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Short communication

Protective role of NF-κB1 (p50) in experimental pneumococcal meningitis

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Abstract

Nuclear factor-kappaB (NF-κB) is a critical regulator of many genes involved in the pathogenesis of bacterial meningitis. Recently, activation of NF-κB was shown to be a key event in the inflammatory host response and the development of intracranial complications during experimental pneumococcal meningitis. Since the p50 subunit of NF-κB lacks a transactivation domain and can therefore act as a transcriptional repressor, we investigated whether NF-κB1 (p50) exerts anti-inflammatory effects in pneumococcal meningitis. p50-deficient mice had higher cerebellar pneumococcal titers (10.06±0.47 vs. 8.51±1.06 log colony-forming units [cfu]/cerebellum), cerebrospinal fluid (CSF) leukocyte counts (11,475±2340 vs. 8444±1405 cells/μl) and brain concentrations of interleukin-1β (125.9±50.3 vs. 58.5±52.2 pg/mg protein) than their wild-type littermates. With ceftriaxone therapy, none of the wild-type mice but 43% of the p50-deficient animals died. In conclusion, lack of NF-κB1 (p50) was associated with impaired bacterial clearing, enhanced inflammatory host response and increased mortality during pneumococcal meningitis.

Keywords: Meningitis; *Streptococcus pneumoniae*; NF-kappaB1; NF-kappaB p50 subunit; Host response

1. Introduction

Nuclear factor-kappaB (NF-κB) is a ubiquitously expressed transcription factor and is thought to act as a transcriptional activator of many genes which are involved in the pathogenesis of pneumococcal meningitis, including tumor necrosis factor (TNF), interleukin-1β and inducible nitric oxide synthase (for a review, see May and Ghosh, 1998; Tauber and Moser, 1999; Koedel et al., 2002a). In vitro studies have also suggested a role for NF-κB in the stimulation of monocytes by pneumococci (Spellerberg et al., 1996). Recently, we have demonstrated that pharmacological NF-κB inhibition markedly attenuated cerebral complications of experimental pneumococcal meningitis, such as blood–brain barrier disruption, intracranial hypertension and cerebrovascular failure (Koedel et al., 2000). The mammalian Rel/NF-κB family of transcription factors comprises five known members: NF-κB1 (p50 and its precursor p105), NF-κB2 (p52 and its precursor p105), RelA (p65), RelB and c-Rel (May and Ghosh, 1998). These proteins can assemble to homo- and heterodimers, such as the p50–p65 heterodimers (the prototypical form of NF-κB) or p50–p50 homodimers. NF-κB is sequestered in the cytosol of unstimulated cells via noncovalent interactions with inhibitory proteins, the inhibitors of NF-κB (IκBs) (May and Ghosh, 1998). Upon stimulation of the cell, phosphorylation and proteasomal degradation of the IκBs result in the release and nuclear translocation of NF-κB. The binding of transactivating complexes, such as p50–p65

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heterodimers, to NF-κB motifs will then activate gene expression. p50–p50 homodimers, however, when bound to DNA cannot transactivate but instead block access of transactivating complexes, because the p50 molecule lacks a transcription activation domain (Ziegler-Heitbrock, 2001; Kastenbauer and Ziegler-Heitbrock, 1999). Since p50–p50 homodimers can act as transcriptional repressors, we hypothesized that p50 may play an anti-inflammatory role in meningitis. Therefore, in the present study, we have studied pneumococcal meningitis in mice lacking the p50 subunit of NF-κB.

2. Materials and methods

2.1. Animals

Experiments were conducted in adult $p50^{-/-}$ mice (Sha et al., 1995) and wild-type littermates (C57BL/6 background). The animal experiments were approved by the government of Upper Bavaria.

2.2. Mouse model of pneumococcal meningitis

We used a well-characterized mouse model of pneumococcal meningitis (Koedel et al., 2002b, 2003; Paul et al., 2003). Briefly, animals were weighed and meningitis was induced by transcutaneous intracisternal injection of 15 μl of 1×10^7 colony-forming units (cfu)/ml of *Streptococcus pneumoniae* type 3 after short-term anesthesia with halothane. Mice were then allowed to wake up. After weighing and measurement of the rectal body temperature, mice were anesthetized with intraperitoneal ketamine/xylazine. A catheter was inserted into the cisterna magna through a burr-hole in the occipital bone, and cerebrospinal fluid (CSF) was removed for the determination of CSF leukocyte counts. After an overdose of thiopental, mice were perfused transcardially and the cerebral hemispheres, lungs and spleen were extracted and rapidly frozen. Pneumococcal titers were determined in serial dilutions of cerebellar homogenates on blood agar plates.

2.3. Experimental groups

Controls received an intracisternal injection of 15 μl sterile phosphate-buffered saline (PBS, pH 7.4) and were examined 24 h after injection. Infected animals were studied either 24 or 48 h post-infection. In experiments of 24-h duration, the mice received no antibiotic treatment. Since untreated animals usually do not survive until 48 h post-infection, mice in the experiments of 48 h duration were treated with 100 mg/kg i.p. ceftriaxone 18 h after infection.

2.4. Determination of cytokines in tissue homogenates

Cryostat-cut tissue sections (brain, lung and spleen) were sonicated in 10 mM HEPES buffer (pH 7.9, 10 mM KCl, 1.5 mM MgCl) containing a proteinase inhibitor cocktail and centrifuged. Total protein concentration of the supernatant was determined according to Bradford. The concentrations of immunoreactive TNF and interleukin-1β were determined by commercial enzyme-linked immunosorbent assay (ELISA) kits (R&D Systems, Wiesbaden, Germany) and expressed as picograms per milligram of protein.

2.5. Statistical analysis

Continuous variables were compared by the Mann–Whitney *U*-test. Mortality was analysed by Fisher's exact test. Only two-sided tests were applied. Wild-type mice 24 h after infection were compared with uninfected controls in order to characterize the alterations due to meningitis. To

Table 1
Experimental data of uninfected controls and infected wild-type or $p50^{-/-}$ mice

Experimental group (*n*)	Mortality	Weight loss (%)	Body temperature (°C)	Log CFU/ cerebellum	CSF leukocyte count (cells/μl)	Brain TNF (pg/mg protein)	Brain interleukin-1β (pg/mg protein)
Uninfected wild-type controls (4)	0 of 4	4.4±0.4[a]	37.2±0.2[a]	negative	225±126[a]	0.5±0.2	1.2±0.5[a]
Wild-type mice 24 h after infection (6)	0 of 6	10.7±1.6	32.5±0.8	8.51±1.06	8444±1405	3.3±3.1	58.5±52.2
$p50^{-/-}$ mice 24 h after infection (6)	0 of 6	12.5±1.9	34.8±2.1	10.06±0.47[a]	11475±2340[a]	32.7±13.4	125.9±50.3[a]
Wild-type mice 48 h after infection (11)	0 of 11	17.8±3.4	34.5±1.5	3.36±0.93	6086±4233	0.4±0.1	4.3±6.5
$p50^{-/-}$ mice 48 h after infection (14)	6 of 14[b]	20.3±5.4	34.5±2.0	3.13±0.62	3167±1385	8.5±22.5	31.7±73.7

In the experiments of 48 h duration, mice were treated with ceftriaxone 18 h post-infection. In order to characterize the alterations due to meningitis, wild-type mice 24 h after infection were compared statistically with uninfected controls. To investigate the role of p50, wild-type and knockout mice from both time points after infection (24 and 48 h) were compared with each other. Continuous variables were compared by the Mann–Whitney *U*-test. Mortality was analysed by Fisher's exact test. All tests were two-sided. Data are expressed as mean±standard deviation.

[a] $P<0.05$ compared with wild-type mice 24 h after infection.

[b] $P<0.05$ compared with wild-type mice 48 h after infection.

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3. Results

3.1. Characterization of meningitis

Twenty-four hours after intracisternal injection of pneumococci, all infected wild-type mice showed clinical signs of meningitis (such as obtundation, piloerection, weight loss and hypothermia). Pneumococci grew from the brains of infected animals but not from controls. The CSF leukocyte counts and the brain concentrations of interleukin-1β were increased compared with uninfected controls (Table 1).

3.2. The role of NF-κB1 (p50) in pneumococcal meningitis

Twenty-four hours after infection, the cerebellar bacterial titers, CSF leukocyte counts, and brain concentration of interleukin-1β were significantly higher in $p50^{-/-}$ mice than in their wild-type littermates ($P<0.05$). Weight loss ($P=0.065$), body temperature ($P=0.065$) and the brain concentrations of TNF ($P=0.093$) were not statistically different. Until 48 h post-infection, none of 11 wild-type littermates but 6 of 14 $p50^{-/-}$ mice died (significant with $P=0.020$). All other parameters did not differ between the surviving animals of both groups. The concentrations of interleukin-1β and TNF in the lungs and spleen were similar in transgenic and wild-type mice from both time points (not shown) (Table 1).

4. Discussion

The main findings of our study were that $p50^{-/-}$ mice had higher cerebellar pneumococcal titers, CSF leukocyte counts and brain concentrations of interleukin-1β and exhibited a higher mortality than their wild-type littermates. A previous study reported similar findings after intraperitoneal challenge of $p50^{-/-}$ mice with *S. pneumoniae*, as they had higher concentrations of pneumococci in their blood and organs and succumbed earlier to the infection than the wild-type animals (Sha et al., 1995). Now, we demonstrate for the first time that p50-deficient mice are also less able to control an intracranial pneumococcal infection.

The reasons why $p50^{-/-}$ mice have an impaired pneumococcal clearing are unclear. In a previous study, peritoneal macrophages and blood neutrophils from p50-deficient mice were present in normal numbers and had normal phagocytic activity when assayed for their ability to ingest opsonized zymosan particles. However, the knockout mice were shown to have markedly decreased resting serum immunoglobulin levels (Sha et al., 1995). Opsonin-mediated phagocytosis is believed to be the major defense mechanism against *S. pneumoniae* (Vitharsson et al., 1994). A lack of opsonizing antibodies, therefore, is one possible explanation why $p50^{-/-}$ mice may have an impaired pneumococcal clearing.

Studies in rabbits and rats have shown that the bacterial titer is a major determinant of complications and mortality of experimental pneumococcal meningitis (Tauber et al., 1992; Giampaolo et al., 1981; Klein et al., 2003). Accordingly, in the present study, $p50^{-/-}$ mice, which had higher cerebellar pneumococcal titers, also had a higher mortality than their wild-type littermates. However, the increased bacterial titers cannot be attributed to a generally impaired host immune response, because the CSF leukocyte counts and brain concentrations of interleukin-1β were even higher than in wild-type animals. The finding of increased bacterial titers in spite of an exaggerated host response seems paradoxical. However, the enhanced inflammation seen in our study is in good agreement with in vitro studies which have shown that NF-κB1 (p50) homodimers can act as transcriptional repressors by blocking the access of transactivating complexes of the NF-κB/Rel family to κB promoter sites, e.g., of the TNF gene (Kastenbauer and Ziegler-Heitbrock, 1999; Ziegler-Heitbrock, 2001).

Therefore, the lack of transcriptional repression may explain higher proinflammatory cytokine levels in $p50^{-/-}$ mice. Only brain levels of interleukin-1β but not those of TNF were significantly increased in $p50^{-/-}$ mice; however, this may be due to the fact that compared with interleukin-1β, TNF brain levels are generally relatively low in our mouse model of pneumococcal meningitis (Koedel et al., 2002b). The role of interleukin-1β during pneumococcal meningitis is not completely clear: Zwijnenburg et al. (2003) reported higher CSF bacterial titers, lower brain cytokine levels and an increased mortality of mice deficient for the interleukin-1 receptor type 1 after intranasal inoculation of pneumococci; Koedel et al. (2002b) also detected lower brain cytokine concentrations but a reduction of intracranial complications and mortality after intracisternal inoculation of pneumococci in mice deficient for caspase-1 (an enzyme which is indispensable for the activation of interleukin-1β). Differences in the animal models used or additional biological functions of interleukin-1 receptor type 1 and caspase-1 might account for the observed differences. Still, the higher degree of brain inflammation evidenced by higher cytokine levels might be another explanation for the higher mortality of the $p50^{-/-}$ mice in the present study.

While pneumococcal meningitis was aggravated in $p50^{-/-}$ mice, pharmacological inhibition of NF-κB activation with *N*-acetyl-leucinyl-leucinyl-norleucinyl (ALLN, an inhibitor of IκB-phosphorylation) or with (2*E*)-3-[[4-(1,1-dimethylethyl)phenyl]sulfonyl]-2-propenenitrile (BAY 11-7085, an inhibitor of the proteasomal degradation of IκB) was previously shown to be beneficial during experimental pneumococcal meningitis (Koedel et al., 2000). In addition, a strong increase of active p65 (i.e., dissociated from IκBs) was demonstrated in the brains and, in particular, in granulocytes infiltrating the leptomeninges of animals with meningitis

(Koedel et al., 2000). This increase of NF-κB activity was attributed to p50–p65 heterodimers (Koedel et al., 2000). Dimers containing the transactivating p65 subunit, such as p50–p65, p65–65, or p65–c-Rel, are strong inducers of many pro-inflammatory genes (May and Ghosh, 1998). Pharmacological inhibitors of NF-κB activation reduced nuclear translocation of transactivating dimers containing p65 and were therefore protective (Koedel et al., 2000). In p50-deficient animals, p50–p65 heterodimers were probably replaced by other dimers, such as p65–p65 or p65–c-Rel, which may be more potent transactivators at κB sites. Furthermore, p50-homodimers, which are transcriptional repressors, could not be formed in the knockout animals. Therefore, the expression of pro-inflammatory genes may have been enhanced and/or disinhibited in $p50^{-/-}$ mice, resulting in a stronger intracranial inflammatory response.

Taken together, our findings suggest that members of the NF-κB family can crucially affect the course of pneumococcal meningitis. Dimers containing the transactivating p65 subunit seem to be essential for triggering the inflammation, and the inhibition of nuclear translocation of p65 containing dimers is a promising strategy for the adjunctive therapy of meningitis. By contrast, expression of the p50 subunit is required for pneumococcal clearing and for control of the inflammatory host response. Possible future adjunctive therapies of bacterial meningitis should therefore not interfere with the expression or nuclear translocation of p50-homodimers.

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