Anti–Interleukin-6 Antibodies Attenuate Inflammation in a Rat Meningitis Model

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Abstract. **Objectives:** Interleukin-6 (IL-6) is elevated in the cerebrospinal fluid (CSF) of humans and animals with bacterial meningitis. This study's hypothesis was that anti-IL-6 antibodies will attenuate meningeal inflammation in a rat model of bacterial meningitis. **Methods:** 14 male Sprague-Dawley rats were inoculated intracisternally (IC) with 0.1 mL of heat-killed pneumococci. At one hour post-inoculation, the rats received intraperitoneal doses of either 1.0 mL phosphate-buffered saline (PBS treatment group, n = 7) or 70 μg anti-IL-6 antibodies in 1.0 mL PBS (anti-IL-6 antibody treatment group, n = 7). Nine rats (normal group, n = 9) had no inoculation, and four rats (surgical sham group, n = 4) had IC inoculations of saline. At six hours post-inoculation, all the animals had CSF removed via IC tap. The CSF protein and white blood cell (WBC) count measures were compared using a t-test. **Results:** Mean CSF WBC for the anti-IL-6 treatment group was 2,458/μL, versus the PBS controls' mean of 9,697/μL ($p = 0.007$). Mean CSF protein for the anti-IL-6 group was 180 mg/dL, versus 296 mg/dL for the controls ($p = 0.032$). The surgical sham and normal animals had normal CSF WBC and protein values. **Conclusions:** In this rat meningitis model, systemic treatment with anti-IL-6 antibodies after the induction of meningitis suppressed both CSF WBC count and CSF protein level, two important indices of meningeal inflammation.
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BACTERIAL meningitis is a serious central nervous system infection that carries significant mortality (5–40%) and neurologic morbidity, including hearing loss, hydrocephalus, mental retardation, and seizures.[1,2] The pathogenesis of this infection involves invasion of the cerebrospinal fluid (CSF) either through the blood–brain barrier by hematogenous pathogens, or by inoculation via direct extension from a parameningeal focus of infection. This invasion is followed by the induction of an intense inflammatory response mediated by a cascade of hormone-like proteins called cytokines. The morbidity of bacterial meningitis is due, in part, to damage from cytokine-mediated release of proteolytic enzymes, toxic free-radicals, and alterations in cerebral metabolism and blood flow.[1]

It has been demonstrated that bacterial cell walls stimulate the release of interleukin-1 (IL-1) and tumor necrosis factor–alpha (TNF-α), which are thought to play important early roles in the initiation of the inflammatory cascade in meningitis, including opening of the blood–brain barrier and cellular toxicity. Both IL-1 and TNF-α are found in high levels in bacterial meningitis in both animals and humans. They, in turn, induce the release of other inflammatory cytokines such as interleukin-6 (IL-6) and interleukin-8. Interleukin-6 is a product of monocytes, endothelial cells, and astrocytes, and is responsible, in part, for complement activation and release of acute phase proteins, as well as inhibition of TNF-α and IL-1.[1,3]

Current treatment of acute bacterial meningitis includes parenteral antibiotics and sometimes corticosteroids to reduce the host's inflammatory response. Recent therapeutic investigations in several different animal models have focused on treatment of various infectious diseases with anti-cytokine therapies, using monoclonal antibodies directed against a particular inflammatory cytokine to attenuate the inflammatory cascade.[4,5]

The present study used a pneumococcal meningitis model in rats to assess the effects of a selective modification of the immunologic response by neutralizing one particular cytokine, IL-6. The activity of IL-6 can be suppressed by the injection of anti-IL-6 antibodies.[4] Our hypothesis was that systemically administered anti-IL-6 antibodies would attenuate meningeal inflammation, as measured by CSF total white blood cell (WBC) count and CSF total protein (TP) level.

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TABLE 1. Cerebrospinal Fluid Results

Group	Number of Animals	WBC/μL (Mean)	(±Standard Error)	Protein (mg/dL) (Mean)	(±Standard Error)
1. Normal	9	2.4	(±2)	28	(±6)
2. Sham	4	7.3	(±4)	50	(±25)
3. Meningitis	7	9,697	(±2,151)	296	(±47)
4. Anti-interleukin-6	7	2,548*	(±573)	180†	(±12)

*p = 0.007, t-test, groups 3 vs 4.
†p = 0.032, t-test, groups 3 vs 4.

Interleukin-6 was chosen as the target for suppression for several reasons. This cytokine is thought to have an important role in the development of the inflammatory cascade in meningitis.[1,3,6] It is known to induce fever and promote the release of acute-phase proteins (such as C reactive protein), to mobilize neutrophils, and to stimulate T-cell and B-cell growth and differentiation.[1,7] Interleukin-6 levels are substantially elevated in bacterial meningitis in humans and animals, while lower levels are found in viral meningitis.[3,6,7] Previous studies have shown that CSF IL-6 titers are significantly correlated with CSF WBC count, CSF protein level, and intracranial pressure.[6,8] Selective suppression of IL-6 might, therefore, be useful in attenuating the inflammatory cascade via a mechanism not used in current standard therapies for bacterial meningitis.

METHODS

Study Design. This study compared outcomes by treatment group of rats challenged with intracisternal (IC) doses of inactivated pneumococcal bacteria. Outcome measures included CSF total WBC counts and CSF TP levels. Groups included: 1) controls with no intervention, 2) controls with a surgical sham procedure and saline IC injection, 3) a group given pneumococcal meningitis intervention only, and 4) active treatment group given pneumococcal meningitis followed by systemic administration of anti-IL-6 antibodies. Care and handling of animals adhered to the *Guide for the Care and Use of Laboratory Animals* published by the U.S. National Institutes of Health (NIH Publication No. 85-23, revised 1996), and the study was approved by the institutional animal care and use committee.

Animal Subjects and Preparation.

Bacterial Preparation and Inoculation. Unencapsulated *Streptococcus pneumoniae* type 3 bacteria were grown in culture, and then heat-killed at 60°C for four hours. This was then diluted to a concentration of 10^8 colony-forming units (CFU)/mL with phosphate-buffered saline (PBS), using photometric measurement to determine density, which correlates with bacterial concentration. Unencapsulated pneumococci were used because it has been noted that heat-killed encapsulated organisms do not reliably induce meningitis after IC injection.[9]

Animal Preparation. Adult male Sprague-Dawley rats weighing 250–350 grams were anesthetized with pentobarbital (60 mg/kg intraperitoneally) prior to procedures. The cisterna magna was surgically exposed using a longitudinal incision just below the occiput. An inoculum of 0.1 mL of inactivated bacteria was injected into the cisterna magna of the rats in groups 3 and 4, at time 0.

Study Protocol. The rats were divided into four treatment groups. Group 1 received no intervention. They had CSF withdrawn by needle aspiration from a surgically exposed cisterna magna. Group 2 (surgical sham group) had the cisterna magna surgically exposed, followed by IC injection of 1.0 mL of PBS only. Group 3 (meningitis group) had IC injection of 0.1 mL of heat-killed bacteria at time 0, and needle aspiration of CSF at six hours. Group 4 (anti-IL-6 antibody group) had IC injection of 0.1 mL of heat-killed bacteria at time 0, followed by an intraperitoneal dose of 70 μg of anti-IL-6 antibodies (Endogen, Boston, MA) one hour after time 0. Needle aspiration of CSF was performed at six hours.

Outcome Measures. All the animals' CSF WBC counts and TP levels were obtained by Rhode Island Hospital's Central Laboratory using standard equipment. Laboratory personnel were blinded with regard to the status of the animals' study group assignments.

Data Analysis. Group means of CSF WBC and protein were tested for significant differences between the interventional groups by t-test, using Stata (College Station, TX) software.

RESULTS

Results for all groups are summarized in Table 1. A total of nine animals were studied in group 1, which received no intervention. Little or no CSF pleocytosis was observed in these animals, and TP

levels were low. Group 2, which had a surgical procedure to expose the cisterna magna at time 0, followed by "placebo" injection of PBS, had a similarly low degree of pleocytosis. Total protein for group 2 averaged slightly higher than that for group 1, but this difference was not statistically significant (Table 1).

The seven animals in group 3, which were given the heat-killed pneumococci and no further intervention, had a pronounced leukocytosis, averaging nearly 10,000 WBCs/μL of CSF, ranging from 2,610/μL to 17,100/μL. The leukocytosis was confirmed by microscopy to consist of almost entirely polymorphonuclear white cells. Total protein levels were also elevated, averaging nearly 300 mg/dL (range 228–571 mg/dL).

Group 4, treated with anti-IL-6 antibody, had lower CSF WBC counts—averaging 2,458/μL (range 450–4,635). This group also had a lower mean CSF TP level of 180 mg/dL (range 131–218 mg/dL). Differences between groups 3 and 4 were calculated using a t-test, with a p-value of 0.007 for CSF WBC count, and 0.032 for protein level.

Discussion

The pathophysiology of bacterial meningitis, and its interplay with a host's response, is an exceptionally complex process involving multiple cytokines and their products. This study attempted to examine the role of one cytokine, IL-6, in a rat model. The results demonstrate that anti-IL-6 antibodies, administered systemically, can suppress inflammation as measured by CSF WBC count and TP level in this model.

Our findings support the notion that IL-6 plays an important role in the development of an inflammatory response in meningitis. The data suggest that selective suppression of IL-6 with systemically administered antibodies to this cytokine will attenuate the inflammatory response as measured by CSF WBC count and protein level. The CSF WBC count and protein level have been used as surrogate markers for the effectiveness of other experimental therapies for bacterial meningitis.[8–10] The white cells reflect, to some degree, the intensity of a host's inflammatory response to a pathogen, and the protein reflects the degree of cell damage from proteolytic enzymes, toxic free-radicals, and other inflammatory mediators.

This study used a rat meningitis model similar to that used in previous studies.[9,10] Although other animal models for meningitis have been used, Koedel et al.[8] used the present model to demonstrate that intraperitoneally injected IL-10, an immunosuppressive cytokine, suppressed indices of inflammation such as CSF WBC count, brain water content, and intracranial pressure, in conjunction with suppression of IL-6. They speculated that IL-10 may work in part by suppression of production of IL-6. We therefore used a similar model in this study to focus on the selective suppression of IL-6 in meningitis.

This was not the first study to examine the effect of selective suppression of IL-6 on an animal model of infectious disease. Starnes et al.[5] investigated the effects of anti-IL-6 antibody in a murine model of lethal *Escherichia coli* infection. Anti-IL-6 antibodies were administered to mice after lethal doses of both *E. coli* and TNF-α, and results demonstrated improved survival relative to untreated controls for both challenges. Gennari and Alexander[4] also demonstrated improved survival using the anti-IL-6 antibodies in a murine study of *E. coli* sepsis using a thermal injury model. To the best of our knowledge, no study has been conducted with anti-IL-6 antibodies in an animal model of meningitis.

Clinical Relevance

This study assessed the effect on inflammation indices of a systemic cytokine suppression treatment in rats induced with meningitis. The treatment used was an antibody to IL-6, and its effect in this model was to reduce CSF WBC counts and TP levels. Although these preliminary findings suggest a possible role for IL-6 suppression in certain cases of bacterial meningitis, it is important to emphasize that the reduction of the CSF leukocytosis and protein might not correlate with clinical benefit. Since the outcome measures in this experiment did not include survival time or any other physiologic measure of morbidity, the actual therapeutic effects of anti-IL-6 antibodies in rats or other species have yet to be proven.

Limitations and Future Questions

Several other limitations of this study should be noted. The effect of systemic anti-IL-6 antibodies on CSF IL-6 levels was not measured in this experiment. It would be useful to confirm that the antibodies actually worked by neutralizing the animals' levels of IL-6. Also, most meningitis is septic and is not due to a direct inoculation. The response to a direct inoculum of a large quantity of heat-killed bacteria may differ significantly from the response to the slow introduction of live bacteria through the blood–brain barrier. It should be noted that cytokine responses in meningitis also vary according to inciting organism, and that the results of experiments such as this one could differ if an organism such as *Haemophilis influenzae* or *Neisseria meningitidis* were used. Finally, the pathogenesis of bacterial meningitis is sufficiently

complex so that the elimination of other pro-inflammatory cytokines (for example, IL-1), augmentation of other inflammatory cytokines, and detailed measurement of morbidity and mortality are necessary before conclusions can be made about the therapeutic use of cytokines or antibodies to them. This is an area in which future research may be fruitful.

CONCLUSIONS

In this rat model of pneumococcal meningitis, systemically administered anti-IL-6 antibodies appeared to attenuate two important indices of inflammation: cerebrospinal fluid total white blood cell count and cerebrospinal fluid total protein level.

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REFLECTIONS

Breakpoint for Forgiveness

Revised dictations and cautiously edited progress notes
Altering the truth would be needed to reliably decree
All our E.D. brothers and sisters to be guilt free.
Many unclothed of self-credited lies we'd facilely demote.
Why do we each define the boundaries of our acceptance?
How can one justify a toiler distracted by family tie,
Or fatigued from illogic shift forward or infirmity,
And residents moonlighting for monetary reward
Then shun the alcohol or drug addicted provider?
All these compatriots are impaired and jeopardous.
Let our maker determine the specificity for misdeed.
I'll accept uncontrolled field testing of the Almighty
Less the transgressors who fail to remain current;
The unread should be prematurely accepted into eternity.

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