The Effect of Interleukin-10 on Meningeal Inflammation in Experimental Bacterial Meningitis

Maria M. Paris,* Sheila M. Hickey,* Mónica Trujillo,* Amina Ahmed, Kurt Olsen, and George H. McCracken, Jr.

Interleukin-10 (IL-10) is a cytokine with antiinflammatory effects. In a rabbit model of meningitis, IL-10 was given intracisternally or intravenously to evaluate the impact on inflammation induced by lipooligosaccharide (LOS), *Haemophilus influenzae* type b (Hib), or *Listeria monocytogenes*. Intracisternal IL-10 in concentrations >1 µg significantly reduced tumor necrosis factor-α (TNF-α) and lactate values in cerebrospinal fluid (CSF). Intravenous IL-10 (1 mg/kg) in two doses after intracisternal LOS significantly reduced CSF TNF-α and lactate. When Hib was used, animals were treated with ceftriaxone and dexamethasone with or without IL-10 (1 mg/kg). TNF-α was significantly reduced in animals treated with IL-10, dexamethasone, or both compared with levels in rabbits receiving ceftriaxone alone. Comparable results were obtained when *L. monocytogenes* was inoculated and animals were treated with ampicillin with or without IL-10, dexamethasone, or nothing. In conclusion, IL-10 modulates CSF TNF-α concentrations in experimental LOS, Hib, or *L. monocytogenes* meningitis. The maximal inhibitory effect was seen when IL-10 and dexamethasone were combined.

Cytokines play an important role in the pathophysiology of meningitis. In animal models [1] and clinical studies [2, 3], we demonstrated strong correlations between tumor necrosis factor-α (TNF-α) and interleukin-1β (IL-1β) and alteration of blood-brain barrier permeability, cerebral edema, and clinical severity of disease in patients with bacterial meningitis. Additionally, concentrations of TNF-α have been correlated with outcome of sepsis in children [4] and of meningococcemia [5]. TNF-α and IL-1α and -1β produce both a local inflammatory response and a systemic vascular endothelial response that includes formation and stabilization of thrombi and production of nitric oxide synthase [6–8].

In recent years, various approaches to modulate the inflammatory response induced by these cytokines have been investigated in animal models. In our laboratory, we demonstrated the modulatory effect of dexamethasone [9, 10], IL-1 receptor antagonist [11], soluble TNF receptor [11], anti-CD18 monoclonal antibody [10], and pentoxifylline [12] on the cerebrospinal fluid (CSF) inflammatory response after intracisternal inoculation of different bacterial strains, homologous cytokines (TNF-α and IL-1β), or endotoxin into rabbits. Both pentoxifylline and anti-CD18 monoclonal antibody alone or combined with dexamethasone decreased inflammation in experimental meningitis [10, 12]. Dexamethasone was later shown in clinical trials to significantly reduce long-term morbidity in children with bacterial meningitis [13–15]. Although dexamethasone can improve the outcome in children with bacterial meningitis, it is not effective in all cases, especially when antibiotics are administered before steroids.

IL-10, a newly discovered cytokine with antiinflammatory activities, has been shown to be produced by T cells, macrophages, keratinocytes, and B cells [16–21]. Multiple in vitro effects have been reported, including inhibition of the synthesis and gene expression in monocytes and neutrophils of IL-1, TNF, IL-6, IL-8, and colony-stimulating factors [22, 23]. In addition to inhibiting the production of cytokines and nitric oxide, IL-10 prevents macrophage cytotoxic activity [24].

Because IL-10 can decrease cytokine production and is present in CSF of children with meningitis [25–28], we evaluated its potential as an antiinflammatory agent in the treatment of experimental meningitis induced by lipooligosaccharide (LOS), *Haemophilus influenzae*, or *Listeria monocytogenes*. In addition, the antiinflammatory effect of IL-10 alone or in combination with dexamethasone was compared with that of dexamethasone alone in this model.

Materials and Methods

*H. influenzae* type b (Hib) lipooligosaccharide (LOS) was provided by Eric Hansen (Department of Microbiology, University
of Texas Southwestern Medical Center at Dallas) and prepared as described previously [29, 30].

**Bacterial strains.** Hib strain DL 42 was isolated from CSF of a child with meningitis [9]. About 10^6 cfu/mL in log-phase growth diluted in 0.5 mL of pyrogen-free PBS was inoculated intracisternally.

*L. monocytogenes* JS-402 was isolated from CSF of an infant with meningitis. A few colonies of *L. monocytogenes* from an overnight culture were added to 5 mL of brain-heart infusion broth and incubated for 9 h at 35–37°C in room air. The inoculum was centrifuged at 5000 g at 5°C for 10 min and washed with endotoxin-free PBS twice. About 10^5 cfu/mL diluted in 0.5 mL of pyrogen-free PBS was intracisternally inoculated.

IL-10. Recombinant human IL-10 was provided by Schering-Plough Research Institute (Kenilworth, NJ). The biologic potency was 80.5 × 10^6 RU/mL. The intracisternal inoculation of IL-10 at different concentrations produced minimal inflammation that was similar to that after pyrogen-free PBS.

**Antibiotics and corticosteroids.** Ceftriaxone (100 mg/kg; Hoffman-La Roche, Nutley, NJ), ampicillin (50 mg/kg; Bristol-Myers Squibb, Princeton, NJ), and dexamethasone (1 mg/kg; Luitpold Pharmaceutical, Princeton, NJ) were used.

**Animal model.** We used our well-characterized animal model of meningitis [1, 2, 31] originally described by Dacey and Sande [32] using New Zealand White male rabbits. Before each procedure, animals received ketamine (50 mg/kg) and acepromazine (3 mg/kg) intramuscularly. At designated times, 200–300 µg/mL CSF was withdrawn. At the end of the experiments, animals were sacrificed by intravenous pentobarbital (50 mg/kg). Two hundred rabbits were used in these studies, 4–8 rabbits for each group.

Four groups of experiments were performed. First, LOS (20 ng) and IL-10 at different concentrations (400 pg–100 µg) were inoculated intracisternally. At time 0, animals received LOS and IL-10 intracisternally. A second dose of IL-10 was repeated in some experiments 1 h later. CSF was withdrawn before and at 2, 4, 6, 9, and 24 h after the initial inoculation.

**Results**

**Intracisternal inoculation of LOS and IL-10.** Increasing concentrations of IL-10, ranging from 400 pg to 100 µg, were

![Table 1. Cerebrospinal fluid tumor necrosis factor-α (TNF-α) concentrations in animals receiving different concentrations of IL-10 intracisternally given concomitantly with lipooligosaccharide.](https://academic.oup.com/jid/article-abstract/176/5/1239/831438)

<table>
<thead>
<tr>
<th>IL-10</th>
<th>2 h</th>
<th>4 h</th>
<th>6 h</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>1890  (543)</td>
<td>73 (527)</td>
<td>72 (37)</td>
</tr>
<tr>
<td>400 pg</td>
<td>1163  (791)</td>
<td>147 (36)</td>
<td>30 (14)</td>
</tr>
<tr>
<td>1 ng</td>
<td>856   (724)*</td>
<td>218 (70)</td>
<td>64 (28)</td>
</tr>
<tr>
<td>10 ng</td>
<td>1695  (68)</td>
<td>560 (470)</td>
<td>79 (61)</td>
</tr>
<tr>
<td>100 ng</td>
<td>1787  (496)</td>
<td>337 (259)</td>
<td>113 (34)</td>
</tr>
<tr>
<td>1 µg</td>
<td>989   (416)</td>
<td>320 (101)</td>
<td>211 (137)</td>
</tr>
<tr>
<td>10 µg</td>
<td>215   (113)*</td>
<td>130 (61)*</td>
<td>108 (20)</td>
</tr>
<tr>
<td>10 µg × 2 doses</td>
<td>89   (62)*</td>
<td>42 (11)*</td>
<td>50 (11)</td>
</tr>
<tr>
<td>100 µg</td>
<td>69    (23)*</td>
<td>55 (13)*</td>
<td>39 (9)</td>
</tr>
</tbody>
</table>

**NOTE.** Data are mean (SD). Second dose of 1 mg/kg IL-10 was given to some rabbits 1 h after initial inoculation.

*P ≤ .05 vs. control.

1 P ≤ .05 vs. others.

Next, LOS (20 ng) was inoculated intracisternally and IL-10 at different concentrations (100 µg–5 mg/kg) was administered intravenously. At time 0, animals received LOS and IL-10. A second dose of IL-10 was administered in some experiments 1 h later. CSF was withdrawn before and at 2, 4, 6, 9, and 24 h after LOS administration.

The next experiments involved intracisternal inoculation of 10^6 cfu/mL Hib and intravenous treatment with ceftriaxone (100 mg/kg), IL-10 (1 mg/kg, two doses 1 h apart), and/or dexamethasone (1 mg/kg). Treatment was initiated 6 h after the intracisternal inoculation of bacteria. CSF was withdrawn before and at 6, 8, 10, 12, and 20 h after Hib inoculation.

The final group of experiments involved intracisternal inoculation of 10^5 cfu/mL *L. monocytogenes* and intravenous treatment with ampicillin (50 mg/kg, two doses 6 h apart), IL-10 (1 mg/kg, two doses 1 h apart), and/or dexamethasone (1 mg/kg). Some animals received no treatment. Treatment was given 14 h after inoculation. CSF samples were withdrawn before and at 14, 16, 20, 24, and 36 h after intracisternal inoculation of *L. monocytogenes*.

Leukocyte concentrations in CSF were determined with a Neubauer hemocytometer (American Optical, Buffalo, NY). Bacterial concentrations were quantified by plating serial dilutions of CSF onto chocolate agar and sheep blood plates when Hib and *L. monocytogenes*, respectively, were inoculated. The remaining CSF was centrifuged at 2500 g for 10 min, and the supernatant was stored at −70°C for further studies.

**TNF-α activity was measured by a cytotoxic assay using L929 cells as previously described [33]. The lower amount of TNF-α detected was 39 pg/mL. Lactate concentrations were measured by a kinetic enzymatic method [1, 34].

**Statistical analysis.** Kruskal-Wallis analysis of variance (Newman-Keuls multiple comparisons test) and one-way analysis of variance (Newman-Keuls multiple comparison test) were used for nonparametric and parametric data, respectively. P ≤ .05 was considered significant.
Table 3. Cerebrospinal fluid lactate concentrations in rabbits inoculated with lipooligosaccharide intracisternally and treated intravenously with different dosages of IL-10.

<table>
<thead>
<tr>
<th>IL-10</th>
<th>2 h</th>
<th>4 h</th>
<th>6 h</th>
<th>9 h</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>26.7 (8.0)</td>
<td>51.3 (6.9)</td>
<td>56.5 (16.4)</td>
<td>44.1 (5.6)</td>
</tr>
<tr>
<td>100 µg/kg</td>
<td>25.8 (5.3)</td>
<td>49.4 (9.5)</td>
<td>66.1 (9.5)</td>
<td>49.2 (11.4)</td>
</tr>
<tr>
<td>1 mg/kg</td>
<td>28.3 (4.8)</td>
<td>46.3 (5.5)</td>
<td>45.8 (2.7)</td>
<td>40.2 (5.4)</td>
</tr>
<tr>
<td>5 mg/kg</td>
<td>22.8 (3.1)</td>
<td>33.1 (3.9)</td>
<td>45.9 (7.0)</td>
<td>37.4 (4.1)</td>
</tr>
<tr>
<td>1 mg/kg x 2 doses</td>
<td>16.7 (3.8)</td>
<td>33.8 (3.5)</td>
<td>33.1 (3.2)</td>
<td>37.9 (7.3)</td>
</tr>
</tbody>
</table>

NOTE. Data are mean (SD). Second dose of 1 mg/kg IL-10 was given to some rabbits 1 h after initial inoculation.  
* P ≤ .05 vs. control, 100 µg, 1 mg.  
† P ≤ .05 vs. control, 100 µg.  
‡ P ≤ .05 vs. control, 100 µg.

Intracisternally inoculated simultaneously with 20 ng of LOS (table 1). White blood cell concentrations between groups were not significantly different (data not shown). Although IL-10 concentrations as low as 1 ng produced some modulation of CSF TNF-α, higher amounts were required to significantly reduce TNF-α concentrations. Lactate concentrations in CSF were also lower in animals treated with large amounts of IL-10, but the differences for the different dosages were not significant except for 6-h values for ≥1 µg of IL-10. Rabbits receiving two doses of IL-10 (at the same time and 1 h after the initial LOS inoculation) had the greatest reduction in lactate concentrations.

Intracisternal inoculation of LOS and intravenous administration of IL-10. Increasing concentrations of IL-10 in the range of 100 µg to 5 mg/kg were administered intravenously. Leukocyte concentrations were not statistically different over this dosage range (data not shown). All dosages of IL-10 decreased CSF TNF-α after inoculation of intracisternal LOS.

Figure 1. Bacterial concentrations (log_{10} cfu/mL, mean ± SD) in cerebrospinal fluid of rabbits intracisternally inoculated with ~10^6 cfu/mL *Haemophilus influenzae* and treated intravenously with ceftriaxone (CRO) with or without dexamethasone (DXM) and IL-10 or not treated (control group). Second dose of IL-10 was given 1 h after ceftriaxone. * P ≤ .05 vs. others; † P ≤ .05 vs. control.
The greater suppression was observed when a second dose of IL-10 was given 1 h after the initial LOS inoculation (table 2). Lactate concentrations in CSF were significantly lower in rabbits treated with larger amounts of IL-10 (1 and 5 mg/kg). The largest reduction in lactate concentrations occurred when two doses of IL-10 were given (table 3).

On the basis of results from the above experiments, a dosage of 1 mg/kg IL-10 was chosen for experiments using live organisms.

**Experiments with intracisternal inoculation of H. influenzae.** Treatment with IL-10 was initiated 6 h after the inoculation of bacteria. IL-10 was given intravenously 15 min before and 1 h after administration of ceftriaxone. Five groups were studied: control animals (no treatment), ceftriaxone alone, ceftriaxone + IL-10, and ceftriaxone + dexamethasone + IL-10. Cerebrospinal leukocyte concentrations were similar between groups, and the only significant difference occurred at one time point (20 h) between animals treated with ceftriaxone and those treated with ceftriaxone + IL-10 + dexamethasone (data not shown). The addition of IL-10 or dexamethasone therapy (or both) to ceftriaxone significantly reduced bacterial killing at 12 h (figure 1). Significant reductions in lactate concentrations occurred in animals receiving IL-10 and ceftriaxone compared with concentrations in untreated rabbits (data not shown). Furthermore, the production of TNF-α was significantly reduced when IL-10 was given, and this effect was enhanced when IL-10 was combined with dexamethasone (figure 2).

**Intracisternal inoculation of L. monocytogenes.** Therapy was initiated 14 h after intracisternal inoculation. Six treatment groups were included: dexamethasone, IL-10, ampicillin alone, ampicillin + dexamethasone, ampicillin + IL-10, and ampicillin + dexamethasone + IL-10. There was also an untreated group. A second dose of IL-10 and ampicillin was given 1 and 6 h after initial treatment, respectively. No significant differences in leukocyte concentrations for the treatment groups were seen (data not shown). Significant differences in CSF bacterial concentrations were observed between ampicillin-treated and nontreated rabbits and at 24 h between control and dexametha-

![Figure 2](https://academic.oup.com/jid/article-abstract/176/5/1239/831438)
sone-treated animals (figure 3). In ampicillin-treated animals, administration of IL-10 or dexamethasone did not adversely affect bacterial concentrations. TNF-α concentrations were significantly reduced in animals treated with ampicillin + IL-10 and/or dexamethasone (figure 4). Decreases in TNF-α occurred when IL-10 was combined with dexamethasone. Lactate concentrations were significantly lower in animals treated with ampicillin alone or in combination with IL-10 or dexamethasone (or both) (table 4).

Discussion

IL-10 has been detected in serum and CSF of patients with sepsis and bacterial meningitis [25–28, 35, 36]. In septic shock, serum concentrations of IL-10 correlated with TNF-α concentrations and with severity of disease. Furthermore, IL-10 concentrations were significantly higher in patients with shock [25, 35, 36]. Serum IL-10 can be detected in healthy subjects only in very low concentrations [36]. In patients with bacterial meningitis, IL-10 was found in CSF [25–28], and concentrations correlated well with TNF-α, IL-6, IL-8, and soluble TNF receptor values in CSF [28]. IL-10 is not detected in CSF of healthy controls or patients with viral meningitis [26–28].

IL-10 has immunosuppressive and antiinflammatory properties. This cytokine indirectly prevents antigen-specific T cell proliferation. By inhibiting IL-2 from T cells, IL-10 down-regulates the production of proinflammatory cytokines and chemokines by monocytes and neutrophils [37]. Because these effects are potentially beneficial for treatment of diseases such as meningitis, in which high concentrations of proinflammatory cytokines have been significantly correlated with adverse outcome [2, 3], we evaluated IL-10 in our animal model of meningitis. In these experiments, IL-10 significantly reduced TNF-α and lactate concentrations induced by intracisternal inoculation of LOS, Hib, and L. monocytogenes. This modulation was greater when IL-10 was given in combination with dexamethasone. Previously in this animal model of meningitis, we demonstrated that TNF-α was released into CSF immediately after intracisternal injection of endotoxin or Hib, and this was followed by activation and migration of leukocytes into CSF, enhancement of blood-brain barrier permeability, and changes in CSF hydrodynamics, cerebral metabolism, and coagulation pathways [1, 10].

Figure 3. Bacterial concentrations (log_{10} cfu/mL, mean ± SD) in cerebrospinal fluid of rabbits intracisternally inoculated with ~10^7 cfu/mL Listeria monocytogenes and treated intravenously with ampicillin (AMP), dexamethasone (DXM), or IL-10 alone and in combinations. Second dose of IL-10 and AMP was given 1 and 6 h after initial therapy. * P ≤ .05 vs. rabbits that did not receive ampicillin.
Figure 4. Tumor necrosis factor-α concentrations (pg/mL, mean ± SD) in cerebrospinal fluid of rabbits that were intracisternally inoculated with ~10^5 cfu/mL Listeria monocytogenes and treated intravenously with ampicillin (AMP), dexamethasone (DXM), or IL-10 alone and in combinations. Second dose of IL-10 and AMP was given 1 and 6 h after initial therapy. * P ≤ .05 vs. others; † P ≤ .05 vs. AMP alone and non–AMP-treated rabbits (nontreated, DXM- and IL-10–treated); ‡ P ≤ .05 vs. non–AMP-treated rabbits (nontreated, DXM- and IL-10–treated).

Table 4. Cerebrospinal fluid lactate concentrations in rabbits intracisternally inoculated with ~10^5 cfu/mL Listeria monocytogenes and treated intravenously with ampicillin (AMP), dexamethasone (DXM), or IL-10 alone or in several combinations or not treated (control group). A second dose of IL-10 and AMP was given 1 and 6 hours after initial therapy.

<table>
<thead>
<tr>
<th>Group</th>
<th>14 h</th>
<th>20 h</th>
<th>24 h</th>
<th>38 h</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>66.4 (7.4)</td>
<td>95.9 (5.3)</td>
<td>99.4 (12.1)</td>
<td>ND</td>
</tr>
<tr>
<td>DXM</td>
<td>71.0 (12.6)</td>
<td>93.5 (15.7)</td>
<td>88.3 (15.6)</td>
<td>ND</td>
</tr>
<tr>
<td>IL-10</td>
<td>74.0 (6.0)</td>
<td>97.2 (5.6)</td>
<td>91.3 (10.7)</td>
<td>ND</td>
</tr>
<tr>
<td>AMP</td>
<td>67.2 (13.0)</td>
<td>83.2 (7.2)</td>
<td>76.0 (8.8)*</td>
<td>66.9 (2.9)</td>
</tr>
<tr>
<td>AMP + DXM</td>
<td>61.5 (8.4)</td>
<td>81.0 (2.9)</td>
<td>71.1 (8.4)*</td>
<td>55.0 (5.1)</td>
</tr>
<tr>
<td>AMP + IL-10</td>
<td>62.4 (9.4)</td>
<td>84.5 (11.8)</td>
<td>68.6 (6.4)*</td>
<td>60.0 (8.8)</td>
</tr>
<tr>
<td>AMP + IL-10 + DXM</td>
<td>53.8 (9.0)*</td>
<td>76.0 (10.1)*</td>
<td>64.4 (8.4)*</td>
<td>49.3 (6.8)</td>
</tr>
</tbody>
</table>

NOTE. Data are mean (SD). Second doses of IL-10 and AMP were given 1 and 6 h after initial therapy. ND = not done (animals were sacrificed after 24 h).

* P ≤ .05 vs. control, IL-10.
† P ≤ .05 vs. IL-10.
‡ P ≤ .05 vs. AMP.
In animal models of sepsis and peritonitis, IL-10 has been shown to reduce release of TNF and increase survival when animals were challenged with endotoxin [38–40]. This effect was prevented by the coadministration of anti–IL-10 [38]. By contrast, in an experimental pneumonia model using live Klebsiella species, survival was increased when animals received anti–IL-10 [41]. In our experiments, non–antibiotic-treated animals infected with L. monocytogenes had higher bacteria counts in CSF when given dexamethasone or IL-10. Similar findings by Frei et al. [26] of decreased killing of L. monocytogenes by CSF macrophages when exposed to IL-10 support the contention that TNF-α enhances macrophage intracellular bacterial activity. Furthermore, in experimental pneumococcal meningitis, IL-10 given intravenously attenuated the increased cerebral blood flow, brain water content, intracranial pressure, and IL-6 concentrations observed in untreated animals [42].

Exogenous administration of IL-10 significantly reduced the TNF-α and lactate concentrations in this model of meningitis when animals were challenged with either Hib or L. monocytogenes. Although administration of IL-10 might be beneficial in modulating the meningeval inflammatory response, it is unlikely to be used clinically, because dexamethasone has been shown to be very effective in this regard and to improve outcome of bacterial meningitis in children [13–15].

References


