the cerebrospinal fluid (CSF) of cerebral ventricles (15), we hypothesized that TLR2 might act as a sensor of pneumococcal CNS infection and as a trigger of the (exaggerated) inflammatory host reaction in pneumococcal meningitis. We used gene-targeted mice lacking TLR2 expression (TLR2−/−) to assess the role of this pattern recognition receptor in experimental pneumococcal meningitis. Thereby, we characterized the influence of TLR2 depletion on 1) pneumococcal outgrowth within the CSF, 2) the inflammatory host response to S. pneumoniae, 3) the development of CNS complications that are assumed to occur as a consequence of an uncontrolled host immune response, and 4) the (short-term) outcome of bacterial meningitis that is dependent predominantly on the extent of CNS alterations.

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3 Abbreviations used in this paper: TLR, Toll-like receptor; CHO, Chinese hamster ovary; MIP, macrophage-inflammatory protein; iNOS, inducible NO synthase; Crry, complement receptor-related protein y; CSF, cerebrospinal fluid; ICP, intracranial pressure; BBB, blood-brain barrier; wt, wild type.
Experimental groups in the mouse model

The following experimental groups were investigated: 1) wild-type (wt) mice injected intracisternally with 15 10^7 CFU/ml S. pneumoniae (n = 16), and 3) TLR2−/− mice injected intracisternally with S. pneumoniae (n = 16). In these groups, mice were killed 24 h after onset of pneumococcal meningitis. At that time, all mice exhibited obvious symptoms of disease, marked intracranial complications, and pronounced meningeal inflammation (16, 17). To determine the impact of TLR2 deficiency on early events associated with pneumococcal meningitis, the following additional subset of experiments was performed: infected wt and TLR2−/− mice (n = 4 for each group; groups 4 and 5) killed 4 h after pneumococcal inoculation. Targeted mice lacking expression of TLR2 were obtained from Tulakir (South San Francisco, CA) and Deltagen (Menlo Park, CA). A portion of the C-terminus of the human TLR2 was transmembrane aa C454 to aa 593 (residues calculated for immature protein) was replaced by a neomycin cassette through homologous recombination (20). TLR2-deficient 129Sv × C57BL/6 mice were bred back against the genetic C57BL/6 background for four generations. Then two heterozygous pairs were used to establish TLR2−/− homozygous breeding colonies with the corresponding genetic background. Matched groups of mice descending from these breeding colonies were applied synchronically for experiments. All of the experiments were approved by the government of Upper Bavaria.

**NF-κB reporter gene assay**

The human embryonic epithelial kidney cell line HEK293 (American Type Culture Collection, Manassas, VA) was maintained in DMEM supplemented with 10% FCS (RecombiTEK, Grenzach-Whylen, Germany) and penicillin/streptomycin; in a phosphothioate protected form; TIB MOLBIOL, Berlin, Germany). After 24 h, cell culture supernatants were sampled and analyzed for TNF-α. Expression of TLRs 2–4 and CD14 were kindly provided by Tularik (24), while the cDNA for TLR9 was a kind gift from Drs. Beutler and Bauer (25, 26).

**TNF-α release from primary macrophages**

Peritoneal macrophages were isolated from TLR2−/− mice (5-fold backcrossed toward the C3H inbred strain background; TLR2−/−TLR4−/−), C3H/HeN mice (wild-type), C3H/HeJ mice (TLR2−/−/TLR4−/−), C3H/HeJ mice lacking both TLR2 and functional TLR4 (TLR2−/−TLR4−/−), and mice lacking both TLR2 and functional TLR4 (TLR2−/−TLR4−/−). Briefly, mice were injected i.p. with 2 ml of 4% thioglycolate (Sigma-Aldrich). After 5 days, peritoneal exudate cells were collected by washing the peritoneal cavity with ice-cold PBS supplemented with 2% FCS. Cells (2 × 10^6 cells/well in 96-well dishes) were washed with PBS for removal of nonadherent cells. Adherent macrophages were stimulated with 10% FCS, standard penicillin/streptomycin, 10 μM monothioglycerol, and murine IFN-γ (50 ng/ml; PeproTech, London, U.K) either with S. pneumoniae (10^7 CFU/ml; applied concomitantly with 100 μg/ml cetuximab for the prevention of bacterial overgrowth) or the following bacterial products: 1) Escherichia coli LPS O111:B4 (10 ng/ml; Sigma-Aldrich), 2) polyinosine-polycytidylic acid (5 μM; Promega, Madison, WI), 3) the synthetic DNA oligonucleotide O2.006 (2 μM; in a phosphothioate protected form; TIB MOLBIOL, Berlin, Germany) for 16 h (26). Then HEK293 cells were lysed for measurement of cytoplasmic luciferase and β-galactosidase activities using agents from Promega (Madison, WI) and ABI (Tropix, Bedford, MA). Luciferase and β-galactosidase activities were determined in a 96-well luminoimeter (Berthold, Weiterstadt, Germany) and normalized by relation to β-galactosidase activities.
Statistical analysis
The principal statistical test was unpaired Student’s t test. Differences were considered significant at \( p < 0.05 \). Data are expressed as mean \( \pm \) SD.

Results
Up-regulation of TLR2 mRNA expression in the brain during pneumococcal meningitis
In brain homogenates from PBS-injected control mice, a low basal expression of TLR2 mRNA was detected by RT-PCR. Pneumococcal infection caused a substantial increase in the mRNA expression of TLR2 in brains obtained from wt mice 4 h as well as 24 h after challenge (Fig. 1a). The relatively high constitutive TLR2 expression in the spleen was not affected by intracisternal pneumococcal inoculation (data not shown).

Effect of TLR2 depletion on the clinical status
Within 24 h after pneumococcal inoculation, all infected wt mice exhibited a similar degree of disease as evidenced by a loss in weight, hypothermia, piloerection, lethargy, and impaired motor functions (Fig. 2a). TLR2 deficiency was associated with a moderate increase in disease severity (Fig. 2a). For example, TLR2 \(^{-/-} \) mice showed a more pronounced reduction in both body temper-

![Figure 1](image1.png)

![Figure 2](image2.png)

FIGURE 1. Brain mRNA expression of TLR2 and meningitis-relevant inflammatory mediators during pneumococcal meningitis. a. In brain homogenates from PBS-injected control mice, a low basal expression of TLR2 mRNA was detected by RT-PCR. Pneumococcal infection caused a significant increase in TLR2 mRNA expression in brains obtained from wt mice either 4 or 24 h after challenge. b–f. After 24 h of intracisternal inoculation, differences in the brain expression of the proinflammatory cytokine TNF-\( \alpha \) (b), the neutrophil chemoattractant MIP-2 (c), the iNOS isoform (d), or C3 (e) were not detectable, except for the brain expression level of the complement inhibitor Crry (f) whose expression level was significantly higher in infected TLR2 \(^{-/-} \) mice as compared with infected wt mice. However, after 4 h of pneumococcal infection, the brain expression of TNF-\( \alpha \) and MIP-2 (but not of iNOS and C3) was significantly lower in TLR2 \(^{-/-} \) mice than in wt mice, whereas Crry expression remained on a higher level.

FIGURE 2. Effect of TLR2 deficiency on the clinical status and CNS complications. a. Clinical examination revealed a similar degree of disease in all infected wt mice. Genetic disruption of the TLR2 gene was associated with a mild, but significant deterioration of the clinical status of infected animals. Control animals had a clinical score of 0 points, representing healthiness. Pneumococcal infection caused a significant increase in ICP (b) and BBB permeability (c), as indicated by an increase in brain albumin levels. At 24 h (but not at 4 h) after pneumococcal challenge, infected TLR2 \(^{-/-} \) mice showed a significantly increased rise in ICP and in brain albumin levels, as compared with infected wt mice.

![Figure 2](image3.png)

None of the control mice injected intracisternally with PBS exhibited signs of infection within the observation period. No significant change in body weight (\(-13.5 \pm 3.3\% \) vs \(-9.9 \pm 3.1\% \) in infected wt mice).

Body temperatures were within the normal range (37.1 \( \pm \) 0.3°C).

Effect of TLR2 depletion on meningitis-induced intracranial complications
Because CNS complications are major determinants of an unfavorable clinical outcome in bacterial meningitis (2, 3), we investigated the impact of TLR2 deficiency on ICP and BBB permeability in our mouse meningitis model.

Pneumococcal infection induced a significant increase in ICP in wt mice. At 24 h after pneumococcal inoculation, TLR2 \(^{-/-} \) mice had significantly higher ICP values than infected wt mice (Fig. 2b). Because vasogenic edema is a predominant cause of the meningitis-associated increase in ICP, we determined the immunoreactivity for the endogenous serum proteins albumin and IgG in mouse brain homogenates by Western blotting. In brain homogenates from PBS-injected control mice, only faint bands for both albumin and IgG were present. Intracisternal injection of pneumococci...
caused an extensive extravasation of albumin (Fig. 2c) and IgG into the brain (data not shown). Infected TLR2\(^{-/-}\) mice exhibited a significantly more pronounced immunoreactivity for albumin (but not IgG) than infected wt mice. These data hint at a slight deterioration of meningitis-induced BBB disruption in mice with a targeted disruption of the TLR2 gene.

**Effect of TLR2 depletion on bacterial titers in blood and organs**

To test whether this worsening of disease resulted from altered bacterial growth in vivo, the bacterial numbers in the cerebellum, blood, and spleen were determined at 4 and 24 h after intracisternal pneumococcal infection. At both time points, the number of bacteria in the cerebellum and blood (but not in the spleen; data not shown) was higher in TLR2-deficient mice than in wt mice (Fig. 3), consistent with the aggravation of disease observed in TLR-deficient mice.

**Effect of TLR2 depletion on the inflammatory host reaction**

To investigate whether the higher susceptibility to pneumococcal infection is due to a defective immune response, we assessed the impact of TLR2 deficiency on the inflammatory host response in our meningitis model.

Pneumococcal infection led to a massive leukocyte infiltration into the subarachnoid space in wt mice. Neither at 4 h nor at 24 h after pneumococcal infection, a significant difference in CSF leukocyte counts between TLR2\(^{-/-}\) mice and wt mice was detectable (e.g., at 24 h postchallenge, 14,373 ± 7,937 cells/μl in TLR2\(^{-/-}\), infected mice vs 11,991 ± 6,175 cells/μl in infected wt mice). Moreover, mouse brain, spleen, and lung homogenates were analyzed for their expression of several meningitis-relevant host factors by RT-PCR (31). After 24 h of intracisternal inoculation, differences in mRNA accumulation of the proinflammatory cytokines IL-1β and TNF-α, the neutrophil chemoattractant MIP-2, the cytokine IL-6, the iNOS isoform, or the complement factor C3 in the brain, spleen, and lung were insignificant in wild-type and TLR2\(^{-/-}\) mice (Fig. 1, b–f; data on IL-1β and IL-6 mRNA expression not shown). Of the mRNA levels analyzed at 24 h upon infection solely, the level of Crry mRNA was significantly higher in infected TLR2\(^{-/-}\) mice as compared with levels in infected wt mice. However, after 4 h of pneumococcal infection, the brain expression of TNF-α and MIP-2 (but not of IL-1β, IL-6, iNOS, and C3) was significantly lower in TLR2\(^{-/-}\) mice than in wt mice whereas the Crry mRNA levels were higher. However, in the spleen, infected TLR2\(^{-/-}\) mice exhibited a higher mRNA expression of both MIP-2 and iNOS (but not the other factors investigated) than infected wt mice (data not shown). In the lung, TLR2 deficiency was associated with a significantly increased expression of TNF-α mRNA, but not of the other mediators determined (data not shown). Combined, this comparative expression analysis revealed rather mild differences in the host immune response of wt and TLR2\(^{-/-}\) mice that were restricted to the early phase of pneumococcal meningitis.

**Role of TLR-2 and TLR-4 in pneumococci-induced cellular activation in vitro**

Because TLR2 deficiency had only a moderate impact on the inflammatory host response in murine pneumococcal meningitis, we further investigated the mechanisms of cellular activation by S. pneumoniae in the cerebellum and blood during experimental pneumococcal meningitis. At 4 and 24 h after intracisternal pneumococcal infection, the number of S. pneumoniae in the cerebellum (a) and blood (b) was higher in TLR2-deficient mice than in wt mice.
pneumoniae using a NF-κB reporter assay. HEK293 cells were cotransfected with a NF-κB-dependent endothelial leukocyte adhesion molecule-1 promoter luciferase reporter, a CD14, and an MD-2 expression construct, as well as expression plasmids for TLR2, TLR3, TLR4, or TLR9. Exposure of HEK293 cells to S. pneumoniae resulted in HEK293 cells responsive to pneumococci, we examined the role of TLR2 and TLR4 in pneumococci-induced TNF-α release by periloneal macrophages isolated from wild-type, TLR2−/−/TLR4−/−, TLR2−/−/TLR4ΔΔ (C3H/HeJ), and TLR2−/−/TLR4Δ4 mice. Surprisingly, no significant differences in TNF-α release from macrophages of all four mice strains were detectable (Fig. 4b). Thus, experiments using HEK293 cells suggest involvement of TLR2 and TLR4, while application of macrophages from gene-targeted mice implicates the involvement of further pattern recognition receptors or combinations of pattern recognition receptors in mediation of immune responses to infection with S. pneumoniae that have not yet been identified.

Discussion
Recent work provided evidence that TLR2 plays a crucial role in the detection of microbial infection and in the activation of inflammatory and antimicrobial innate immune responses. In vitro, TLR2 was shown to respond to a variety of microbial products, such as lipoteichoic acid specific for Gram-positive bacteria, peptidoglycan particularly from Gram-positive bacteria, lipoprotein from bacteria such as Gram-negatives and particularly spirochetes, as well as from mycoplasma, and mycobacterial products such as lipoarabinomannan (for reviews, see Refs. 11, 12, 32, and 33). The function of TLR2 in cerebral innate immunity is still unclear. For example, neither mice deficient in TNF-α, both of its receptors, caspase-1, or iNOS, nor animals treated with matrix metalloproteinase inhibitors, iNOS inhibitors, or antioxidants showed any differences in CNS bacterial growth from infected wt littermates or infected, untreated animals (46–50). These observations supported the concept that the subarachnoid space is a localized area of host immunodeficiency allowing unrestrained proliferation of pneumococci which, if untreated, overwhelms the host until death occurs. This functional deficit is assumed to be due to the lack of sufficient Ig and complement concentrations to achieve opsonic and bactericidal activity (51, 52). In contrast, Tuomanen et al. (53) reported that complement depletion (by treatment with Egyptian cobra venom factor) resulted in a diminished opsonophagocytosis of encapsulated S. pneumoniae and consequently to increased bacterial titers in the CSF of rabbits with pneumococcal meningitis. Thus, complement factors (e.g., C3b) appear to mediate partial killing, although not clearance of S. pneumoniae from CSF. According to previous reports by Stahl et al. (54, 55), we observed a marked up-regulation of the brain C3 mRNA expression during meningitis. Brain C3 mRNA levels did not differ between infected TLR2−/− mice and wt littermates. However, TLR2 deficiency was accompanied by an increased brain expression of Crry. Murine Crry is a known inhibitor of the activation of the third component of complement (56). Combined, it seems conceivable that the higher cerebellar bacterial titers in TLR2−/− mice correlate with increased brain Crry expression rather than with the slightly reduced initial host immune response, which was evidenced by decreased brain expression of TNF-α and MIP-2 in infected TLR2−/− mice at 4 h postchallenge.

In our study, we also detected a (slight) increase in both spleen MIP-2 and iNOS mRNA and lung TNF-α expression in TLR2−/− mice during early meningitis. This was associated with a marked increase in blood bacterial titers. Previous studies have clearly demonstrated that secondary bacteremia is a common occurrence of disease observed in infected TLR2−/− mice was associated with significantly higher bacterial titers in the CNS. Previous studies in animal models of pneumococcal meningitis have shown that the bacterial inoculum size/bacterial titer is an important factor predetermining severity of the disease (37, 38). The pathologic alterations (e.g., BBB disruption or neuronal cell death) in S. pneumoniae-induced meningitis are suggested to be a two-pronged problem. On the one hand, the host inflammatory response to the pathogen was found to contribute substantially to the development of CNS complications and the unfavorable clinical outcome (e.g., Refs. 17, 39, and 40). In contrast, pneumococcal toxins such as pneumolysin and hydrogen peroxide were reported to act as direct inducers of cell death and as immune activators in vitro and in vivo (41–43). Our observation that, in established meningitis (24 h postchallenge), the immune response did not differ between TLR2−/− mice and wt littermates, hints at higher pneumococcal toxin concentrations as a major factor for deterioration to more severe CNS alterations and clinical symptoms in TLR2−/− mice. Moreover, the increased release of bacterial toxins due to an increased bacterial burden as observed in infected TLR2−/− mice may also contribute to the abrogation of the potential anti-inflammatory properties exerted by TLR2 deficiency (13). Both toxins can nonspecifically induce the production of different inflammatory mediators such as cytokines (44, 45).

Our observation that TLR2 deficiency was associated with higher cerebellar bacterial titers is remarkable considering that, in numerous animal studies, pneumococcal growth in the CNS remained unaltered by “anti-inflammatory” treatment strategies or by targeted disruption of genes involved in the inflammatory cascade. For example, neither mice deficient in TNF-α, both of its receptors, caspase-1, or iNOS, nor animals treated with matrix metalloproteinase inhibitors, iNOS inhibitors, or antioxidants showed any differences in CNS bacterial growth from infected wt littermates or infected, untreated animals (46–50). These observations supported the concept that the subarachnoid space is a localized area of host immunodeficiency allowing unrestrained proliferation of pneumococci which, if untreated, overwhelms the host until death occurs. This functional deficit is assumed to be due to the lack of sufficient Ig and complement concentrations to achieve opsonic and bactericidal activity (51, 52). In contrast, Tuomanen et al. (53) reported that complement depletion (by treatment with Egyptian cobra venom factor) resulted in a diminished opsonophagocytosis of encapsulated S. pneumoniae and consequently to increased bacterial titers in the CSF of rabbits with pneumococcal meningitis. Thus, complement factors (e.g., C3b) appear to mediate partial killing, although not clearance of S. pneumoniae from CSF. According to previous reports by Stahl et al. (54, 55), we observed a marked up-regulation of the brain C3 mRNA expression during meningitis. Brain C3 mRNA levels did not differ between infected TLR2−/− mice and wt littermates. However, TLR2 deficiency was accompanied by an increased brain expression of Crry. Murine Crry is a known inhibitor of the activation of the third component of complement (56). Combined, it seems conceivable that the higher cerebellar bacterial titers in TLR2−/− mice correlate with increased brain Crry expression rather than with the slightly reduced initial host immune response, which was evidenced by decreased brain expression of TNF-α and MIP-2 in infected TLR2−/− mice at 4 h postchallenge.
in meningitis, is directly related to the concentration of the microorganisms within the CSF, and is dependent on active bacterial multiplication within the subarachnoid space (57). Therefore, it is conceivable that the higher brain bacterial titers lead to higher concentrations of \emph{S. pneumoniae} in the blood, which in turn induces a more pronounced initial immune response in TLR2–/– mice.

In conclusion, our study showed that TLR2 plays a significant role in a murine model of experimental pneumococcal meningitis. Nonetheless, a robust immune response was established in TLR2–/– mice upon infection, pointing at TLR2-independent cellular recognition of \emph{S. pneumoniae} cell-wall products. Ectopic expression-dependent cell activation implicates TLR4 as another potential candidate for sensing pneumococcal infection. However, its role in vivo remains to be analyzed, and further pattern recognition receptors remain to be implicated in recognition of pneumococcal infection of the host organism.

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References


