Interleukin-10 and Soluble Tumor Necrosis Factor Receptors in Cerebrospinal Fluid of Children with Bacterial Meningitis


The antiinflammatory mediators interleukin (IL)-10 and soluble tumor necrosis factor (TNF) receptors p55 (sTNFR-55) and sTNFR-75 in cerebrospinal fluid (CSF) from 37 children with bacterial meningitis were studied. CSF concentrations of IL-10, sTNFR-55, and sTNFR-75 and of the proinflammatory cytokines TNF-α, IL-6, and IL-8 were markedly elevated and were, with the exception of the sTNFRs, significantly higher in CSF than in serum. CSF concentrations of sTNFR-55 and sTNFR-75 were only associated positively with IL-10 levels. CSF glucose levels correlated highly with levels of IL-10, sTNFR-55, and sTNFR-75 and weakly with TNF-α and IL-6. Cytokine levels in CSF decreased rapidly, while sTNFR levels remained elevated for at least 24 h.

Proinflammatory cytokines (tumor necrosis factor-α [TNF-α], interleukin [IL]-6, IL-8) are released within the cerebrospinal fluid (CSF) compartment of patients with bacterial meningitis upon recognition of bacterial products. The production of proinflammatory cytokines and the extent of the inflammatory response are partly controlled by antiinflammatory compounds, such as IL-10, and naturally occurring antagonists of TNF-α, including soluble extracellular domains of the 55- and 75-kDa membrane-bound TNF receptors (sTNFR-55 and sTNFR-75).

IL-10 is produced by monocytes/macrophages, the Th2 subset of T helper lymphocytes, and B lymphocytes and suppresses the synthesis of proinflammatory cytokines by T cells [1], polymorphonuclear leukocytes [2], and monocytes/macrophages [3]. IL-10 protects mice against TNF-mediated lethality in murine models of endotoxemia [4]. CSF levels of IL-10 are significantly increased in patients with bacterial meningitis, suggesting a role for IL-10 in the control of the inflammatory response in the CSF compartment [5].

The biologic activity of TNF-α is also neutralized by sTNFR-55 and sTNFR-75 [6]. Release of sTNFR-55 and sTNFR-75 may provide a mechanism for modulation of excessive TNF-α activity in response to injury or infection. sTNFRs are shed from the cell surface of polymorphonuclear cells and monocytes in response to many of the same inflammatory stimuli that are known to induce TNF-α [7]. Of interest, IL-10 induces in vitro an increased release of sTNFRs from monocytes [8, 9].

To investigate the role of antiinflammatory compounds (IL-10, sTNFR-55, sTNFR-75) and their relation to proinflammatory cytokines (TNF-α, IL-6, IL-8) in children with bacterial meningitis, serum and CSF levels of these mediators were measured in 37 patients. The kinetics of these mediators in the CSF compartment and the association between mediators and CSF characteristics were also studied.

Methods

Patients and controls. Patients between the ages of 3 months and 18 years diagnosed with bacterial meningitis between August 1992 and September 1994 were included. The patients were admitted to the Departments of Pediatrics of Sophia Children’s Hospital, Zuiderziekenhuis, Reinder de Graaf Gasthuis, or Juliana Children’s Hospital. Bacterial meningitis was defined as the presence of a positive bacterial culture from CSF or a positive blood culture in combination with clinical evidence of meningitis and a CSF white blood cell (WBC) count >10 × 10⁹/L. Patients with prior antibiotic treatment were excluded.

Initial CSF and serum samples were collected in most patients. A subset of patients had a second lumbar puncture done in a randomized fashion at 6, 12, or 24 h after initiation of antibiotic treatment. These patients were treated with intravenous ceftriaxone (150 mg/kg/day in three doses). The remaining patients were treated with cefotaxime or cefotaxime, but a second lumbar puncture was not done.

Paired control samples of serum and CSF were obtained from patients with cancer who were in remission or did not have meningitis. The lumbar punctures in these children were done as part of diagnostic protocols.

Laboratory studies. CSF samples from patients and controls were examined for WBC count and levels of glucose and protein. Samples of blood and CSF were cultured before antibiotic treat-
ment and were processed according to standard procedures. Antibiotic susceptibility was determined by disk diffusion method. CSF and serum samples were stored at -70°C until used for different assays.

Serum and CSF levels of TNF-α, IL-6, IL-8, IL-10, sTNFR-55, and sTNFR-75 were assayed with ELISA kits (Medgenix, Fleurus, Belgium), according to the manufacturer’s instructions, with the following detection limits (lowest positive standard): TNF-α, 15 pg/mL; IL-6, 30 pg/mL; IL-8, 7 pg/mL; IL-10, 11 pg/mL; sTNFR-55, 0.4 ng/mL; and sTNF-75, 1.0 ng/mL.

Statistical analysis. Differences between groups in continuous variables were tested for significance with the Mann-Whitney test. Pearson’s (r) and Spearman’s (r_s) correlation coefficients were used to evaluate the relation between variables. A regression analysis for repeated measurements evaluated the change in concentration of the mediators with time. P ≤ .05 (two-tailed) was considered significant.

Results

Patients and controls. The mean age of the 37 patients was 3.3 years (range, 0.3–13.0). There were 17 boys and 20 girls. The causative pathogens were Haemophilus influenzae (n = 16), Neisseria meningitidis (n = 14), Streptococcus pneumoniae (n = 6), and Fusobacterium necrophorum (n = 1). Twenty-one patients had a second lumbar puncture at 6 (n = 8), 12 (n = 4), or 24 h (n = 9) after initiation of antibiotics. All strains were susceptible to ceftriaxone and cefotaxime.

Control subjects were 10 patients who were in remission from acute lymphatic leukemia. All CSF samples from the controls were sterile and had normal CSF characteristics. Chemotherapy had been discontinued for at least 3 months. The children in the control group were 2.7–16.5 years old.

Proinflammatory cytokines (TNF-α, IL-6, IL-8). Median CSF concentrations of TNF-α, IL-6, and IL-8 during the acute phase of bacterial meningitis were 555, 89,590, and 2604 pg/mL, respectively. These levels were significantly (P < .001) elevated compared with those of control subjects (figure 1). Levels of TNF-α, IL-6, and IL-8 correlated with each other by significantly positive (P < .001) correlation coefficients between .57 and .77.

IL-10 in initial serum and CSF samples. IL-10 was detected in 33 of 35 initial CSF samples from patients with bacterial meningitis (figure 1). CSF IL-10 levels were below the detection limit (11 pg/mL) in all control subjects. The median CSF concentration of IL-10 in patients was 701 pg/mL (range, <11–4000), which was significantly higher than in the control population (P < .001). CSF levels of IL-10 correlated positively with TNF-α (r = .71, P < .001), IL-6 (r = .60, P < .001), sTNFR-55 (r = .53, P = .003), and sTNF-75 (r = .61, P < .001) but not with IL-8 (r = .28, P = .10). Serum levels of IL-10 in 22 patients were also significantly elevated (median, 78 pg/mL; range, <11–20,000) in comparison with levels in the control subjects (median, <11 pg/mL; P < .001).

sTNFR-55 and sTNFR-75 in initial serum and CSF samples. CSF concentrations of both sTNFRs were measured in 31 patients (figure 1). The median CSF level of sTNFR-55 was 3.6 ng/mL (range, 1.1–25.0) versus 0.6 ng/mL (range, <0.4–0.9) in control subjects (P < .001). Median CSF sTNFR-75 concentrations were 13.0 ng/mL (<1.0–140.4) in patients and below the detection limit in control subjects (P < .001).

A positive correlation was observed between sTNFR-55 and sTNFR-75 levels (r = .88, P < .001). CSF levels of sTNFR-55 and sTNFR-75 correlated significantly with IL-10 concentrations (r = .53, P = .003, and r = .61, P < .001, respectively) but not with TNF-α, IL-6, or IL-8 levels. Median serum levels...
of the sTNFRs were significantly higher in patients (n = 23) than in control subjects (sTNFR-55, 6.0 ng/mL [range, 2.3–18.3] vs. 1.5 ng/mL [range, 1.2–2.1], P < .001; sTNFR-75, 19.7 ng/mL [range, 5.3–126.0] vs. 4.1 ng/mL [range, 3.7–4.4], P < .001).

TNF-α, IL-6, IL-8, IL-10, sTNFR-55, and sTNFR-75 in initial paired serum-CSF samples. The median concentrations of TNF-α, IL-6, IL-8, and IL-10 were significantly higher in initial CSF specimens than in serum. In contrast, median concentrations of both sTNFRs were similar in the 23 available pairs of serum and CSF samples on admission (sTNFR-55, 6.0 ng/mL [range, 2.3–18.3] vs. 3.7 ng/mL [range, 1.1–25.0, P = .43]; sTNFR-75, 19.7 ng/mL [range, 5.3–126.0] vs. 13.0 ng/mL [range, <1.0–140.4], P = .88).

Correlation between CSF inflammatory parameters and levels of TNF-α, IL-6, IL-8, IL-10, sTNFR-55, and sTNFR-75. CSF glucose concentrations were highly correlated with levels of IL-10 (r5 = −.87, P < .001), sTNFR-55 (r5 = −.81, P < .001), and sTNFR-75 (r5 = −.85, P < .001), whereas correlations with TNF-α and IL-6 were relatively low. In contrast, IL-8 concentrations were not significantly associated with CSF glucose concentrations. The CSF concentrations of the mediators were, with the exception of IL-8, significantly correlated with the WBC count. CSF levels of protein were significantly associated with concentrations of TNF-α (r5 = .54, P = .002), IL-6 (r5 = .52, P = .002), IL-8 (r5 = .48, P = .006), IL-10 (r5 = .53, P = .002), and sTNFR-75 (r5 = .37, P = .05) but not with sTNFR-55.

Kinetics of TNF-α, IL-6, IL-8, IL-10, sTNFR-55, and sTNFR-75. CSF levels of TNF-α, IL-6, IL-8, and IL-10 declined 46% (95% confidence interval [CI], 39%–57%; P < .001), 52% (95% CI, 39%–63%; P < .001), 58% (95% CI, 45%–67%; P < .001), and 50% (95% CI, 35%–61%; P < .001) per 6 h, respectively. However, levels of the sTNFRs did not significantly decrease but remained markedly elevated, at least during the initial 24 h of treatment (figure 2).

Discussion

In the present study, proinflammatory cytokines (TNF-α, IL-6, IL-8) and antiinflammatory mediators (IL-10, sTNFR-55, sTNFR-75) were markedly elevated in serum and CSF of children with bacterial meningitis. The concentrations of the proinflammatory cytokines were significantly higher in CSF, suggesting a compartmentalized release in the subarachnoid space of patients with bacterial meningitis. This has been reported by others [10].

Antiinflammatory compounds IL-10, sTNFR-55, and sTNFR-75 down-regulate the host response. Administration of recombinant IL-10 effectively inhibits systemic LPS-induced synthesis of proinflammatory cytokines. In addition, IL-10 suppresses the synthesis of nitric oxide and reactive oxygen intermediates, which are involved in the pathophysiology of bacterial meningitis [11]. In agreement with Lehnmann et al. [5], we observed significantly elevated levels of IL-10 in CSF of children with bacterial meningitis. IL-10 is mainly produced in the CSF compartment, since CSF levels are significantly higher than serum levels [5]. Endogenous IL-10 production in the CSF compartment may thus play an important role as a natural regulatory and antiinflammatory cytokine.

We observed significantly elevated levels of sTNFR-55 and sTNFR-75 in CSF and serum of patients with bacterial meningitis, as reported by others [12]. TNF-α induces release of sTNFR-55 and sTNFR-75 from neutrophils in suspension in a time- and dose-dependent manner [13]. The shedding of these receptors and the resultant acute decrease of TNFRs on the cell surface may serve to transiently desensitize cells, thereby providing a mechanism for inhibition of TNF-α activity. This process may have implications in vivo, since soluble receptors may inhibit TNF-α bioactivity by binding to the molecule and preventing ligand binding to the cellular TNFRs. Of interest, levels of sTNFRs in this study were exclusively associated with concentrations of IL-10 but not with concentrations of TNF-α, IL-6, or IL-8. This is in agreement with the recent observation that IL-10 induces an increase in the synthesis and cell-surface turnover of sTNFRs from monocytes [8, 9]. IL-10 thus may suppress TNF activity in the CSF compartment by an inhibition of TNF-α secretion, stimulation of the release of sTNFRs, and down-regulation of the expression of surface TNFRs [8, 9].

In our study, serum and CSF levels of sTNFR-55 and sTNFR-75 were similar. The relatively low CSF levels of sTNFRs may be partly explained by a release of sTNFRs from polymorphonuclear leukocytes before entry of these cells into the CSF compartment. Alternatively, shedding of sTNFRs in the CSF compartment may be lower because of the ~10-fold lower WBC count in CSF than in peripheral blood in these patients. The significantly higher stimulus by TNF-α and IL-10 for release of sTNFRs in the CSF compartment apparently does not compensate for this inequality.

CSF inflammatory characteristics were significantly associated with the levels of inflammatory mediators. CSF glucose levels correlated strongly with levels of IL-10 and IL-6, sTNFR-75, and weakly with levels of TNF-α.

The CSF levels of TNF-α, IL-6, IL-8, and IL-10 decreased rapidly. This feature is similar to that observed in consecutive serum samples of experimental models of bacteremia or endotoxemia and in patients with sepsis [14]. The elevated levels of sTNFR-55 and sTNFR-75 in the present study persisted for a remarkably long time (at least 24 h), as is observed in bacteremia [15]. This may be due to slow clearance from the CSF compartment or to continuing release.

We conclude that IL-10, sTNFR-55, and sTNFR-75 were released along with proinflammatory cytokines in the CSF compartment of patients with bacterial meningitis. IL-10 may have been partly responsible for the release of sTNFRs in the CSF compartment. CSF levels of TNF-α, IL-6, IL-8, and IL-10 rapidly decreased, while CSF levels of the sTNFRs remained elevated.
Figure 2. Kinetics of tumor necrosis factor-α (TNF-α, n = 20) and anti-inflammatory compounds interleukin (IL)-10 (n = 20), soluble TNF receptor p55 (sTNFR-55, n = 19), and sTNFR-75 (n = 19) in cerebrospinal fluid compartments of patients with bacterial meningitis. Dashed lines = detection limits of some mediators.

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Vitamin C for the Treatment of Recurrent Furunculosis in Patients with Impaired Neutrophil Functions

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The effect of vitamin C treatment on 23 patients with a history of recurrent furunculosis with negative nasal cultures was studied. Neutrophil functions (chemotaxis, phagocytosis, or superoxide generation) of 12 patients were significantly lower than those of the matched controls. In this group, treatment with vitamin C (1 g/day) caused a dramatic clinical response as well as a significant improvement of neutrophil functions, reaching values similar to those of the controls. Two patients remained vitamin C-dependent. In the patients with normal neutrophil functions, vitamin C treatment neither affected neutrophil activity nor caused a clinical response. Therefore, patients suffering from recurrent furunculosis with defective neutrophil functions may be treated successfully with vitamin C, contributing to both neutrophil function recovery and a dramatic clinical response.

Materials and Methods

Patients. Forty-one patients (18–47 years old) were referred to the Infectious Disease Clinic with a history of recurrent furunculosis (lasting 6 months to 2 years). All patients had frequent and severe attacks (6–24 episodes/year) in the groin, axilla, face, or back. Repeated cultures from furuncles in these areas were positive for Staphylococcus aureus. All patients received repeated courses of systemic antibiotics, surgical drainage, and various topical cosmetic treatments. None of their family members had similar disorders. Physical examination was unremarkable except for skin scarring. Eighteen patients had positive nasal cultures for S. aureus. They were treated with topical pseudomonic acid and excluded from the study. In the 23 patients with negative nasal cultures, neutrophil functions were studied. These functions in patients were tested only during therapy-free intervals. Age- and sex-matched healthy volunteers, not taking any medication, were selected as controls.

Vitamin C treatment. Vitamin C (1 g/day) was given orally for 4–6 weeks. No other systemic or topical treatments were recommended.

Preparation of neutrophils. Neutrophils at 95% purity were obtained by ficoll-hypaque centrifugation, dextran sedimentation, and hypotonic lysis of erythrocytes, as previously described [5]. Cells were counted and their viability was determined by trypan blue exclusion.

Superoxide generation. The production of superoxide anion was measured as the superoxide dismutase–inhibitable reduction of ferricytochrome c by the microtiter plate technique, as previously described [5].

Chemotaxis. Chemotaxis was assessed on agarose plates according to the method of Nelson et al. [6] and modified as described [5]. Chemotaxis was defined as the ratio of the linear migration toward the chemotactrant, FMLP, and migration toward the control medium, modified Eagle medium.

Phagocytosis. Phagocytosis was determined [5], by microscope, in cells with differential Wright-Giemsa stain as the percent-