Prophylactic and Therapeutic Targeting of the Neurokinin-1 Receptor Limits Neuroinflammation in a Murine Model of Pneumococcal Meningitis

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There is increasing evidence that the tachykinin substance P (SP) can augment inflammatory immune responses within the CNS. We have recently demonstrated that resident CNS cells express high-affinity receptors for this neuropeptide (neurokinin-1 receptors [NK-1R]), and we have shown that SP can significantly augment glial inflammatory responses to clinically relevant Gram-negative bacteria. Furthermore, we provided evidence that endogenous SP/NK-1R interactions are an essential component in the initiation and/or progression of CNS inflammation following in vivo exposure to these pathogens. In this study, we demonstrate that SP similarly enhances inflammatory glial responses to the major Gram-positive causative agent of bacterial meningitis, *Streptococcus pneumoniae*, and show that endogenous SP/NK-1R interactions play a critical role in the development of CNS inflammation in an in vivo model of pneumococcal meningitis. Importantly, we provide the first demonstration, to our knowledge, that pharmacological targeting of the NK-1R not only prevents the development of damaging inflammation when administered prophylactically, but can also limit or reverse neuroinflammation associated with an established streptococcal CNS infection when delivered therapeutically. We show that an NK-1R antagonist attenuates increases in CNS inflammatory cytokine levels and decreases in immunosuppressive cytokine production associated with an ongoing *S. pneumoniae* infection. Furthermore, we demonstrate that such a therapeutic intervention reverses infection-associated gliosis and demyelination in the absence of changes in CNS bacterial burden. Together, these results suggest that targeting SP/NK-1R interactions is a strategy worthy of further study for the treatment of microbially induced neuroinflammation. *The Journal of Immunology*, 2011, 186: 7255–7263.
with meningitis. This organism was grown on tryptic soy agar with 5% defibrinated sheep blood and cultured overnight in Todd-Hewitt broth (Difco Microbiology, Lawrence, KS) at 37°C with 5% CO2 as previously described by our laboratory (4).

**SP receptor-deficient mice**

Mice genetically deficient in the expression of the NK-1R (NK-1R−/−) that have a high affinity for SP for SP were bred for >10 generations onto a C57BL/6 background at the University of Iowa Medical Center (12). These mice were originally derived from induced mutations made by insertion of the lacZ gene into exon 1 of the NK-1R (13). NK-1R−/− mice were routinely screened by PCR to confirm disruption of the NK-1R−/− gene as previously described (13, 14) using the positive and negative strand primers 5′-CCAACACCTC-CCAAGACTCTG-3′ and 5′-GCCACAGCTATGGAGTAGAT-3′ for wild-type and 5′-TCCAGACTGCTCCTGAAA-3′ and 5′-GCCACAGCTGT-TCATGGAGTAGAF-3′ for SP receptor deficiency, respectively.

**Intracerebral administration of bacteria**

Viable *S. pneumoniae* were administered via intracerebral (i.c.) injection at a nonlethal dose (1 × 10⁴ bacteria) as we have previously described (4) into NK-1R−/− and wild-type 6- to 8-wk-old female C57BL/6 mice (The Jackson Laboratory). To assess the prophylactic effects of an NK-1R antagonist, a group of infected wild-type animals additionally received s.c. doses of the specific NK-1R antagonist L703,606 (cis-2-[diphenylmethyl]-N-[2-(isonicotinyl)methyl]-1-azabicyclo(2.2.2)octan-3-amine oxidate; Sigma-Aldrich, St. Louis, MO), at a dose of 5 mg/kg or vehicle at days −1, +1 and +2 relative to infection as we have previously described (11) and sacrificed at 3 d postinfection. This potent and specific competitive nonpeptide NK-1R antagonist inhibits binding of SP to the NK-1R with an IC₅₀ of 2 nM (15). To assess the therapeutic effects of this NK-1R antagonist, a group of infected wild-type animals received L703,606 (5 mg/kg s.c. daily) beginning at day 3 postinfection, a time point empirically determined to be following the onset of CNS inflammatory but prior to significant demyelination and continued through day 7 prior to euthanasia at day 8 postinfection.

To infect mice, anesthetized animals (four to five animals per group) were secured in a stereotactic platform and stand and received an i.c. injection containing 1 μl of a 10⁹ colony-forming unit (CFU) of *S. pneumoniae* in a volume of 1 μl of vehicle only 1 mm lateral and 1 mm posterior to the bregma using a Hamilton positive displacement syringe (7001 series; Hamilton) with a 25-gauge needle and a tubing guard to ensure constant depth of administration (3–3.5 mm) as described by our laboratory (16). Such an approach allows bacteria in an aqueous solution to reflux into the subarachnoid space via the needle tract, leading to meningitis formation. Mice displaying complications such as seizures, abnormal gait/gaitaxia, or failure to open eyes immediately following intracranial administration were euthanized.

Body temperatures were monitored using s.c.-implanted heat sensor transponders (Biomedical Data Systems, Seaford, DE). At 0, 2, 3, 4, 6, or 8 d postinfection, animals were euthanized, and all brain tissue was removed and weighed. In some experiments, Evan’s blue dye (Sigma-Aldrich) was administered i.v. 30 min prior to euthanasia to assess blood–brain barrier (BBB) permeability as previously described (17). To control for mechanical disruption of the BBB following intracranial bacterial administration, changes in Evan’s blue content were compared with those seen in sham-infected animals receiving sterile Ringer’s solution. Isolated brain tissue was mechanically disrupted in a glass homogenizer, washed, and then exposed to bacteria at multiplicities of infection (MOI) between 25:1 and 250:1 bacteria to cells in media without antibiotics for 2 h at 37°C. After allowing this period of infection, cultures were washed and incubated in media with 10% FBS supplemented with 25 μg/ml gentamicin to kill remaining extracellular bacteria. At 12–24 h following this procedure, culture supernatants were collected.

**Bacterial counts**

Postinfection, 100 μl isolated brain homogenates were cultured on tryptic soy agar plates with 5% defibrinated sheep blood for 24 h at 37°C and *S. pneumoniae* colonies subsequently counted.

**Locomotor activity following i.e. bacterial challenge**

Locomotor activity was assessed in mice following bacterial infection as an index of neurologic impairment as previously described by our laboratory (21). Locomotor activity according to a modified protocol of Levin and colleagues (21) using a radial arm maze (Coulbourn Instruments, Allentown, PA). Briefly, wild-type and NK-1R−/− mice (n = 4) were acclimated to the handler and the maze apparatus for several days prior to bacterial challenge (22, 23). Mice were allowed to move freely within the apparatus for 5 min, during which time the total number of arms visited was counted. Values were obtained for 3 consecutive d prior to bacterial administration and at various time points up to 3 d following bacterial challenge.

**Immunoblot analysis of glial NK-1R expression**

Immunoblot analyses for the presence of the NK-1R in acutely isolated glial cells or cultured primary murine microglia and astrocytes were performed essentially as previously described (9). The primary Ab used was a commercially available rabbit polyclonal Ab directed against murine NK-1R (clone 77516; Abcam, Cambridge, MA).

**Acute isolation and cytometric analysis of CNS cells**

Mixed CNS cells were acutely isolated from infected and uninfected animals using a protocol modified from Campanella and coworkers (24) and previously employed by our laboratory (20). Briefly, brains were rapidly removed and mechanically disrupted in a glass homogenizer, washed, and resuspended in RPMI 1640 plus 20% FBS/30% Percoll (Fluka; Sigma-Aldrich) solution. This was overlaid on a gradient containing 37 and 70% Percoll solutions and centrifuged at 600 ×g for 20 min at room temperature. Cells were then collected from the 37/70% Percoll interface and washed with PBS plus 20% FBS. Cells were staining with an FITC-conjugated monoclonal rat Ab directed against murine CD11b (clone M1/70; BD Pharmingen, San Diego, CA) and a PE-conjugated monoclonal rat Ab directed against murine F4/80 (clone C1:A3-1; Serotec, Raleigh, NC).

These cells were analyzed using a FACSAria flow cytometer (BD Biosciences, San Jose, CA) to determine the proportion of cells expressing both CD11b and F4/80. In each experiment, a minimum of 50,000 cells were counted, and data are reported as the percentage of cells that were positive for both CD11b and F4/80 (CD11b⁺/F4/80⁺). In some experiments, microglia and astrocytes were isolated by FACS of permeabilized cells using an FITC-conjugated anti-CD11b Ab (clone M1/70) or an Alexa Fluor 488-conjugated monoclonal mouse Ab directed against murine glial fibrillary acidic protein (GFAP; clone C1-17719; Invitrogen, Carlsbad, CA), and protein isolates were prepared from 200,000 sorted cells.

**Isolation of primary murine glia and in vitro infection with *S. pneumoniae***

Mouse neonatal brain microglia and astrocytes were isolated as described previously by our laboratory (9). Microglia were cultured in RPMI 1640 with 10% FBS and 20% conditioned medium from LADMAC cells (ATCC number CRL-2420; American Type Culture Collection) as a source of M-CSF. Astrocytes were cultured in RPMI 1640 containing 10% FBS. Isolated cells were >95% authentic microglia as assessed by their characteristic morphology and expression of CD11b and F4/80 as determined by immunofluorescent microscopy and >97% authentic astrocytes as assessed by their distinct morphology, expression of GFAP, and the absence of CD11b expression.

For in vitro exposure of isolated glia to *S. pneumoniae*, bacteria were harvested by centrifugation and washed twice in PBS. Confluent cell layers of glia were washed three times with 4 ml PBS to remove growth media and then exposed to bacteria at multiplicities of infection (MOI) between 25:1 and 250:1 bacteria to cells in media without antibiotics for 120 min at 37°C. Following this period of infection, cells were washed and incubated in media with 10% FBS supplemented with 25 μg/ml gentamicin to kill remaining extracellular bacteria. At 12–24 h following this procedure, cell culture supernatants were collected.

**Histochmical and immunofluorescent analysis of brain sections**

Infected and sham-infected mice were euthanized at 3 and 8 d postinfection as indicated, and brains were perfused with PBS followed by Prefer fixative...
Interestingly, the absence of appropriate using commercially available software (Prism; GraphPad, La Jolla, CA) as previously described by our laboratory (4, 11, 20). Briefly, paraffin-embedded sections were sliced and dehydrated with decreasing concentrations of ethanol and then washed with xylene. Finally, the slides were placed in Cresyl Echt violet stain for 60 min and differentiated with alcohol. After dehydration, slides were washed in xylene, and coverslips were mounted using permanent mounting media and myelination of the corpus callosum visually assessed in a low power (×10 objective) microscope field in animals from each experimental group.

Astrogliosis was assessed in deparaffinized coronal sections blocked with goat serum (Zymed Laboratories, San Francisco, CA) according to GFAP expression. GFAP was detected using a monoclonal mouse Ab directed against murine GFAP (Invitrogen) and an Alexa Fluor 488-conjugated chicken anti-mouse secondary Ab (Invitrogen), and the coverslip was mounted using Fluoro-Gel with Tris buffer (Electron Microscopy Sciences, Hatfield, PA). GFAP expression was assessed in multiple microscopy fields (×20 objective) of the same cortical locations in animals from each experimental group using an Olympus IX70 Fluoroview confocal microscopy system (Olympus). Fluorescence intensity was quantified using Fluoview 500 Version 4.3 and National Institutes of Health ImageJ software to acquire microscopic field section images and to determine mean gray values for multiple fluorescent fields.

Statistical analyses
All results are presented as the mean ± SEM and were tested statistically by Student t test or one-way ANOVA with Tukey’s post hoc test as appropriate using commercially available software (Prism; GraphPad, La Jolla, CA). Results were considered to be statistically significant at p < 0.05.

Results

Endogenous SP/NK-1R interactions are required for increased BBB permeability following CNS S. pneumoniae infection

To begin to determine the role of SP in Gram-positive bacteria-induced CNS inflammation, we have measured BBB permeability following S. pneumoniae infection in the presence or absence of endogenous SP/NK-1R interactions. As shown in Fig. 1A, i.e., administration of a nonlethal dose of S. pneumoniae raised BBB permeability as evidenced by an elevated Evans’s blue dye entry into brain tissue over that seen in sham-infected mice. This increase in BBB permeability was abolished by prophylactic treatment with the specific NK-1R antagonist L703,606. To confirm that this effect was directly attributable to the inhibition of SP/NK-1R interactions, we have performed parallel experiments in mice genetically deficient in the expression of the SP receptor (NK-1R−/−). As shown in Fig. 1A, BBB permeability was not increased in NK-1R−/− mice following S. pneumoniae infection. Interestingly, the absence of S. pneumoniae-induced increases in BBB permeability in NK-1R−/− mice or wild-type animals following L703,606 treatment was not attributable to changes in CNS SP levels or differences in bacterial burden (Fig. 1B, 1C, respectively). Although there was a trend for the bacterial burden to be lower in infected NK-1R−/− mice than the infected wild-type animals (Fig. 1C), this effect failed to reach statistical significance.

SP/NK-1R interactions are required for increased inflammatory and decreased immunosuppressive cytokine levels within the CNS following S. pneumoniae infection

To further evaluate the role of SP/NK-1R interactions in S. pneumoniae-induced neuroinflammation, we have assessed the effect of prophylactic NK-1R antagonist treatment on pro- and anti-inflammatory mediator levels in the CNS. As shown in Fig. 2, S. pneumoniae administration significantly elevated levels of the inflammatory cytokines TNF-α and IL-6 in the CNS at 3 d postinfection with a concomitant decrease in the level of the immunosuppressive cytokine IL-10. Interestingly, prophylactic L703,606 treatment abolished increases in TNF-α levels, and IL-6 and IL-10 levels in antagonist-treated infected mice were not significantly different from those seen in uninfected animals (Fig. 2). Again, we have confirmed that these effects were attributable to NK-1R blockade in parallel experiments employing NK-1R−/− mice. Although TNF-α and IL-6 tended to increase in the CNS of infected NK-1R−/− animals, these effects failed to reach statistical significance (Fig. 2). Furthermore, TNF-α levels in these animals were significantly lower than those seen in infected wild-type mice (Fig. 2A). Finally, CNS IL-10 levels were not decreased in infected NK-1R−/− mice and were significantly higher than those in infected wild-type mice (Fig. 2C). Indeed, IL-10 levels in infected NK-1R−/− mice tended to be higher than those seen in uninfected NK-1R−/− animals, although this elevation failed to reach statistical significance. Taken together, these data suggest that endogenous SP/NK-1R interactions can augment the initiation and/or progression of S. pneumoniae-associated neuroinflammation by promoting proinflammatory mediator expression and limiting immunosuppressive cytokine production.

![Image](http://www.jimmunol.org/Downloaded_from/http://www.jimmunol.org/)
S. pneumoniae-associated gliosis and behavioral changes following in vivo bacterial administration are reduced in the absence of endogenous SP/NK-1R interactions

We have also investigated the importance of endogenous SP/NK-1R interactions on the gliosis associated with in vivo S. pneumoniae challenge. As shown in Fig. 3A, S. pneumoniae administration elicits a marked astrogliosis as rapidly as 3 d postinfection as indicated by the increased number of GFAP-positive cells and the higher immunofluorescence intensity of each (16.56 ± 0.88 versus 6.70 ± 1.36 arbitrary fluorescence units per micrograph quadrant in infected versus uninfected animals, respectively; n = 4; p < 0.05). Importantly, such infection-induced astrogliosis was abolished by prophylactic L703,606 treatment (Fig. 3A) with an average GFAP-associated fluorescence of 2.36 ± 0.68 arbitrary fluorescence units per micrograph quadrant (n = 4; p < 0.05) and was also significantly attenuated in NK-1R−/− mice (8.72 ± 1.37 arbitrary fluorescence units per micrograph quadrant; n = 4; p < 0.05).

Finally, we have assessed the effects of prophylactic L-703,606 treatment on S. pneumoniae-induced changes in locomotor activity as a means to assess the role of endogenous SP/NK-1R interactions in the development of infection-associated neurologic sequelae. As shown in Fig. 3B, S. pneumoniae elicits a significant increase in locomotor activity in wild-type mice as determined by the number of arms of the radial arm maze apparatus visited during the observation period. Importantly, prophylactic L-703,606 treatment completely abolished these S. pneumoniae-induced behavioral changes (Fig. 3B).

SP/NK-1R interactions augment microglial responses to S. pneumoniae

To begin to assess the role played by SP/NK-1R interactions in the inflammatory immune responses of resident CNS cells, we have examined the effect of S. pneumoniae challenge on NK-1R expression by microglia and astrocytes. As shown in Fig. 4A, NK-1R expression was upregulated in whole brain in S. pneumoniae-infected mice. Interestingly, NK-1R levels were not readily discernible in microglia isolated from uninfected brain tissue but demonstrated marked expression in an equal number of cells purified from the brains of S. pneumoniae-infected animals (Fig. 4A). In contrast, astrocytes exhibited robust NK-1R expression even in uninfected animals, and such expression was only modestly increased in S. pneumoniae-infected animals (Fig. 4A). To confirm these findings, NK-1R expression was assessed in isolated glial cell cultures. As shown in Fig. 4B, microglia demonstrated very...
low levels of NK-1R expression but showed up to a 12.3-fold increase following in vitro *S. pneumoniae* exposure. In contrast, isolated astrocytes showed robust NK-1R expression levels that showed little or no increase following infection (Fig. 4B).

Further evidence of an important role for SP/NK-1R interactions in *S. pneumoniae*-induced glial responses comes from the observation that the brains of infected wild-type mice contained significantly higher numbers of CD11b^+^F4/80^+^ microglia/macrophages, but such increases were not seen following prophylactic L703,606 treatment (Fig. 4C) or in NK-1R^−/−^ animals following *S. pneumoniae* administration.

Finally, we have investigated the effects of exogenous SP on the inflammatory responses of isolated primary glial cells to *S. pneumoniae*. As shown in Fig. 4, in vitro *S. pneumoniae* challenge markedly upregulated IL-6 production by both microglia and astrocyte cultures. Interestingly, exogenous SP (5 nM) significantly elevated IL-6 production by *S. pneumoniae*-challenged microglia but failed to increase IL-6 production by similarly challenged astrocytes (Fig. 4D). Taken together, these data suggest that SP/NK-1R interactions can augment the inflammatory responses of resident myeloid cells to *S. pneumoniae* and could therefore be an important component in the rapid CNS inflammation associated with pneumococcal meningitis.

**Pharmacological inhibition of SP/NK-1R interactions ameliorates disease severity following the establishment of an *S. pneumoniae* CNS infection**

To begin to determine the therapeutic potential of targeting the NK-1R to limit *S. pneumoniae*-induced neuroinflammation, we have investigated the effect of L703,606 on infection-associated sequelae in mice after the administration of a nonlethal dose of bacteria. As shown in Fig. 5, *S. pneumoniae*-infected animals showed a significant CNS bacterial burden at 2 d postinfection (Fig. 5A) that was associated with a reduction in body weight (Fig. 5B) and a marked increase in BBB permeability (Fig. 5C). Although increases in BBB permeability peaked at 2 d following *S. pneumoniae* administration (Fig. 5C), infected mice demonstrated lower body weights up to 6 d postinfection (Fig. 5B) with a maximal bacterial burden at 4 d (Fig. 5A) and a significant drop in body temperature at 6 d postinfection (Fig. 5D). Importantly, infection-associated decreases in body temperature were abolished in mice that began receiving L703,606 at 3 d postinfection (Fig. 5D) and a marked increase in BBB permeability (Fig. 5C). In addition, the body weights of infected animals rapidly recovered following the onset of NK-1R antagonist therapy and were not statistically different from uninfected control mice after only 24 h of treatment (Fig. 5B). Interestingly, L703,606 did not significantly alter BBB permeability in infected animals and did not increase bacterial burden in these animals (Fig. 5A, 5C).

Finally, we have investigated the effect of L703,606 treatment on the morphological changes associated with *S. pneumoniae* infection. As shown in Fig. 6, *S. pneumoniae* administration elicited marked demyelination by 8 d postinfection as evidenced by reduced Luxol blue staining of the corpus callosum and pronounced astroglia in mice after the administration of a nonlethal dose of bacteria. As shown in Fig. 5, *S. pneumoniae*-infected animals showed a significant CNS bacterial burden at 2 d postinfection (Fig. 5A) that was associated with a reduction in body weight (Fig. 5B) and a marked increase in BBB permeability (Fig. 5C). Although increases in BBB permeability peaked at 2 d following *S. pneumoniae* administration (Fig. 5C), infected mice demonstrated lower body weights up to 6 d postinfection (Fig. 5B) with a maximal bacterial burden at 4 d (Fig. 5A) and a significant drop in body temperature at 6 d postinfection (Fig. 5D). Importantly, infection-associated decreases in body temperature were abolished in mice that began receiving L703,606 at 3 d postinfection (Fig. 5D) and a marked increase in BBB permeability (Fig. 5C). In addition, the body weights of infected animals rapidly recovered following the onset of NK-1R antagonist therapy and were not statistically different from uninfected control mice after only 24 h of treatment (Fig. 5B). Interestingly, L703,606 did not significantly alter BBB permeability in infected animals and did not increase bacterial burden in these animals (Fig. 5A, 5C).

**NK-1R antagonist treatment attenuates *S. pneumoniae*-induced changes in inflammatory and immunosuppressive cytokine levels within the CNS**

To further assess the therapeutic potential of this NK-1R antagonist, we have investigated the effect of L703,606 on pro- and anti-inflammatory mediator levels within the CNS following the
administration of a nonlethal dose of *S. pneumoniae*. As shown in Fig. 7, bacterial administration elicits significant elevations in CNS levels of the inflammatory chemokine MCP-1, with subsequent significant elevations in IL-6 and then TNF-α and a corresponding decrease in IL-10 levels. Importantly, L703,606 treatment starting at 3 d postinfection completely abolished infection-associated changes in IL-6 and IL-10 production and attenuated elevations in MCP-1 and TNF-α (Fig. 7). As such, NK-1R antagonist treatment appears to limit streptococcal neuroinflammation by simultaneously inhibiting proinflammatory mediator expression and limiting decreases in immunosuppressive cytokine production.

**Discussion**

Bacterial infections of the CNS constitute a group of highly damaging and often life-threatening diseases that have shown dramatic increases in incidence over the last two decades. What makes the etiology of these diseases so perplexing is that severe CNS inflammation can be initiated by bacterial species that are generally regarded to be of low virulence (25). Although such responses may represent protective immune responses to certain pathogens, inflammation elicited by infectious agents often results in progressive CNS damage. A hallmark of developing immune responses is the synergistic interaction between cells and their products that can amplify the response. Such amplification and positive-feedback loops serve to recruit cells to the site of infection while promoting activation signals that propagate the response. It is now widely accepted that SP can exacerbate the responses of myeloid immune cells via high-affinity NK-1Rs (7). SP is found throughout the CNS (8), and in the current study, we have confirmed the robust expression of this neuropeptide in the brains of both infected and uninfected mice. Furthermore, glial cells have long been known to express NK-1Rs (12, 26, 27), and in this study, we have determined that such expression is markedly upregulated in microglia following in vivo bacterial challenge. Importantly, we have recently demonstrated that SP can augment glial inflammatory immune responses to *Neisseria meningitidis* and *Borrelia burgdorferi* (11). Consistent with this observation, we have shown that endogenous NK-1/SP interactions are required for maximal neuroinflammation and CNS damage following in vivo Gram-negative bacterial infection.

In the current study, we have confirmed that endogenous SP/NK-1R interactions are similarly required for maximal in vivo inflammatory immune responses to the principle Gram-positive causative agent of meningitis, *S. pneumoniae*. We have demonstrated that bacterially induced gliosis is markedly reduced in mice following prophylactic NK-1R antagonist treatment, and infection-induced changes in BBB permeability are abolished following such treatment or in animals genetically deficient in the expression of the NK-1R. In addition, we have assessed the effect of this antagonist on bacterially induced changes in locomotor activity as an index of neurologic impairment. We show that *S. pneumoniae* infection elicits a marked increase in locomotor activity, and this effect is consistent with previous demonstrations of hyperactivity in mice following CNS infection with some bacterial and viral pathogens (19, 28, 29), which may correlate with the long-term hyperactivity observed in children following bacterial meningitis (30). Importantly, we have found that L703,606 was
bacterial pathogens (11). In the current study, we show that SP can also augment the types such as macrophages and dendritic cells (6, 7). However, SP can only augment glial responses in a similar manner to that seen in macrophages. For example, SP was reported to be insufficient to induce IL-1 production by murine microglia, but synergistically augments LPS-induced IL-1 production (34). In addition, stereotaxic injection of SP into the brainstem has been reported to increase IFN-γ-mediated MHC class II upregulation in parenchymal microglia (40). Furthermore, SP has been reported to augment LPS-induced TNF-α production by astrocytes (26) and can enhance the ability of IL-1β to induce the production of IL-6 and PGE2 by these cells (41). Finally, the present findings are in agreement with our own more recent studies demonstrating that SP augments inflammatory cytokine production and prostanoid synthesis by murine glia in response to Gram-negative bacterial pathogens (10, 11).

Nonpeptide NK-1R antagonists are known to exert central effects (42), and inhibitors of this tachykinin receptor have been the subject of extensive study for the clinical treatment of depression and anxiety (43–46). In the current study, we have provided evidence that therapeutic intervention with a specific NK-1R inhibitor can ameliorate inflammation and disease signs in a murine model of established streptococcal meningitis. We have shown that systemic L703,606 administration attenuates S. pneumoniae-induced increases in inflammatory cytokine levels within the CNS while preventing decreases in immunosuppressive cytokine production. Such an effect is consistent with the ability of this NK-1R antagonist to abolish the astrogliosis observed following bacterial infection and its ability to attenuate S. pneumoniae-induced neuronal damage as assessed by the degree of demyelination. Furthermore, we have shown that NK-1R antagonist treatment reverses infection-associated effects on body weight and prevents the development of hypothermia in S. pneumoniae-challenged animals. Interestingly, and in contrast to prophylactic treatment and our studies in NK-1R−/− animals, therapeutic L703,606 administration following infection failed to significantly alter BBB permeability. Although the reason for this is unclear, it should be noted that changes in BBB permeability occur rapidly following bacterial administration, and so the inability of this NK-1R antagonist to reverse this effect may reflect a role for SP in initiating increases in BBB permeability rather than maintaining such changes. Importantly, pharmacological inhibition of the NK-1R does not significantly affect bacterial burden within the CNS, suggesting that the ability of this agent to attenuate as the bacterial stimuli. Although the reasons for this difference are presently unclear, it is interesting to note that we have previously found that SP augments inflammatory mediator production by microglia exposed to B. burgdorferi lysates, but it failed to enhance astrocyte responses to this stimulus (11). In addition, it should be noted that although exogenous SP significantly augmented microglial responses to S. pneumoniae, these in vitro effects were somewhat modest compared with the in vivo effects of negating SP/NK-1R interactions. This discrepancy may result, in part, from a direct effect of SP on infiltrating leukocytes such as macrophages. Alternatively, it is possible that our in vitro experiments underestimate the importance of this neuropeptide, as they do not mimic chronic SP effects on glial cell function. Finally, the use of isolated cells may negate the cumulative effect that SP could have on microglia and astrocyte responses and the cross-talk that is likely to occur in vivo between these cell types and/or infiltrating leukocytes. These issues are the subjects of ongoing investigations in our laboratory.

The present study is in agreement with previous work demonstrating that SP can exacerbate the immune responses of activated glial cells. Although some evidence exists that SP is a full and sufficient stimulus to induce microglial and astrocyte responses (33–39), several studies indicate that SP can only augment glial responses in a similar manner to that seen in macrophages. For example, SP was reported to be insufficient to induce IL-1 production by murine microglia, but synergistically augments LPS-induced IL-1 production (34). In addition, stereotaxic injection of SP into the brainstem has been reported to increase IFN-γ-mediated MHC class II upregulation in parenchymal microglia (40). Furthermore, SP has been reported to augment LPS-induced TNF-α production by astrocytes (26) and can enhance the ability of IL-1β to induce the production of IL-6 and PGE2 by these cells (41). Finally, the present findings are in agreement with our own more recent studies demonstrating that SP augments inflammatory cytokine production and prostanoid synthesis by murine glia in response to Gram-negative bacterial pathogens (10, 11).

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neuroinflammation does not appear to significantly compromise the ability of the host to mount protective immune responses against this pathogen. As such, our studies suggest that the targeting of SP/NK-1R interactions may represent a new therapeutic strategy for the treatment of bacillary induced CNS diseases.

Taken together, the current study indicates that SP is an important component in the generation of Gram-positive bacteria-induced CNS inflammation and suggests that this neuropeptide may contribute to the inflammatory neuronal damage that is often disproportionate to the bacterial stimulus. Although it remains to be seen whether the net result of such exacerbation is helpful or harmful to the host, the present observation that pharmacological inhibition of SP/NK-1R interactions fails to significantly alter the bacterial burden within the CNS while attenuating meningitis-like disease severity would appear to support the latter conclusion. Accordingly, the ability of a systemically administered NK-1R antagonist to limit Streptococcus pneumococcal meningitis-like disease may represent an exciting new therapeutic target for bacillary induced CNS disorders.

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Disclosures
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