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The production of IL-8 in cerebrospinal fluid in aseptic meningitis of children

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SUMMARY

Neutrophils accumulate initially in the cerebrospinal fluid (CSF) of aseptic meningitis, perhaps because of increased levels of granulocyte colony-stimulating factor (G-CSF), macrophage inflammatory protein-1α (MIP-1α), and IL-8 in the subarachnoid space. We studied levels of these cytokines in children with aseptic meningitis using ELISA. When meningeal symptoms existed, IL-8 levels (1399 ± 1600 ng/l, $n = 32$) in the CSF were significantly higher than those either after meningeal symptoms disappeared (61 ± 56 ng/l, $n = 18$) or in controls (44 ± 63 ng/l, $n = 27$) ($P < 0{\cdot}0001$). High levels of IL-8 on admission dropped sequentially. Significant correlations were found between IL-8 levels and either neutrophil counts ($r = 0{\cdot}612$), G-CSF levels ($r = 0{\cdot}873$) or MIP-1α levels ($r = 0{\cdot}623$) in the CSF of the affected patients ($P < 0{\cdot}0001$). IL-8 values in serum were lower than in the corresponding CSF samples from all individuals with meningeal symptoms. The IL-8 mRNA was detectable by reverse-transcribed polymerase chain reaction (PCR)-assisted amplification in fresh leucocytes from the CSF, but not from the peripheral blood of a healthy volunteer. The culture of CSF mononuclear cells produced high levels of IL-8 (~ 2750 ng/l). These data indicate that IL-8 levels rise transiently at the initial stage of aseptic meningitis, and that mononuclear cells that migrate into the CSF are a cellular source of this chemokine. We suppose that IL-8, in addition to G-CSF and MIP-1α, contribute to the localized neutrophil accumulation during the disease.

Keywords IL-8 meningitis neutrophils cerebrospinal fluid children

INTRODUCTION

IL-8 is a C-X-C chemokine that specifically activates human neutrophils and attracts them to acute inflammatory sites [1,2]. In a C-X-C motif, any amino acid exists between two cysteines. Most types of cells produce little, if any, IL-8 constitutively. When stimulated with either IL-1, tumour necrosis factor-alpha (TNF-α) or lipopolysaccharide (LPS), IL-8 is produced predominantly by monocytes, but also by fibroblasts and endothelial cells. This chemokine may play a role in various inflammatory and infectious diseases, e.g. psoriasis, rheumatoid arthritis (RA) and adult respiratory distress syndrome.

A variety of cytokines play critical roles in local inflammatory responses in bacterial and aseptic meningitis [3–6]. We [7–10] and other investigators [3–6,11] showed that levels of the following cytokines increase in the cerebrospinal fluid (CSF) of aseptic meningitis: IL-1, IL-6, IL-10, interferon-gamma (IFN-γ), macrophage inflammatory protein-1α (MIP-1α) and colony-stimulating factors, but not TNF-α. Aseptic meningitis is characterized by an initial accumulation in the CSF of neutrophils followed by an elevation of mononuclear cells [8,9]. It has been suggested that granulocyte colony-stimulating factor (G-CSF) and MIP-1α (a C-C chemokine) function to recruit neutrophils to the subarachnoid space [8,9,11,12]. Previous studies have shown elevated IL-8 levels in the CSF in meningitis, with the elevation being greater for bacterial meningitis than aseptic meningitis [13–18]. Results in aseptic meningitis have been variable; IL-8 levels in the CSF were higher than [13,14] or similar to [15,16] those in subjects without meningitis. Thus, IL-8, in addition to G-CSF and MIP-1α, may act in meningeal inflammatory responses in aseptic meningitis. However, in the CSF during the disease, little is known about IL-8 kinetics and the cellular origin of this chemokine has not yet been identified. In this study on childhood aseptic meningitis, we show that intrathecal IL-8 levels are transiently increased at the initial stage, and mononuclear cells in the CSF are a cellular source of this chemokine.

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SUBJECTS AND METHODS

Study subjects

We studied 27 patients with aseptic meningitis and 27 controls without meningitis (Table 1). They ranged in age from 1 month to 15 years and were admitted to our hospital from June 1991 to March 1994. The patients with the disease fit the following criteria: (i) fever, headache, vomiting and stiff neck; (ii) cell counts >35/μl in the CSF; (iii) sterile CSF found in bacteriologic studies. The pathogens, examined as previously described [8], included echovirus 30 in six patients, mumps virus in six, echovirus 9 in two and uncertain causes in 13 individuals. The symptomatic stage was designated as the period when any of the meningeal symptoms or signs existed, and the recovery stage as the period after all of the symptoms and signs disappeared. The first day of the illness was determined as the day when all of the meningitis symptoms had first occurred. The controls fit the following criteria: (i) cell counts <5/μl in the CSF; (ii) CSF negative for bacteriologic and viral studies. They consisted of two with epilepsy, four with febrile convulsions, three with relapse-free acute lymphoblastic leukaemia, two with Guillain–Barré syndrome, three with tension headache and 12 individuals with fever, vomiting and headache.

Sample collection

We obtained institutional approval from the responsible committee and a fully informed consent from the patients' parents. To reduce the patients' risk throughout this study, we did lumbar punctures only when clinically indicated as previously shown [10]. When neutrophils in the CSF were dominant on admission, discontinuation of antibiotic therapy was decided by an additional puncture at the symptomatic stage. During the recovery stage, three individuals received an additional evaluation of the CSF, since the leucocyte counts in the prior CSF analysis were higher than in the previous tap. The CSF samples were spun down and the supernatant was stored at −30°C [10]. Serum specimens were collected simultaneously [19].

Cell culture

Mononuclear cells were separated by Ficoll–Hypaque gradient centrifugation from the CSF or peripheral blood. The mononuclear cells (1×10^6/ml) were incubated in RPMI 1640 supplemented with 10% fetal calf serum (FCS) for 24 h [10]. The culture supernatant was obtained by centrifugation and stored.

ELISAs for measuring cytokines

Duplicate samples were measured for IL-8 by ELISA (Toray-Fuji Bionics, Tokyo, Japan) as previously reported [20]. Briefly, 50 μl of CSF, serum, culture supernatant or standard IL-8 were dispensed into the plate precoated with a polyclonal antibody against human IL-8. The IL-8 was then sandwiched with an EL139 anti-IL-8 MoAb conjugated with horseradish peroxidase. This ELISA system specifically recognized human IL-8 of both the longer form (77 amino acids) and shorter form (72 amino acids), and its measuring range was 12–300 ng/*l*. Concentrations of G-CSF were assessed by a chemiluminescent enzyme immunoassay and the minimal detectable concentration of this cytokine was 1 ng/*l* (Chugai Pharmaceutical Co., Ltd., Tokyo, Japan) [19]. MIP-1α values were quantified by ELISA (Amersham, Aylesbury, UK) and the lower limits of detection were 6 ng/*l* [9].

Reverse-transcribed polymerase chain reaction

Reverse-transcribed polymerase chain reaction (RT-PCR) was done as previously described [10,21]. Briefly, first-strand cDNA was synthesized from total RNA collected from fresh leucocytes in the CSF or peripheral blood and amplified by PCR. Human IL-8 primers were designed as 5′-AACATGACTTCCAAGCTGGC-3′ (sense, nt 99–118) and 5′-CTGGCATCTTCACTGATTC-3′ (antisense, nt 428–446) [22]. An IL-8 oligonucleotide probe was 5′-TTGAGAGTGGACCACACTGCGCCAACACAG-3′ (nt 265–294) [22]. The primers and the cDNA probe for β-actin were made as previously shown [21]. The reaction consisted of 29 cycles (IL-8) or 21 cycles (β-actin) in a thermal cycler. The PCR products were electrophoresed and transferred onto nylon filters and then hybridized with the probes.

Statistical analysis

Results are expressed as means ± s.d. unless otherwise stated. Variables were transformed to common logarithms before statistical analyses. Probability of a significant difference was determined using the Mann–Whitney *U*-test. Relationships between IL-8 levels and other indices were assessed using Pearson's correlation coefficients. Differences were considered significant when the two-tailed *P* value was <0·05.

RESULTS

Study subjects

Gender and age were not significantly different in the three groups (Table 1). Counts for total leucocytes, neutrophils and mononuclear cells in CSF samples from meningitis were significantly higher than those from controls. Levels of glucose and protein in the CSF during the symptomatic stage were not significantly different from those in controls.

IL-8 levels in the CSF

IL-8 was detected in all CSF samples from patients with aseptic meningitis and in 25 of 27 samples from controls (Table 2). When meningeal symptoms existed, IL-8 levels in the CSF were high (1399 ± 1600 ng/*l*). These values were significantly higher than those either during the recovery stage or in controls ($P < 0{\cdot}0001$). After meningeal symptoms disappeared, IL-8 levels in 16 of 18 samples were low (<170 ng/*l*, the mean (44) + 2 s.d. (2 × 63) of controls). Figure 1 illustrates the kinetics of IL-8 levels in the CSF in aseptic meningitis. Elevated levels of IL-8 were found in 25 of

Table 1. Clinical characteristics of study subjects

	Aseptic meningitis		
	Symptomatic stage	Recovery stage	Non-meningitis (controls)
Patient no. (M/F)	27 (19/8)		27 (17/10)
Age (years)	6·8 ± 3·9		6·6 ± 5·1
CSF samples (*n*)	32	18	27
Leucocytes (10^6/*l*)	154 ± 240†‡	68 ± 85‡	2 ± 2
Neutrophils (10^6/*l*)	66 ± 152*‡	16 ± 47‡	1 ± 1
MNC (10^6/*l*)	87 ± 201‡	52 ± 48‡	2 ± 2
Glucose (mmol/*l*)	3·7 ± 1·2†	3·2 ± 0·4	3·5 ± 0·8
Protein (g/*l*)	0·20 ± 0·11	0·17 ± 0·10	0·18 ± 0·20

Data are expressed as the means ± s.d. MNC, Mononuclear cells.

*$P < 0{\cdot}001$; †$P < 0{\cdot}05$ (symptomatic stage *versus* recovery stage); ‡$P < 0{\cdot}001$ (meningitis *versus* non-meningitis).

Table 2. IL-8 levels in the cerebrospinal fluid (CSF) and serum

	Aseptic meningitis		
	Symptomatic stage	Recovery stage	Non-meningitis (controls)
CSF (ng/*l*)	1399 ± 1600 (32)	61 ± 56 (18)*	47 ± 64 (25) *†
			<12 (2)
Serum (ng/*l*)	32 ± 14 (19)*	33 ± 25 (6)	17 ± 10 (5)
			<12 (5)
Culture medium	2315 ± 715 (7)	ND	ND

Data are expressed as the means ± s.d. (*n*). Culture medium was produced from CSF mononuclear cells in aseptic meningitis. ND, not done.
*$P < 0{\cdot}0001$ *versus* CSF at symptomatic stage of aseptic meningitis; †$P = 0{\cdot}0269$ *versus* CSF at recovery stage of aseptic meningitis.

27 samples before day 4 of the illness, but they decreased thereafter in 20 of 23 samples. The mean IL-8 level on the first day of the disease (2864 ± 2118 ng/*l*) was the highest. In all of 17 corresponding individuals examined longitudinally, IL-8 levels were the highest on admission and then fell sequentially.

Relationships between IL-8 levels and clinical indices
Figure 2 shows a significant correlation between IL-8 levels and neutrophil counts ($r = 0{\cdot}612$) in the CSF of aseptic meningitis ($P < 0{\cdot}0001$); but the correlations were not significant between IL-8 levels and counts of total leucocytes or mononuclear cells ($r = 0{\cdot}248$, $r = 0{\cdot}152$). Significant relationships were found between IL-8 levels and levels of both G-CSF ($r = 0{\cdot}873$, Fig. 2) and MIP-1α ($r = 0{\cdot}623$, $n = 34$) in the CSF ($P < 0{\cdot}0001$). IL-8 values in the CSF were not significantly correlated with clinical features such as either the grade or duration of fever, headache or vomiting, or the concentrations of glucose or protein in the CSF. Only the correlation between IL-8 levels and body temperature was significant ($r = 0{\cdot}691$, $P < 0{\cdot}0001$).

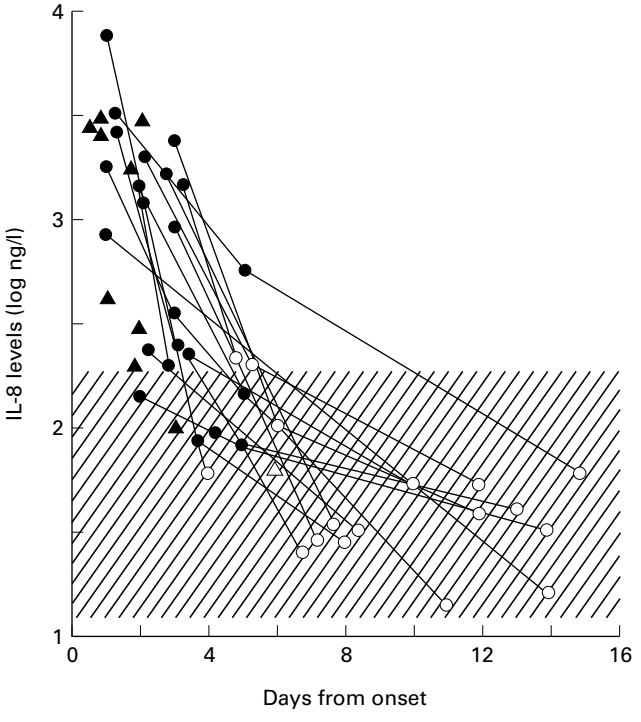

Fig. 1. Kinetics of IL-8 levels in the cerebrospinal fluid (CSF) of individuals with aseptic meningitis. Values of the same individuals are connected. Filled symbols, symptomatic stage; open symbols, period without meningeal symptoms; circles, values in patients who received sequential lumbar punctures; triangles, values in patients who received a single lumbar puncture; hatched area, IL-8 values from the lower limit of detection to the mean + 2 s.d. in the CSF of controls.

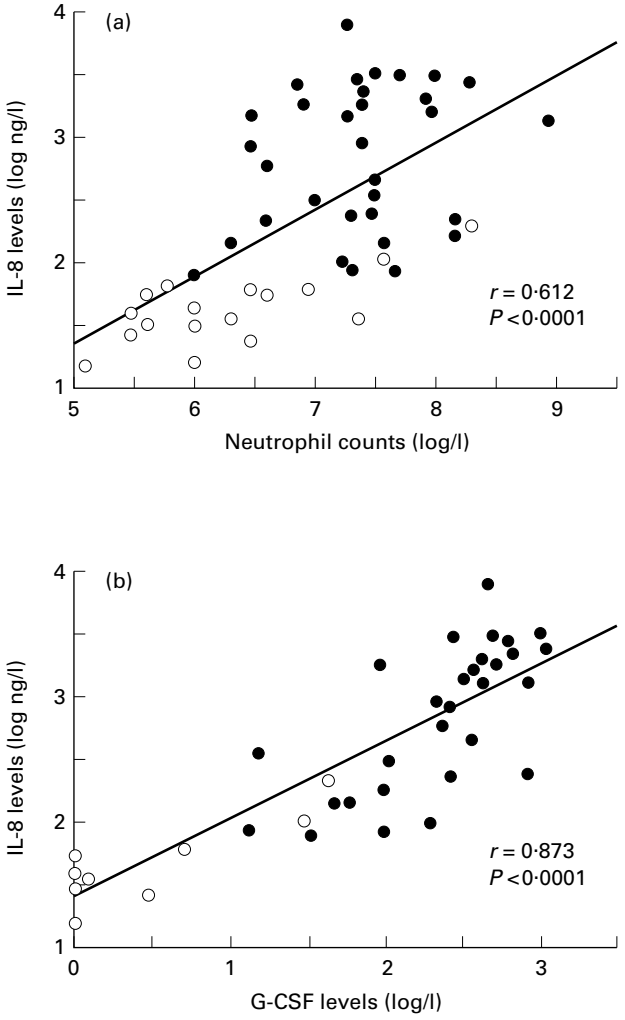

Fig. 2. Relationships between IL-8 levels and both neutrophil counts (a) and granulocyte colony-stimulating factor (G-CSF) levels (b) in the cerebrospinal fluid (CSF) of patients with aseptic meningitis. ●, Symptomatic stage; ○, stage without meningeal symptoms; solid line, linear regression line.

Serum IL-8 levels
While IL-8 was detected in all serum samples in aseptic meningitis, mean levels were similar during the symptomatic and the recovery stage (Table 2). They were much lower than in the CSF from all individuals with meningeal symptoms ($P < 0{\cdot}0001$).

Production of IL-8 by CSF cells
The expression of IL-8 mRNA in leucocytes from the CSF or peripheral blood was examined by PCR amplification. The CSF leucocytes were collected on admission, and total RNA was

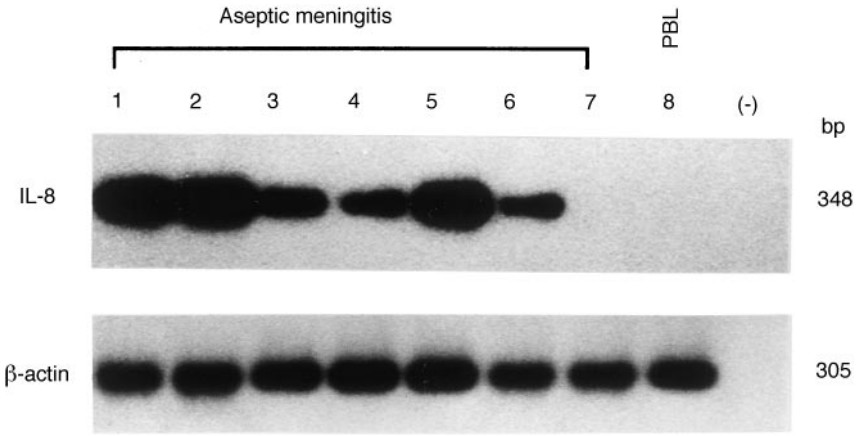

Fig. 3. Expression of IL-8 mRNA by cerebrospinal fluid (CSF) leucocytes without culture in individuals with aseptic meningitis. Signals for human IL-8 and β-actin mRNA were shown by reverse-transcribed polymerase chain reaction (PCR) amplification and hybridization. Lane 8, control sample obtained from peripheral blood leucocytes (PBL) of a healthy volunteer.

directly isolated from them. Figure 3 demonstrates the human IL-8-specific hybridization signals: prominent bands were observed in six of seven patients with aseptic meningitis (lanes 1–7). In contrast, IL-8 mRNA was not detected in blood leucocytes from a healthy volunteer (lane 8). Studies of IL-8 production *in vitro* by CSF mononuclear cells revealed high levels of IL-8 protein (738–2750 ng/*l*) in the culture supernatant from all of seven affected children (Table 2). However, IL-8 was not detected (<12 ng/*l*) in the supernatant from blood mononuclear cells of two healthy volunteers.

DISCUSSION

Our study revealed that IL-8 levels were high in 25 of 27 CSF samples (93%) at the initial stage (before day 4 from onset) of aseptic meningitis. IL-8 values were the highest on admission and then dropped sequentially with the improvement of meningeal signs or symptoms. This pattern of IL-8 kinetics is similar to recent observations in bacterial meningitis [13,18], suggesting that IL-8 concentrations in the CSF in aseptic meningitis had peaked before or on admission, at the same time as the neutrophils accumulate in the CSF. The significant correlations between IL-8 levels and neutrophil counts in the CSF of the illness were shown by us and López-Cortés *et al.* [15]. We found significant relationships between IL-8 levels and levels of both G-CSF and MIP-1α in the CSF. In previous studies, elevated levels of G-CSF, MIP-1α and IL-1 have been detected in the CSF [5,8,9,11]. In addition, intracerebral injections of either IL-1, MIP-1α or IL-8 cause neutrophil recruitment to the meninges and CSF [4,12,23]. The IL-8 can modulate adherence of neutrophils to vascular endothelium via integrin expression [2]. Thus, we conjecture that transiently increased IL-8, perhaps in concert with G-CSF, MIP-1α and IL-1, leads to neutrophil accumulation in the CSF during human aseptic meningitis.

Prominent levels of intrathecal IL-8 have been shown in bacterial meningitis [13–18], but results in aseptic meningitis have been variable [13–16]. Seki *et al.* did not detect IL-8 (30 ng/*l*) in CSF samples of aseptic meningitis [16]. However, we detected IL-8 (>79 ng/*l*) in all CSF samples at the symptomatic stage of the disease, as reported by several other investigators [13–15]. Significant differences in intrathecal IL-8 levels between affected patients and control subjects were shown by us and others [13,14], but not by López-Cortés *et al*. [15]. The controls of López-Cortés *et al.* included individuals with AIDS-related complex and ischaemic stroke, having high levels of intrathecal IL-8 [15]. In any case, we suggest that IL-8 levels in the CSF transiently increase in most patients with aseptic meningitis.

We showed a significant correlation between IL-8 levels in the CSF and body temperature. Intraventricular infusions of IL-8 at concentrations seen in meningitis in rats suppress food intake and induce mild hyperthermia [24]. Fever can be caused by hypothalamic stimulation with IL-1 or IL-6; these cytokines are produced locally during meningitis [3–5]. Astrocytes in the central nervous system can secrete IL-8 and IL-6 following IL-1 stimulation [25]. Thus, IL-8, in coordination with IL-1 and IL-6, might induce fever in patients with aseptic meningitis.

The new information that has emerged from our study is that IL-8 levels were high in the CSF but low in sera during the symptomatic stage of aseptic meningitis. These data are consistent with a previous observation in meningococcal meningitis [18]. Serum IL-8 levels did not fluctuate throughout the course of the illness. Thus, the intrathecal IL-8 is unlikely to be derived from the blood, but may be produced locally. Macrophages, obtained from the inflammatory sites in pulmonary fibrosis and RA, express IL-8 mRNA and release this chemokine [1,2]. We found that IL-8 mRNA was expressed in freshly isolated CSF leucocytes, and that mononuclear cells collected from the CSF produced large amounts of this chemokine *in vitro*. Because CSF samples from recovering patients and controls had too few cells to analyse, peripheral blood cells from healthy volunteers were used as controls. We detected neither IL-8 mRNA in blood leucocytes nor IL-8 protein in the culture supernatant from blood mononuclear cells. These results provide evidence that mononuclear cells that migrate into the CSF are a cellular source of IL-8 found in the CSF, in addition to local astrocytes and microglial cells [25].

Because LPS strongly stimulates IL-8 production by monocytes/macrophages and endothelial cells [1,2], it can act in bacterial meningitis. In the CSF of individuals with aseptic meningitis, elevated levels of proinflammatory cytokines have been detected, including IL-1, G-CSF and granulocyte-macrophage colony-stimulating factor (GM-CSF) [3–5,8]. These cytokines can stimulate monocytes/macrophages to release IL-8 [1,2]. IL-1 can also induce IL-8 production by endothelial cells and

fibroblasts. In addition, viruses can stimulate IL-8 production by many types of cells, such as macrophages [2], fibroblasts [1,2] and T lymphocytes [26]. These viruses include respiratory syncytial virus [2], measles [1], rubella [2], hepatitis C [26], influenza A [27], and rhinovirus [28]. Thus, IL-8 production may be induced directly by viruses or indirectly via the cytokine network during aseptic meningitis.

Although accumulated neutrophils can provide the first line of defence against bacterial infections, it is unclear whether the acute neutrophil response functions against viruses [3,5]. The fact that activated neutrophils can produce IL-8 suggests that neutrophils may induce their further recruitment [1,2]. IL-8 can also attract monocytes and lymphocytes to a lesser extent [1,2]. Neutrophils can, in addition, produce MIP-1α and -1β, which are potent chemoattractants for monocytes, lymphocytes and neutrophils [29]. Our previous study showed a transient increase in intrathecal MIP-1α levels in aseptic meningitis [9]. These chemokines thus may promote the subsequent accumulation and activation of monocytes and lymphocytes. In addition, studies *in vitro* have shown that IL-8 can support the survival of neural cells, and enhance the growth of astrocytes and microglia that are major immunocompetent cells in the central nervous system [30]. IL-8 also induces the production of leukotrienes, arachidonate metabolites and platelet-activating factor, which can function in inflammatory responses [3,5]. Taking all data together, we speculate that IL-8 plays an important role in the inflammatory responses in concert with neutrophil accumulation in the central nervous system during aseptic meningitis.

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