Innate Immunity to Pneumococcal Infection of the Central Nervous System Depends on Toll-Like Receptor (TLR) 2 and TLR4

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Background. Recent studies have suggested that, in addition to Toll-like receptor (TLR) 2, other pattern recognition receptors mediate activation of the immune response after infection of the central nervous system (CNS) with Streptococcus pneumoniae (SP).

Methods. Using a mouse meningitis model, we investigated the influence of TLR4 single deficiency (TLR4/H11002/H11002), TLR2/TLR4 double deficiency (TLR2/4/H11002/H11002), and TLR2/TLR4/TLR9 triple deficiency (TLR2/4/9/H11002/H11002) on the immune response of the CNS to SP infection. To identify the cell populations that mediate the responses to SP, we generated TLR2/4/H11002/H11002–wild-type (wt) bone marrow (BM) chimeras.

Results. Compared with infected wt mice, infected TLR2/4/H11002/H11002 and TLR2/4/9/H11002/H11002 mice had similar reductions in brain cytokine levels, pleocytosis, and cerebral pathologic findings, whereas no such effect was noted in infected TLR4/H11002/H11002 mice. The attenuated immune response was paralleled by an impaired host defense that resulted in worsening of disease. Analysis of the chimeric mice after infection showed that mere TLR2/4 deficiency, either of radioresistant cells or of transplanted BM-derived cells, was sufficient to mount a substantial cerebral immune response, such as that noted in wt mice.

Conclusion. In murine SP meningitis, TLR2 and TLR4 expressed on radioresistant and transplanted BM-derived cells were major cellular sensors of invading SP inducing inflammatory responses.

Despite targeted antibiotic therapy, adjunctive treatment with dexamethasone, and supportive intensive care, pneumococcal meningitis is fatal in 15%–33% of affected patients [1, 2]. The poor prognosis of pneumococcal meningitis is, in part, due to the inability of the cerebral immune response to efficiently control and clear Streptococcus pneumoniae (SP) from the central nervous system (CNS) [3, 4]. Several reasons might account for this “local immunodeficiency,” including (1) a low number of antigen-presenting cells, (2) the near absence of opsonizing components, and (3) the prevention of intracerebral influx of most blood components as a result of the blood-cerebrospinal fluid (CSF) barrier excluding the CSF space from the blood. Consequently, once SP reaches the CSF, its replication is barely restricted. Subsequently, high amounts of proinflammatory bacterial components are released at the time of autolysis. In pneumococcal meningitis, subsequent pattern recognition results in an extraordinarily strong inflammatory response, which contributes to intracranial complications and tissue damage [3, 4]. In addition, specific bacterial toxins, such as pneumolysin, are released that most likely further aggravate the cerebral damage [5–7].

Recently, we demonstrated a central role for MyD88 in the host immune response to pneumococcal meningitis [8, 9]. MyD88 is a signal-transducing adapter molecule recruited by interleukin (IL)–1 type cytokine receptors and most Toll-like receptors (TLRs) at the time of ligand binding. To date, only TLR2 has been shown to
be involved in immune activation in murine pneumococcal meningitis [10–12]. However, the phenotype of intracisternally infected TLR2−/− mice is relatively mild: despite reduced bacterial clearance in TLR2-deficient (TLR2−/−) mice, the immune response of TLR2−/− and wild-type (wt) mice differed during the early stage of the disease (4 h after infection) only but not during established meningitis (24 h after infection) [10]. In contrast, the host immune response to intracisternal pneumococcal infection was almost ablated in infected MyD88−/− mice [8]. This finding suggests the involvement of additional TLRs as pattern recognition receptors for SP sensing by the host during meningitis. Results of recent studies have indicated that TLR4 and TLR9 are possible candidates. Although peptidoglycan, lipoteichoic acid, and lipoproteins have been implicated as TLR2 ligands [13, 14], pneumococcal pneumolysin and DNA were shown to be sensed through TLR4 and TLR9, respectively [10, 15–17]. Mouse studies revealed that, in addition to TLR2 [18], TLR4 or TLR9 is also necessary to systemically activate the inflammatory cascade during pneumococcal infection in vivo [16, 19, 20]. However, these findings depended on the application of experimental models of primary intraperitoneal or intranasal infection, but not CNS infection, with its specific characteristics.

In the present study, we evaluated the influence of TLR2/TLR4 double deficiency (TLR2/4−/−) and TLR2/TLR4/TLR9 triple deficiency (TLR2/4/9−/−) in our mouse model of pneumococcal meningitis. To determine whether resident brain cells or infiltrating hematopoietic cells contribute to immune activation in meningitis, we also studied infected bone marrow (BM) chimeras in which TLR2 and TLR4 expression was limited to either the radioresistant or radiosensitive compartments of the host.

MATERIALS AND METHODS

TLR signaling response to SP in vitro. Peritoneal TLR2/4−/− and TLR2/4/9−/− macrophages were prepared as described elsewhere [10]. In brief, mice were injected intraperitoneally with 2 mL of 4% thioglycolate in PBS. After 5 days, peritoneal exudate cells were collected by washing the peritoneal cavity with PBS supplemented with 2% fetal calf serum and were incubated for 1 h under standard cell culture conditions in Dulbecco’s modified eagle medium supplemented with 10% fetal calf serum, standard penicillin/streptomycin, 10 μmol/L monothioglycoler, and 20 ng/mL murine interferon (IFN)–γ (all from Sigma). Subsequently, macrophages were stimulated with ethanol-inactivated SP (1.7 × 10^6 cfu/mL) in penicillin/streptomycin-free medium. Twenty-four h later, cell culture supernatants were sampled and analyzed for IL-6 and tumor necrosis factor (TNF)–α content by use of ELISA (R&D Systems). These experiments were run on 2 days with different collections of peritoneal macrophages.

Experimental pneumococcal meningitis. A well-described mouse model that closely mimics the typical characteristics of pneumococcal meningitis was used [21, 22]. In brief, mice were weighed and clinically examined by a blinded observer. The clinical score [22–24] resulted from the evaluation of body temperature (assigned 0–2 points), spontaneous motor activity (0–3 points), proprioception (0–2 points), capacity of beam balancing (0–3 points), postural reflexes (0–3 points), weight loss (0–2 points), and vigilance, tremor, piloerection, and seizures (1 point each). A score of 0 was assigned to healthy animals. Animals that died within the observation period were assigned a score of 20.

Animals were infected by transcutaneous injection of 15 μL of a suspension containing 1 × 10^7 cfu/mL of SP type 3 into the cisterna magna while they were under halothane anesthesia. Twenty-four h after infection, the clinical score was reassessed. Next, mice were anesthetized by intraperitoneal application of 100 mg/kg ketamine and 5 mg/kg xylazine, so that a catheter could be placed into the cisterna magna. A CSF sample was obtained and evaluated for pleocytosis (by determination of the white blood cell [WBC] count). After receiving a lethal injection of thiopental, animals were perfused transcardially with 15 mL of PBS. The brain of each animal was removed and frozen immediately. All animal experiments were approved by the government of Upper Bavaria, Germany, according to the recommendations of an independent commission.

Bacterial titers. The cerebellum was dissected and homogenized in 1 mL of sterile PBS. Cerebellar homogenates and blood samples were diluted serially, plated on blood agar plates, and cultured for 24 h at 37°C in an atmosphere containing 5% CO₂.

Blood-brain barrier integrity, cerebral bleeding, and hydrocephalus. The albumin content in brain homogenates was measured by ELISA to assess blood-brain barrier integrity, as described elsewhere [8]. Cerebral bleeding and hydrocephalus were evaluated by measurement of the area of the brain, ventricles, and bleeding spots in serial sections, followed by mathematical calculation of the respective volumes of these areas [22].

Cytokine expression. Concentrations of the following 14 cytokines and chemokines in mouse brains were determined using a custom-made mouse cytokine antibody array (RayBiotech) [22]: IL-6, IL-12p40p70, keratinocyte chemoattractant (CXCL-1), macrophage inflammatory protein (MIP)–1γ (chemokine CC ligand [CCL]–9), granulocyte–colony-stimulating factor (G-CSF), macrophage–colony-stimulating factor (M-CSF), monocyte chemoattractant protein (MCP)–1 (CCL-2), MCP-5 (CCL-12), CXCL9, CXCL4 (platelet factor 4), stem cell factor, CXCL16, Axl, and L-selectin. Cytokine levels in the brains of TLR2/4−/− and TLR2/4/9−/− mice were compared with cytokine levels in the brains of infected wt mice, by calculating the ratio of both optical densities (OD). An OD ratio >2 denotes higher cytokine levels, and an OD ratio <0.5 denotes lower cytokine levels. Furthermore, IL-1β and L-selectin protein content tests were assessed quantitatively by ELISA (R&D Systems).
Experimental groups. For in vivo analysis, TLR2/4−/− (n = 12), TLR2/4/9−/− (n = 11), and C57BL/6 (wt) mice (n = 21) that were challenged intracisternally with pneumococci were compared. TLR2/4−/− mice were backcrossed 9 times, whereas TLR2/4/9−/− mice were backcrossed onto the C57BL/6 background for 6 generations. To assess the role of TLR4 alone, additional experiments were conducted using TLR4-deficient mice (n = 6; backcrossed onto the C57BL/6 background 9 times). Four wt animals (“uninfected controls”) received an intracisternal injection of 15 μL of sterile PBS and subsequently underwent the same procedures as did infected animals.

To distinguish the roles of radiosensitive brain cells and radiosensitive hematopoietic cells in the recognition of pneumococci at the time of invasion, BM chimeric mice were used. BM chimeric mice were generated as described elsewhere [25]. Eight-week-old wt or knockout mice were exposed to a Cesium-137 beam source (Amersham) to receive a radiation dose of 900 cGy 24 h before supplementation with the BM of untreated mice. BM cells (5 × 10⁶ cells per recipient mouse) derived from the tibiae and femurs of either wt or knockout mice were injected into the tail veins (i.e., intravenously) of recipients (wt>wt [n = 6], wt>TLR2/4−/− [n = 7], TLR2/4−/−>wt [n = 6], and TLR2/4−/−>TLR2/4−/− [n = 5]). Six weeks after grafting, reconstitution was assessed, by means of a whole-blood bioassay. Therefore, blood samples were obtained from 2 mice in each group by means of retrobulbar bleeding [26]. A total of 100 μL of whole blood was blended with 100 μL of RPMI 1640 medium with or without 100 ng/mL Escherichia coli 0111:B4 lipopolysaccharide (LPS; Sigma). After incubation for 16 h, supernatants were sampled and assayed for IL-6 containment by use of ELISA (R&D Systems). Challenge with LPS induced the release of IL-6 from the whole blood of wt>wt and wt>TLR2/4−/− mice but not from the blood cells of TLR2/4−/−>wt and TLR2/4−/−>TLR2/4−/− mice, compared with controls (unchallenged mice) (data not shown). Chimeric mice were used for experiments performed 8 weeks after radiation.

Statistical analysis. The designated sample size was calculated using PS Power and Sample Size Calculations Software (version 2.1.30; Department of Biostatistics, Vanderbilt University), focusing on the CSF WBC count (α = 0.05; power, 0.8), on the basis of observations published elsewhere [22]. For calculation of the sample size for BM chimera experiments, results from infected wt and TLR2/4−/− mice were used. Data were analyzed using SYSTAT 9 software (SPSS) with the application of analysis of variance and an unpaired Student’s t-test. Alpha adjustment was used for multiple comparisons and sample size calculations. Data are expressed as the mean value ± SD.

RESULTS

Detection of SP by macrophages through TLR2, TLR4, and TLR9. Challenge with ethanol-inactivated SP induced the release of TNF-α and IL-6 from wt macrophages (TNF-α, 2.45 ± 0.04 ng/mL; IL-6, 6.02 ± 0.04 ng/mL), which was not observed for unchallenged macrophages of any of the 3 genotypes analyzed. Release from macrophages of TLR2/4−/− mice after pneumococcal challenge was reduced (TNF-α, 1.77 ± 0.08 ng/mL [P = .009, if related to wt]; IL-6, 2.61 ± 0.12 ng/mL [P < .001, if related to wt]), whereas release from macrophages of challenged TLR2/4/9−/− mice was barely detectable (TNF-α, 0.60 ± 0.11 ng/mL [P = .002, if related to wt]; IL-6, 0.11 ± 0.00 ng/mL [P < .001, if related to wt]), compared with release from cells from wt mice.

Increased susceptibility to SP displayed by TLR2/4−/− and TLR2/4/9−/− mice. All animals that were intracisternally challenged with SP developed clinical signs of infection. TLR2/4−/− and TLR2/4/9−/− mice were affected more intensely than were infected wt control animals, as was documented by their significantly higher clinical scores (for TLR2/4−/− vs. wt, P = .027; for TLR2/4/9−/− vs. wt, P = .002) (figure 1A). The worsening of the clinical outcome of mice lacking TLR2 and TLR4 (and TLR9) was associated with a reduction in pleocytosis (for TLR2/4−/− vs. wt, P = .027; for TLR2/4/9−/− vs. wt, P = .002) (figure 1B) and impaired bacterial killing (as determined by evaluation of cerebellar and blood bacterial titers; for TLR2/4−/− mice vs. wt, P = .016 and P < .001, respectively; for TLR2/4/9−/− vs. wt mice, P = .007 and P = .004, respectively) (figure 1C and 1D). Contrasting with the results of in vitro analysis, differences in susceptibilities to intracisternal pneumococcal infection in TLR2/4−/− and TLR2/4/9−/− mice were not detected (figures 1A–D). A TLR4 single deficiency was not associated with a significant worsening of disease (clinical score for TLR4−/− mice vs. wt mice, 12.2 ± 4.9 vs. 11.0 ± 1.4; P = .80), a reduction in pleocytosis (for TLR4−/− mice vs. wt mice, 10,704 ± 2,873 vs. 13,466 ± 1,454 cells/μL; P = .18), or an increase in cerebellar bacterial titers (for TLR4−/− mice vs. wt mice, 6.57 ± 0.84 vs. 6.86 ± 0.39 log cfu per cerebellum; P = .70).

Reduction in intracranial complications in infected TLR2/4−/− and TLR2/4/9−/− mice. Breaking of the blood-brain barrier in infected animals, as indicated by an increased cerebral albumin content, was reduced in both TLR2/4−/− and TLR2/4/9−/− mice, compared with infected wt controls (cerebral albumin content: in uninfected wt mice, 14 ± 5 ng/mL; in infected wt mice vs. infected TLR2/4−/− mice, 180 ± 126 ng/mL vs. 36 ± 15 ng/mL [P < .004]; in infected wt mice vs. infected TLR2/4/9−/− mice, 80 ± 46 ng/mL [P = .02]). Accordingly, both infected TLR2/4−/− and TLR2/4/9−/− animals developed less brain edema than did wt animals, as was indicated by their smaller brain volumes (for TLR2/4−/− vs. wt mice, P = .027; for TLR2/4/9−/− vs. wt mice, P = .002) (figure 2B). Moreover, a tendency was observed for TLR2/4−/− and TLR2/4/9−/− animals to have an increase in ventricle volume (an indicator of hydrocephalus formation) smaller than that in infected wt controls.
intracranial pneumonia, and impairments in clearance were not observed (figure 2A, B, E, F). This was also evident from the markedly reduced bleeding volumes noted (figure 2C–F). Again, significant differences between TLR2/4−/− and TLR2/4/9−/− animals, with respect to the parameters analyzed, were not observed (figure 3). Reduction in cerebral cytokine production in infected TLR2/4−/− and TLR2/4/9−/− mice. The following proteins were induced in the brains of infected wt animals but not in uninfected wt mice (as measured by protein array analysis): IL-6, keratinocyte chemoattractant, G-CSF, MCP-1, MCP-5, CXCL9, Axl, and L-selectin. IL-12, CXCL4, and CXCL16, which were already present in uninfected wt mice were further up-regulated in infected brains. In infected animals lacking either TLR2 and TLR4 or TLR2, TLR4, and TLR9, elevation of CXCL4, CXCL16, and L-selectin levels was reduced, compared with the levels noted in the brains of infected wt mice. The data from the protein array analysis were confirmed by analysis of L-selectin expression by ELISA (figure 3A). Also, less IL-1β was present in the brain homogenates of infected TLR2/4−/− and TLR2/4/9−/− mice than in those of infected wt controls (figure 3B). However, the levels of CXCL4, CXCL16, L-selectin, and IL-1β in the brains of TLR2/4−/− and TLR2/4/9−/− mice did not differ to a substantial degree.

Triggering of the immune response toward SP by TLR2/TLR4 in either radioresistant or radiosensitive cells. With respect to all parameters analyzed (e.g., pleocytosis, bacterial killing, and cytokine levels), the responsiveness of mice lacking TLR2 and TLR4 in their radioresistant, nonhematopoietic cells (wt>TLR2/4−/−) or in radiosensitive, hematopoietic cells only (TLR2/4−/−>wt) did not differ from that of wt>wt chimeras to intracranial pneumococcal infection. However, infected TLR2/4−/−>TLR2/4−/− chimeras developed markedly reduced pleocytosis, compared with infected wt>wt chimeric mice (TLR2/4−/−>TLR2/4−/− mice vs. wt>wt mice, 9080 ± 1378 vs. 17,222 ± 2353 cells/μL [P = .01]). This finding resulted in impaired bacterial clearance (data not shown). Similarly, only the brains of infected TLR2/4−/−>TLR2/4−/− mice but not those of wt>TLR2/4−/− or TLR2/4−/−>wt mice, contained lower levels of IL-1β, MIP-2, and L-selectin than did the brains of infected wt>wt mice (figure 4A) (data not shown). Also, intracranial complications like intracranial bleeding and hydrocephalus were reduced only in TLR2/4−/−>TLR2/4−/− mice but not in wt>TLR2/4−/− or TLR2/4−/−>wt chimeras (figure 4B–F).
DISCUSSION

In the present study, we investigated the involvement of TLR4 and/or TLR9, in addition to that of TLR2, in the creation of beneficial and/or adverse immune responses to cerebral pneumococcal infection. Although a role for TLR9 in the cellular recognition of SP was implied by the results of in vitro analysis, TLR2/TLR4 double deficiency and TLR2/TLR4/TLR9 triple deficiency led to equal reductions in the reactivity to pneumococcal meningitis in vivo. The presence of TLR2 and TLR4 in either radioresistant brain cells or radiosensitive hematopoietic cells alone was sufficient to trigger the immune response in our mouse model of pneumococcal meningitis.

Numerous studies have assessed mediation of the signals that initiate activation of the host innate immune system in response to SP infection. NF-κB–dependent activation of human embryonic kidney cells that overexpress TLRs on pneumococcal infection and subsequent application of ceftriaxone depended on TLR2 and TLR4 but not TLR9 [10]. However, TLR9 has been found to mediate cellular recognition of heat-inactivated pneumococci in vitro [15]. This finding is in accord with our observation of severely reduced responsiveness of TLR2/4/9−/− macrophages but only gradually reduced responsiveness of TLR2/4−/− macrophages to challenge with ethanol-killed SP. Of note, ethanol-inactivated pneumococci have been shown to elicit TLR4 signaling [16], whereas heat-inactivated pneumococci have failed to do so [15]. Antibiotic treatment, heat inactivation, and ethanol inactivation might lead to distinctive patterns of liberation of pathogen-associated molecular patterns (PAMPs) from intact bacteria and, thus, to varying stimulatory capacities. Indeed, it has been shown that heat inactivation of bacteria can lead to unwanted inactivation of PAMPs [27], such as a TLR4 ligand, possibly pneumolysin [16]. Also, the pneumococ-

Figure 2. Attenuation of intracerebral complications in mice with pneumococcal meningitis that are lacking Toll-like receptor (TLR) 2 and TLR4. Twenty-four h after infection, mice of the genotypes indicated were analyzed for blood-brain barrier destruction (A) and brain edema development (B), as well as for intracerebral bleeding (C–F). The arrow denoted uninfected wild-type (wt) mice as negative controls. TLR2/4−/−, TLR2/TLR4 double deficiency; TLR2/4/9−/−, TLR2/TLR4/TLR9 triple deficiency. *P < .05.
Homogenized brains were analyzed by ELISA for detection of L-selectin wt, deficiency; [10], early during the disease (4 h after infection), intracisternal pneumococcal meningitis in vivo? As has been shown elsewhere intracisternally infected with pneumococci can activate cells through TLR2, TLR4, and TLR9 in vitro. These results demonstrate that pneumococci can activate additional cellular pneumococci sensors [30].

Using BM chimeric mice, we addressed the question of which cell population detects pneumococci and triggers inflammation in meningitis. The first possibility is that radiosensitive brain parenchyma cells may contribute to the detection of pathogens in cerebral infections. Expression of the mRNA of several TLRs, nally infected TLR2−/− mice expressed brain levels of inflammatory cytokines that were lower than those expressed by wt mice. Also, in a mouse model of intracerebral pneumococcal infection, TLR2 played an important role in the regulation of cerebral immunity [11, 12, 29]. However, later during the disease (24 h after infection), clinical score, pleocytosis, bacterial titers, blood-brain barrier damage, and cytokine release did not differ between infected TLR2−/− animals and infected wt control animals, whereas MyD88−/− animals were prone to bacterial overgrowth resulting from diminished inflammation [8, 10]. These results suggest the involvement of additional pattern recognition receptors. In this article, we provide clear evidence that, in addition to TLR2, TLR4 also contributes to pneumococcal detection in meningitis. Compared with wt mice, TLR2/4−/− mice displayed less pleocytosis and reduced expression of inflammatory cytokines in advanced pneumococcal infection (24 h after infection). The reduced cerebral inflammation noted in TLR2/4−/− mice was associated with a substantial reduction in intracranial complications, such as blood-brain barrier damage, brain edema formation, hydrocephalus, and intracerebral bleeding. Furthermore, the power of TLR2/4−/− mice to clear bacteria from the CSF was severely reduced, compared with that of wt mice. Associated was a significant increase in blood bacterial titers, which was reflected in an elevation of the clinical score. These findings imply that TLR4 is a host sensor of SP, in addition to TLR2. Moreover, our data suggest that TLR2 can compensate for TLR4 deficiency, and vice versa, because the phenotypes of TLR2−/− and TLR4−/− mice did not differ from those of wt mice at 24 h after infection [10].

In our model of pneumococcal meningitis, the immune system of TLR2/4−/− mice was still active to a stronger degree (with a 50% reduction in the CSF WBC count in TLR2/4−/− mice vs. wt mice) than that of MyD88−/− mice (with an 80% reduction in the CSF WBC count in MyD88−/− mice vs. wt mice [8]). Surprisingly, in our in vivo analyses did not imply that TLR9 was an additionally important receptor, because the phenotypes of TLR2−/− and TLR4−/− mice did not differ (not even a trend toward a difference was observed). The missing effect of additional TLR9 deficiency in vivo might be the result of the release of only limited amounts of bacterial DNA released by live pneumococci. Also, it could be that TLR9 is not expressed in the cell population that detects pneumococci in the brain. However, the current data set does not provide evidence for one or the other possibility. Aside from other TLRs, intracellular pattern recognition receptors, such as those belonging to nucleotide-binding oligomerization domain (Nod) proteins, are candidates for additional TLR9 deficiency in vivo might be the result of the release of only limited amounts of bacterial DNA released by live pneumococci. Also, it could be that TLR9 is not expressed in the cell population that detects pneumococci in the brain. However, the current data set does not provide evidence for one or the other possibility. Aside from other TLRs, intracellular pattern recognition receptors, such as those belonging to nucleotide-binding oligomerization domain (Nod) proteins, are candidates for additional TLR9 deficiency in vivo might be the result of the release of only limited amounts of bacterial DNA released by live pneumococci.
including TLR4, in the brains of uninfected mice has been reported [31–33], and it has been shown that microglial cells can be activated by TLR2 and TLR4 agonists in vitro [34]. In the present study, a specific lack of TLR2 and TLR4 in radioresistant cells did not lead to an impaired cerebral immune response. This finding argues against a major role for resident brain parenchyma cells in TLR2- and TLR4-dependent activation of the immune system during pneumococcal meningitis. A second possibility is that pneumococci are detected mainly in the bloodstream by radiosensitive BM-derived cells. However, mice lacking TLR2 and TLR4 only in blood cells were still able to establish a robust cerebral inflammatory response. The third possibility is that, in meningitis, pneumococci are detected by perivascular and leptomeningeal macrophages, which migrate from the bloodstream into the perivascular space. It is known that, 6 weeks after reconstitution, only ~30% of perivascular cells are replaced by donor cells [35]. Thus, in our chimeric mice, at the time of infection, this compartment consisted of both reconstituted, immigrated BM-derived cells and resident perivascular cells that survived radiation [35]. All mixed chimeras established a cerebral inflammatory reaction similar to that of infected wt mice. Only mice lacking TLR2 and TLR4 in both radioresistant and radiosensitive cells failed to develop a robust immune response. In conclusion, our findings imply that a reduced number of perivascular and leptomeningeal macrophages is sufficient to activate the immune system after pneumococcal infection of the subarachnoid space.

The results of the present study show that both TLR2 and TLR4 are important in initiating the immune response at the time of pneumococcal invasion of the subarachnoid space. Administration of antibiotics is implicated in aggravating antigen liberation and inflammation. Thus, it is possible that blockage of

**Figure 4.** Association of Toll-like receptor (TLR) 2 and TLR4 expression either in resident brain cells or on invading bone marrow–derived cells with full immunoreactivity to pneumococcal meningitis in the absence of dual TLR expression from the opposed compartment. Chimeric mice of the genotypes indicated were infected and analyzed after 24 h for brain levels of interleukin (IL)–1β (A), bleeding (B), and brain pathologic findings (C–F). TLR2/4−/−, TLR2/TLR4 double deficiency; wt, wild type. *P < .05.
TLRs (e.g., by antibodies) could lead to a reduction in the inflammatory burst. The data from this study suggest that only simultaneous blockage of TLR2 and TLR4 could have such an effect. However, further experimental studies focusing on adjunctive therapy are required to answer this question. Patients with pneumococcal meningitis currently are treated with adjunctive corticosteroid therapy, which has been shown to reduce case-fatality rates. In vitro experiments provide the first evidence that corticosteroids can increase the expression of TLR2 and TLR4 [36, 37], but they also impair TLR signaling during challenge with various stimulants [38, 39]. Thus, investigations of whether the benefit of treatment with dexamethasone is mediated, in part, through interference with TLR-mediated immune activation would be interesting.

Our results show that the immune response of TLR2/4−/− mice to intracisternal pneumococcal infection was more severely impaired than that of mice lacking 1 of the 2 TLRs alone. Thus, TLR4 is a major SP sensor, like TLR2. In contrast to our in vitro data implicating TLR9, the susceptibility of TLR2/4/9−/− and TLR2/4−/− mice to pneumococcal meningitis was similar, negating a central role for TLR9 in recognition of SP in vivo. Of note, our data suggest the importance of perivascular and leptomeningeal cells as mediators of the cerebral inflammatory reactivity to pneumococcal meningitis, which depends on expression of TLR2 and TLR4.

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References


