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TLR2 Mediates Neuroinflammation and Neuronal Damage

Olaf Hoffmann,* Johann S. Braun,* Doreen Becker,* Annett Halle,* Dorette Freyer,* Emilie Dagand,* Seija Lehhardt,† and Joerg R. Weber,*+†

Innate immunity relies on pattern recognition receptors to detect the presence of infectious pathogens. In the case of Gram-positive bacteria, binding of bacterial lipopeptides to TLR2 is currently regarded as an important mechanism. In the present study, we used the synthetic bacterial lipopeptide Pam3CysSk4, a selective TLR2 agonist, to induce meningeal inflammation in rodents. In a 6-h rat model, intrathecral application of Pam3CysSk4 caused influx of leukocytes into the cerebrospinal fluid (CSF) and induced a marked increase of regional cerebral blood flow and intracranial pressure. In wild-type mice, we observed CSF pleocytosis and an increased number of apoptotic neurons in the dentate gyrus 24 h after intrathecal challenge. Inflammation and associated neuronal loss were absent in TLR2 knockout mice. In purified neurons, cytotoxicity of Pam3CysSk4 itself was not observed. Exposure of microglia to Pam3CysSk4 induced neurotoxic properties in the supernatant of wild-type, but not TLR2-deficient microglia. We conclude that TLR2-mediated signaling is sufficient to induce the host-dependent key features of acute bacterial meningitis. Therefore, synthetic lipopeptides are a highly specific tool to study mechanisms of TLR2-driven neurodegeneration in vivo. The Journal of Immunology, 2007, 178: 6476–6481.

The innate immune system recognizes bacterial molecules and generates an inflammatory response to invading pathogens. In recent years, important progress has been made in understanding how specific receptors of the immune system recognize pathogen-associated molecular patterns to induce immune response (1, 2). A highly relevant class of pattern recognition receptors is the family of TLRs, of which 12 have been found in mammals (2). In the case of Gram-positive bacteria, components of the bacterial cell wall interact with TLR2. Although the exact molecular motif required for binding has not been identified, it appears that bacterial lipopeptides and teichoicated peptidoglycans (PGs)4 are the major ligands. Controversy exists whether TLR2 is also capable of sensing lipoteichoic acid-depleted PG. However, other components of Gram-positive bacteria may be recognized by other TLRs, e.g., bacterial nucleic acids via TLR9, or they may bind to non-TLRs, e.g., the muramyl dipeptide of PGs to NOD2 receptors (3, 4). Diversity at the level of the intracellular adaptor proteins adds further complexity. Most of the evidence discussed above was gathered in vitro, mostly involving overexpressing systems, which may not truly model the in vivo situation. Indeed, experiments in TLR2 knockout mice and several infection models often failed to show a pronounced anti-inflammatory phenotype, suggesting redundancy in microbial pattern recognition (5–9).

Lipoproteins are present in the cell wall of all bacteria and may represent a general principle for how bacteria induce an immune response and tissue damage. Other than the complex macromolecular structure of the cell wall backbone, the basic structure of lipopeptides, an unusual S-propyl-substituted cysteine carrying three lipid residues plus a variable amino acid chain, has been chemically synthesized. Synthetic lipopeptides allow us to study TLR2-driven inflammation without the confounding effects of contaminants such as bacterial toxins, metabolic products, or other cell wall components. In previous studies, the synthetic bacterial lipopeptide Pam3CysSk4 (N-palmitoyl-S-(2,3-bis(palmitoyloxy)-(2R,S)-propyl)-(R)-cysteinyl-seryl-(lysyl)3-lysine) has been identified as a specific TLR2 agonist (10) acting mainly through TLR2/1 heterodimeric receptors (11). In the present study, we used Pam3CysSk4 to study TLR2-driven inflammation and neuronal damage in experimental meningitis as an important example of invasive bacterial infection. For comparison with a viable pathogen, meningitis was induced in further animals by intrathecal inoculation with live encapsulated Streptococcus pneumoniae, the most common microorganism in bacterial meningitis.

Materials and Methods
Bacterial culture
D39, an encapsulated strain of S. pneumoniae serotype 2 originally derived from a clinical isolate, was grown in standard casein plus yeast medium with 5% CO2 at 37°C. During log phase growth, bacteria were pelleted by centrifugation and resuspended in pyrogen-free 0.1 M PBS. CFU per milliliter were then determined photometrically (absorption at 620 nm) using a standard curve. Adequate dilutions in pyrogen-free PBS were used to produce defined inocula. CFU calculations were verified by plating of serial dilutions.

Rat experiments
All animal experimental procedures were reviewed by institutional and state authorities. The general experimental procedure was as described earlier (12). The experiments were performed on male Wistar rats (280–350 g) anesthetized with i.p. thiopental sodium (Byk Gulden). A dose of 100 mg/kg was used for induction of anesthesia, while 20 mg/kg was given for maintenance at ~2-h intervals (total dose per animal, 160–180 mg/kg). Adequate anesthesia was verified by absence of response to a painful stimulus. Animals were tracheotomized and mechanically ventilated (model AP-10; K. Effenberger). End-tidal CO2 was monitored continuously (Heyer Ariema MM204). Body temperature was measured by a rectal probe and maintained at 37.8 ± 0.4°C using a heating pad. Mean arterial blood pressure was measured continuously by a transducer (Statham P109-EZ; Spectramed) connected to a catheter placed in the left femoral artery. From this
catheter, arterial blood samples were analyzed for PaO₂, PaCO₂, and pH at 0, 2, 4, and 6 h. The left femoral vein was cannulated for infusion of saline as a volume replacement. A 3 × 3-mm area of the parietal bone lateral to the sagittal suture was thinned to allow laser Doppler flow (LDF) measurements (Perifux 4001 Master; Perimed) in cortical blood vessels. A catheter was placed into the cisterna magna through an occipital burr hole and connected to a pressure transducer (Statham P1009-EZ; Spectramed) for continuous intracranial pressure (ICP) measurement. The 100 μl of cerebrospinal fluid (CSF) removed from this catheter was replaced with 100 μl of Pam3CysSK₄ (1 mg/ml; EMRC Microcollections) or with 100 μl of bacterial suspension containing 10⁵ CFU. Controls received 100 μl of endotoxin-free PBS. At the end of the experiment, CSF samples were obtained to determine the CSF leukocyte count. In experiments involving live bacteria, CFU in the CSF were determined by the plating of serial dilutions on blood agar plates. Animals were then killed by i.v. injection of 3 M potassium chloride to induce cardiac arrest.

Mouse model of meningitis

Mouse experiments were conducted using a previously published model (12, 13). In brief, male C57BL/6 mice (∼20 g) or TLR2−/− mice (14) were anesthetized with i.p. ketamine (100 mg/kg; DeltaSelect) and xylazine (20 mg/kg; Bayer). A skin incision was made to expose the lumbar spine. Using a 30-gauge needle and a microcatheter syringe, 40 μl of PBS, Pam3CysSK₄ solution or bacterial suspension containing 5 × 10⁵ CFU were slowly injected into the spinal canal at vertebræ L2 or L3. The skin incision was closed using dermal clips. Animals were then allowed to wake up and given free access to food and water. Ability of paresis and adequate waking were verified. After 24 h, the animals were again deeply anesthetized. To obtain a CSF sample, a skin incision was made over the head and neck. After dissection of the suboccipital muscles under a stereomicroscope, the cisterna magna was punctured and CSF withdrawn using a 27-gauge butterfly cannula connected to a microliter syringe. White blood cells were then counted in the CSF using a Fuchs-Rosenthal chamber using a 20X objective. The cisterna magna was thinned to allow laser Doppler flow (LDF) measurement, and the mean threshold cycle was used for analysis. All reactions were performed in duplicate, and the mean threshold cycle was used for analysis. The expression of each sample was normalized on the basis of its β-actin and GAPDH mRNA content. The following sequence-specific primers were used: TLR2 forward, 5'-GATATTCTGAGTTCGTGAG-3' and reverse, 5'-TTGATTACTCTTTATTAC-3'; β-actin forward, 5'-ACCACACGTGGCCATCTA-3' and reverse, 5'-GCCACAGATCCATACACCA-3'; and GAPDH forward, 5'-AGATGTGAGCCTGACTTCGTCG-3' and reverse, 5'-TCTTTAGTGTCACTGATCTGG-3'.

Immunocytochemistry for TLR2

Cells were fixed and immunostained as previously described (17). To identify neurons and microglia, cells were stained with the Ab microtubule-associated protein-2 (Chemicon International) or with IB4-Alexa (Invitrogen Life Technologies), respectively. The mouse TLR2 Ab 6C2 was obtained from eBioscience. Specificity of the TLR2 Ab was confirmed by the absence of signal in TLR2−/− microglia.

Effects of Pam₃CysSK₄ in cell culture

Primary rat neuronal cultures were challenged by addition of Pam₃CysSK₄ to the medium at a final concentration of 0.1 μg/ml. At 24, 48, and 72 h, cell death was assessed by the measurement of lactate dehydrogenase (LDH) in the supernatant, an indicator of cell death. Moreover, cells were stained with the intercalating dyes acridine orange and ethidium bromide (2 μg/ml) to identify neurons and microglia. Cells were fixed and immunostained as previously described (17). To identify neurons and microglia, cells were stained with the Ab microtubule-associated protein-2 (Chemicon International) or with IB4-Alexa (Invitrogen Life Technologies), respectively. The mouse TLR2 Ab 6C2 was obtained from eBioscience. Specificity of the TLR2 Ab was confirmed by the absence of signal in TLR2−/− microglia.

Statistics

Data are presented as mean ± SD. Two group comparisons were performed with Student’s t tests after ensuring normal distribution; otherwise, Mann-Whitney U tests were conducted. Multiple group comparisons on samples with normal distribution were performed with ANOVA followed by Student-Newman-Keuls post hoc testing. Otherwise, nonparametric ANOVA (Kruskal-Wallis) and Dunn’s post hoc analysis were used. The impact of TLR2 genotype on the survival rate of D39-challenged mice was examined using Fisher’s exact test.

Results

Intrathecal administration of Pam₃CysSK₄ induces the pathophysiological hallmarks of bacterial meningitis

In anesthetized rats (n = 3), intracisternal application of pyrogen-free PBS did not induce relevant changes of regional cerebral blood flow as measured by LDF or ICP during the experimental period of 6 h (Fig. 1, A–C). At the end of the experiment, LDF was
103 ± 13% of the individual baseline (Fig. 1A), and ICP was increased by 0.5 ± 1.5 cm H₂O (Fig. 1B). Leukocyte count in the CSF at 6 h was 25 ± 15 leukocytes/μl (Fig. 1C). In contrast, instillation of Pam₃CysSK₄ into the cisterna magna of anesthetized rats (n = 3) was followed by a gradual increase of ICP and regional cerebral blood flow. At 6 h after application, LDF was 155 ± 17% of the individual baseline (p < 0.05; Fig. 1A), whereas ICP was 11.9 ± 0.7 cm H₂O above baseline (p < 0.01; Fig. 1B). By comparison, inoculation with viable pneumococci resulted in a more pronounced increase of LDF (at the end of the experiment: 216 ± 23% vs 155 ± 17% of the individual baseline; p < 0.05 vs Pam₃CysSK₄ and p < 0.05 vs control), whereas the increase of ICP was not different between the two challenges (at 6 h: 12.3 ± 0.3 cm H₂O with D39 vs 11.9 ± 0.7 cm H₂O with Pam₃CysSK₄, p was not significant vs Pam₃CysSK₄ and p < 0.01 vs control). At the end of the experiment, the CSF contained 3278 ± 355 leukocytes/μl in Pam₃CysSK₄-challenged rats, whereas controls had 25 ± 15 leukocytes/μl (p < 0.01 vs control; Fig. 1C). In D39 infected rats, the CSF revealed 3984 ± 1656 leukocytes/μl (p < 0.01 vs control and p is not significant vs Pam₃CysSK₄) and a bacterial concentration of 5.4 ± 1.6 × 10⁶ CFU/ml. In all experimental groups, arterial blood gas analysis and invasive blood pressure monitoring revealed normal values throughout the entire experimental period (data not shown).

Pam₃CysSK₄ causes leukocyte influx into the CSF in wild-type but not in TLR2-deficient mice

Wild-type mice (n = 4) receiving intrathecal pyrogen-free PBS had 11 ± 2 leukocytes/μl CSF at 24 h after surgery. Intrathecal challenge with Pam₃CysSK₄ caused a dose-dependent influx of leukocytes into the CSF. At 24 h after application of 2 μg of Pam₃CysSK₄ per animal (n = 3), 2607 ± 349 leukocytes/μl were counted in the CSF, while a higher dose of 20 μg of Pam₃CysSK₄ per animal (n = 6) increased pleocytosis to 6741 ± 1969 leukocytes/μl (p < 0.05; Fig. 1D). The leukocyte influx was absent in TLR2−/− mice (n = 8) 24 h after application of 20 μg of Pam₃CysSK₄ (24 ± 35 leukocytes/μl, p < 0.05 vs wild-type mice) (Fig. 1E). Compared with the 24-h interval, a higher number of leukocytes (20913 ± 5667/μl) were detected at 12 h after application of 20 μg of Pam₃CysSK₄ in wild-type mice (n = 5; p < 0.05), whereas TLR2-deficient mice (n = 4) again showed no invasion of leukocytes (15 ± 7/μl; Fig. 1E). In meningitis induced by live D39 pneumococci, two of seven wild-type mice died within the experimental period compared with no deaths in six TLR2−/− mice (p was not significant using Fisher’s exact test). CSF was not available from the two wild-type mice that died. In the remaining wild-type mice, challenge with live pneumococci resulted in a similar CSF pleocytosis at 24 h as was observed with challenge by 20 μg of Pam₃CysSK₄ (5103 ± 2451 leukocytes/μl, n = 5; p was not significant vs Pam₃CysSK₄ and p < 0.01 vs control). Unlike Pam₃CysSK₄, D39 also caused leukocyte influx (2428 ± 830 leukocytes/μl) in TLR2-deficient mice (n = 6; p < 0.01). D39-induced pleocytosis was less pronounced in TLR2−/− than in wild-type mice (p = 0.05).

Pam₃CysSK₄ induces neuronal damage in a TLR2-dependent fashion in vivo

In the dentate gyrus, TUNEL-positive cells were found in all animals 24 h after challenge (Fig. 2). In wild-type control mice (n = 3), 73 ± 18 apoptotic cells/mm² were present following intrathecal application of pyrogen-free PBS. Meningitis induction with 20 μg of Pam₃CysSK₄ increased apoptotic cell number to 120 ± 18 mm² (n = 7; p < 0.05), whereas D39-induced meningitis resulted in 146 ± 40 apoptotic cells/mm² (n = 5; p < 0.05 vs control and p was not significant vs Pam₃CysSK₄). In TLR2−/− control mice (n = 3) receiving intrathecal PBS, 81 ± 3 apoptotic cells/mm² were present (p was not significant vs wild-type control mice). No significant increase of this number was observed following challenge in TLR2−/− control mice (n = 8) with Pam₃CysSK₄.
Studies of neurodegeneration in the murine dentate gyrus. A–D, Merged TUNEL (green) and Hoechst (chromatin, blue) staining of the dentate gyrus of wild-type mice (A and C) and TLR2−/− mice (B and D) 24 h after challenge with intrathecal Pam3CysSK4 (A and B) or live pneumococci (C and D). E, Quantitative analysis of TUNEL-positive nuclei in the dentate gyrus per area on multiple sections per animal. * p<0.05 determined by one-way ANOVA followed by Student-Newman-Keuls post hoc analysis.

(74±11 apoptotic cell/mm²) or with live pneumococci (92±15 apoptotic cell/mm²; n=5).

Expression of TLR2 is cell type-specific

In purified primary cultures from rat cortex, real-time PCR demonstrated minor levels of TLR2 mRNA in neurons or astrocytes with a 50- to 600-fold higher presence in microglia (Fig. 3A). Accordingly, immunocytochemistry revealed the presence of TLR2 Ag on microglial cells, but not on cortical neurons (Fig. 3, B–J).

Pam3CysSK4 does not induce neuronal cell death in vitro

In unchallenged cortical rat neurons, the proportion of apoptotic cells did not increase during the experimental period. We observed 32.3±8.7% apoptotic neurons at 24 h, 31.0±7.4% at 48 h, and 30.7±6.8% at 72 h (Fig. 4A). Accordingly, the concentration of LDH in the supernatant remained essentially unchanged with 10.1±3.0 U/ml at 24 h, 9.1±2.6 U/ml at 48 h, and 12.3±5.0 U/ml after 72 h (Fig. 4B). Challenge with 0.1 μg/ml Pam3CysSK4 did not result in a significant increase of apoptotic neurons (35.5±7.7% at 72 h; p=0.12 vs control at 72 h; Fig. 4A) or of LDH release into the supernatant (12.0±4.4 U/ml at 72 h; p=0.86 vs control at 72 h; Fig. 4B). Conversely, exposure to 0.5 μM staurosporine resulted in apoptosis of 97.6±2.1% of the neurons (p<0.01 vs control at 72 h; Fig. 4A) and a strong increase of LDH in the supernatant (38.3±10.9 U/ml; p<0.01 vs control at 72 h; Fig. 4B). Cells exhibiting a necrotic staining pattern were not observed in any of the cultures.

Next, primary mouse microglia was stimulated with 0.1 μg/ml Pam3CysSK4 (Fig. 4, C and D). Supernatant was collected at 6, 24, 48 or 48 h and used to challenge cortical neurons as described (Fig. 4C). Supernatant from unstimulated microglia was collected at identical times to be used as a control. Supernatant from microglia after 6 h of incubation with Pam3CysSK4 did not induce an increase in LDH release from neurons compared with unstimulated microglia. LDH concentrations after incubation with “stimulated” vs “unstimulated” supernatant were 53.2±17.9 U/ml vs 52.3±16.2 U/ml at 24 h, p=0.89; 63.8±37.3 U/ml vs 64.1±33.7 U/ml at 48 h, p=0.98; and 80.5±37.5 U/ml vs 69.1±37.0 U/ml at 72 h, p=0.46. Supernatant from microglia stimulated for 24 h with Pam3CysSK4 induced a time-dependent LDH release into the neuronal culture medium (incubation of neurons for 24 h: 64.1±13.7 U/ml; 48 h: 78.4±31.1 U/ml; 72 h: 89.8±37.1 U/ml) that was significantly different from the effect of the supernatant collected from unstimulated microglia after 24 h (incubation of neurons for 24 h: 48.0±14.1 U/ml, p<0.01; 48 h: 53.5±25.8 U/ml, p=0.04; 72 h: 61.3±30.6 U/ml, p=0.05). Similar results were observed with the supernatant from stimulated microglia after 48 h (incubation of neurons for 24 h: 68.1±19.1 U/ml vs 41.3±6.4 U/ml, p<0.01; 48 h: 85.0±40.7 U/ml vs 49.5±19.5 U/ml, p<0.01; 72 h: 84.3±35.8 U/ml vs 55.2±23.0 U/ml, p=0.03). Fig. 4C shows LDH release from neurons after 48 h of supernatant exposure. Pam3CysSK4 did not induce neurotoxic properties in the supernatant from TLR2−/− microglia (Fig. 4D).

FIGURE 2. Studies of neurodegeneration in the murine dentate gyrus. A–D, Merged TUNEL (green) and Hoechst (chromatin, blue) staining of the dentate gyrus of wild-type mice (A and C) and TLR2−/− mice (B and D) 24 h after challenge with intrathecal Pam3CysSK4 (A and B) or live pneumococci (C and D). E, Quantitative analysis of TUNEL-positive nuclei in the dentate gyrus per area on multiple sections per animal. * p<0.05 determined by one-way ANOVA followed by Student-Newman-Keuls post hoc analysis.

FIGURE 3. Cell type-specific expression of TLR2 in the CNS. A, Real-time PCR analysis of TLR2 mRNA expression (in arbitrary units) in rat neurons, astrocytes, and microglia using GAPDH (■) and β-actin (●) as internal standards. Immunocytochemical localization of TLR2 (C, F, and I). Murine primary microglia from wild-type (B–D) and TLR2−/− mice (E–G) is identified by IB4 immunostaining (B and E). Murine primary neurons (H–J) are identified by microtubule-associated protein-2 immunoreactivity (H). Merged images (D, G, and J) are shown. Scale bar, 50 μm.
FIGURE 4. Microglia-mediated toxicity of Pam3CysSK4 in primary rat cortical neurons. A and B, Neuronal cultures were left untreated (□) or incubated for 24–72 h with 0.1 μg/ml Pam3CysSK4 (●) or with 0.5 μM staurosporine (STS) as a positive control (△). A, Proportion of apoptotic neurons after 24, 48, and 72 h. B, Concentration of LDH in the supernatant at 24, 48, and 72 h. C and D, Neuronal cultures were incubated for 48 h with supernatant that had been obtained from murine primary microglia upon stimulation with 0.1 μg/ml Pam3CysSK4 for 6–48 h. Data are the LDH concentration at 48 h in the supernatant of the neuronal cultures. Controls (□) were incubated with medium from unstimulated microglia collected at identical times. In these experiments, supernatant was either obtained from wild-type microglia (C) or from TLR2−/− microglia (D). * p < 0.05; ** p < 0.01 determined by Student’s t test.

Discussion

The major finding of our study is that the selective activation of TLR2 by a synthetic bacterial lipopeptide in vivo causes inflammatory changes typically associated with experimental bacterial meningitis. Lipopeptide-induced intracranial inflammation has all the hallmarks of bacterial meningitis, i.e., influx of leukocytes, increase of blood flow, development of intracranial hypertension as well as neuronal cell death (21–26). In particular, the time course of the inflammatory response to the synthetic bacterial lipopeptide in the mouse experiments resembles an earlier study with purified pneumococcal cell wall components (22). In this study, challenge also resulted in a self-limiting acute influx of leukocytes into the CSF with high leukocyte concentrations at 6 h and receding numbers at 24 h. In our rat model, the maximum increases of LDF and ICP after challenge with Pam3CysSK4 were similar to previous findings in meningitis induced by purified pneumococcal cell wall components (12, 23). Although the effects of Pam3CysSK4 on leukocyte influx and ICP increase were not different from those of viable pneumococci, we observed a significantly stronger LDF increase with live D39. As the likely basis of this effect, we have previously identified release of hydrogen peroxide by S. pneumoniae as an independent vasodilator during early experimental meningitis (27). Studies of neuronal apoptosis induction in vivo by bacterial cell wall components have not previously been published. Earlier studies using viable pneumococci have argued that neuronal damage during meningitis is the combined effect of bacterial toxins and the inflammatory host response (13, 28, 29). Our present findings show that stimulation of the TLR2 pathway alone induces neuronal apoptosis in vivo.

Our data present clear evidence that TLR2 signaling is highly sufficient to trigger meningitis in vivo, including the induction of neuronal apoptosis in the dentate gyrus. The clinical relevance of this finding is underlined by the fact that pneumococcal cell wall components, a known TLR2 agonist (30, 31), are excessively released after antibiotic-induced lysis of bacteria and that patient outcome is correlated to their concentration in the CSF (32). The ubiquitous presence of lipoparticles on bacterial surfaces, including other meningitis-inducing pathogens, further broadens the clinical importance.

Conversely, the relative impact of TLR2 on inflammation and host damage in bacterial meningitis caused by a viable pathogen has not been clearly delineated. In vitro, ectopic expression of TLR2 confers responsiveness to PG and LTA of Gram-positive S. pneumoniae in Chinese hamster ovary fibroblasts (30, 33) and human embryonic kidney HEK 293 cells (8, 33, 34). In vivo, several studies report a reduced ability of TLR2−/− mice to limit the growth of Gram-positive bacteria (5–7, 35, 36). In a previous study (8), higher titers of S. pneumoniae in CSF and blood were found in TLR2−/− mice compared with wild-type mice 24 h after meningitis induction. Typical CNS complications, i.e., ICP increase and formation of brain edema, were more pronounced and clinical scores were worse in the knockout mice. However, CSF leukocyte count and tissue levels of proinflammatory cytokines were not significantly changed, and aggravation of disease in TLR2−/− mice may have resulted from pronounced cytotoxic effects of the pneumococcal metabolism. Reduced CSF pleocytosis was later observed in pneumococcal meningitis of mice lacking the adaptor protein MyD88, which is situated downstream of both TLR2 and TLR4 (37). Similarly, aggravation of the clinical course, higher bacterial titers in brain tissue, enhanced brain edema, and increased mortality were reported in TLR2−/− mice in meningitis due to S. pneumoniae or Listeria monocytogenes (7). These studies suggest that TLR2 plays an important role in the host defense during Gram-positive meningitis. However, these reports do not allow differentiation between effects of an inadequate suppression of bacterial growth and consequences of the absence of TLR2 on the inflammatory host response, nor can meningitis-associated phenomena be traced back to specific receptors. Indeed, our own findings in D39-induced meningitis do not support a more severe clinical course in TLR2-deficient mice in the presence of equivalent bacterial concentrations in the CSF. Rather, reduced CSF pleocytosis and prevention of excess neuronal damage in the knockout mice despite an identical bacterial load suggest a prominent contribution of TLR2 signaling to inflammation and host damage during meningitis.

Neuronal loss, predominantly in hippocampal structures, is a hallmark of bacterial meningitis (21, 24, 38–40). In pneumococcal meningitis, at least two distinct pathways leading to neuronal loss have been demonstrated: live pneumococci induce an early caspase-3-independent, AIF-dependent wave of apoptosis, while later, the release of bacterial cell wall components leads to a different type of cell death involving release of cytochrome c, formation of the apoptosome, and ultimately activation of caspase-3 (21, 24, 28, 29, 41). It has been argued that induction of apoptosis by bacterial cell wall components occurs secondary to the activation of immune competent host cells (28). In vitro, bacterial cell wall components induce cell death in organotypical slices and in neuronal/glial coculture, but not in primary neurons cultured alone, and NO release from activated glia has been suggested as the relevant cytotoxic principle (42). We did not observe an induction of cell death in neuronal monocolonies exposed to Pam3CysSK4. These findings are conclusive and are supported by the absence of TLR2 in neurons. As demonstrated in another model, our experiments confirm that TLR2-dependent release of neurotoxins by microglia causes neuronal cell death (43). Additionally, initiation of
TLR2-mediated and caspase-dependent cell death by pneumococcal cell wall has only been reported in TLR2-expressing cells such as endothelial cells or dendritic cells (41, 44). Our finding of low TLR2-mRNA expression in resting astrocytes does not argue against an additional contribution of these cells in vivo because astrocytes have been shown to up-regulate TLR2 upon activation by bacterial compounds (45). Taken together, we have established a model of meningitis induced by a synthetic TLR2 ligand. Synthetic bacterial lipopeptides offer an opportunity to study TLR2-dependent events independently of bacterial metabolism and other bacterial cell wall components and thus enable further dissection of the complex mechanisms of intracranial immune response.

Disclosures

The authors have no financial conflict of interest.

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