Cerebrospinal fluid concentrations of interleukin-1 β , tumour necrosis factor- α , and interferon gamma in bacterial meningitis

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Abstract

To investigate the role of the inflammatory cytokines, the cerebrospinal fluid concentrations of interleukin (IL)-1 β , tumour necrosis factor- α (TNF- α), and interferon gamma (IFN- γ) were measured in 11 children with bacterial meningitis and two with mycoplasmic meningoencephalitis and compared with those in 50 children with aseptic meningitis and 15 with non-pleocytotic cerebrospinal fluid. Concentrations of IL-1 β and TNF- α were each significantly higher in the cerebrospinal fluid of patients with bacterial meningitis than in those with aseptic meningitis or those with non-pleocytotic cerebrospinal fluid. IFN- γ was detected at low concentrations in the cerebrospinal fluid of only 2/11 of those with bacterial meningitis. On the other hand, the IFN- γ concentration was the highest in the cerebrospinal fluid of patients with aseptic meningitis. These results suggest that the inflammatory cytokines are differently released in the intrathecal space infected with viruses or bacteria.

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Interleukin (IL)-1 β , tumour necrosis factor- α (TNF- α), IL-6, IL-8, and interferon (IFN) are known as the inflammatory cytokines. They act not only as essential soluble cofactors in immunological responses, but are also responsible for inducing fever and the acute phase response.¹⁻⁵ Sensitive immunoassays have shown appreciable concentrations of these

cytokines in serum/plasma or effusions from the patients with some reactive or neoplastic disorders.⁵⁻⁹ Recent reports indicate that IL-1 β , TNF- α , platelet activating factor, IFN- γ , and IL-6 are increased in the cerebrospinal fluid of patients with meningitis,¹⁰⁻¹³ however, their clinical and biological roles are not fully elucidated.

We measured the cerebrospinal fluid concentrations of IL-1 β , TNF- α , and IFN- γ in children with bacterial meningitis and compared them with those in children with aseptic meningitis using immunoassays.

Patients and methods

PATIENTS

Eleven children with bacterial meningitis and two with mycoplasmic meningoencephalitis who had been treated at our related hospitals between 1985 and 1992 were eligible for the study (table). All patients survived. There were nine boys and four girls with mean age of 5.3 years, range 1.2 to 14 years. Patients 2 and 3 were diagnosed as having C7 and C5 deficiency, respectively. Patients 8 and 13 had recurrent episodes of bacterial meningitis and were demonstrated to have liquorrhoea and IgG₂ deficiency, respectively. The causal agents were *Haemophilus influenzae* (n=4), *Neisseria meningitidis* (n=3), *Streptococcus pneumoniae* (n=3), *Mycoplasma pneumoniae* (n=2), and one undetermined. Bacteria were isolated from the cerebrospinal fluid in all but patient 1, in whom *N meningitidis* was isolated from a biopsy specimen of skin lesions typical of meningococcal eruption. Mycoplasmic

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Concentrations of IL-1 β , TNF- α , and IFN- γ in the cerebrospinal fluid of 13 patients at the time of diagnosis of bacterial or mycoplasmic meningoencephalitis

Case No	Age (years)	Sex	Underlying disorder	Causal agent	Use of steroid	IL-1 β (pg/ml)	TNF (pg/ml)	IFN- γ (U/ml)	Sequelae
1	6.3	F	-	<i>N meningitidis</i>	-	255	85	<0.1	-
2	7.0	F	C7 deficiency	<i>N meningitidis</i>	-	430	60	<0.1	-
3	14.0	M	C5 deficiency	<i>N meningitidis</i>	-	120	25	<0.1	-
4	1.0	F	-	<i>H influenzae</i>	-	185	288	<0.1	-
5	2.0	M	-	<i>H influenzae</i>	-	80	20	<0.1	-
6	2.6	M	-	<i>H influenzae</i>	-	405	1700	<0.1	PMR†
7	1.3	M	-	<i>S pneumoniae</i>	-	5550	10500	2.8	Epilepsy, PMR†
8	15.0	M	Liquorrhoea	<i>S pneumoniae</i>	-	300	265	<0.1	-
9	1.4	M	-	Unknown	-	80	110	<0.1	-
10	1.3	F	-	<i>M pneumoniae</i>	-	3000	ND*	3.0	-
11	14.0	M	-	<i>M pneumoniae</i>	-	ND*	70	<0.1	-
12	2.1	M	-	<i>H influenzae</i>	+	1610	2650	<0.1	-
13	1.2	M	IgG ₂ deficiency	<i>S pneumoniae</i>	+	125	1950	2.1	Deafness
Control non-pleocytotic cerebrospinal fluid (n=15)						111 (44)	29 (21)	<0.1	

*ND: not done, †PMR: psychomotor retardation, -: no, +: yes.

Each cytokine concentration was determined by immunoradiometric assay. The minimum detectable level of IL-1 β , or TNF- α is 5 pg/ml, and that of IFN- γ is 0.1 U/ml.

infection was diagnosed based on the increased indirect haemagglutination titres of *M pneumoniae* in the paired sera of patients 10 and 11. However, the former was suspected of having concomitant bacterial meningitis. Dexamethasone was administered to patients 12 and 13 according to the previous method.¹⁴

A specimen of cerebrospinal fluid was taken from each patient at the initial diagnosis. As controls, we obtained specimens of cerebrospinal fluid from 50 children with aseptic meningitis and 15 febrile children without meningitis, as described in a previous report.¹⁵ Cerebrospinal fluid samples were stored at -30°C and assayed for cytokines.

IL-1 β , TNF- α , AND IFN- γ ASSAYS

IL-1 β and TNF- α were measured by an immunoradiometric assay (IRE-Medgenix, Fleurus, Belgium). IFN- γ was measured by a radioimmunoassay test kit (Centocore, Malvern, PA) according to a previously reported method.¹⁵ The minimum detectable concentration for both IL-1 β and TNF- α was 5 pg/ml, and that of IFN- γ was 0.1 U/ml.

STATISTICAL ANALYSIS

Group means were compared by the Mann-Whitney test. An undetectable concentration of a cytokine was regarded as 0 for data analysis. Calculations were performed using a statistical package Stat Flex (ViewFlex, Tokyo, Japan) on a PC9801 system (NEC, Tokyo, Japan).

Results

IL-1 β , TNF- α , AND IFN- γ IN CEREBROSPINAL FLUID OF BACTERIAL MENINGITIS

The concentrations of IL-1 β , TNF- α , and IFN- γ in the cerebrospinal fluid of children with bacterial meningitis or mycoplasmic meningoencephalitis are shown in the table. The IL-1 β concentration was higher in 8/12

patients (67%) (cases 1, 2, 4, 6, 7, 8, 10, and 12), and the TNF- α was higher in 10/12 patients (83%) (cases 1, 2, 4, 6, 7, 8, 9, 11, 12, and 13), than that in the control children with non-pleocytotic cerebrospinal fluid. IFN- γ was detected at a low concentration in only three cases (7, 10, and 13).

CYTOKINES IN CEREBROSPINAL FLUID OF BACTERIAL AND ASEPTIC MENINGITIS

We compared the concentrations of these cytokines in the cerebrospinal fluid of patients with bacterial meningitis and compared them with those from patients with aseptic meningitis and in the control cerebrospinal fluid (figure). In the 13 specimens from patients with bacterial/mycoplasmic meningitis, the median concentration of IL-1 β was 278 (range 80–5, 550) pg/ml and that of TNF- α was 188 (range 22–10 500) pg/ml, both of which significantly exceeded those of the aseptic and control cerebrospinal fluid (IL-1 β $p < 0.05$, TNF- α $p < 0.01$). The cerebrospinal fluid of the patients with aseptic meningitis showed significantly higher concentrations of IFN- γ than either the bacterial ($p < 0.005$) or the control group ($p < 0.001$).

Discussion

We found an increase of IL-1 β and/or TNF- α in the cerebrospinal fluid of patients with bacterial meningitis, but not in those with aseptic meningitis. Concentrations of IFN- γ were much higher in the cerebrospinal fluid of patients with aseptic meningitis than in those with bacterial meningitis. The differing cytokine rise in aseptic or purulent cerebrospinal fluid was remarkable. Significant concentration of TNF- α were detected both in cerebrospinal fluid and serum studied, and the concentrations in cerebrospinal fluid always exceeded those in serum (data not shown). This raises the possibility that these cytokines do not overflow the peripheral circulation to

Concentrations of inflammatory cytokines in the cerebrospinal fluid of children with infectious meningitis. The concentrations are shown of IL-1 β , TNF- α , and IFN- γ in cerebrospinal fluid at diagnosis obtained from 13 children with bacterial meningitis (11 bacteria and two mycoplasma), 50 with aseptic meningitis, and 15 with fever but without a central nervous system infection. The minimum detectable concentration of IL-1 β , TNF- α , and IFN- γ was 5 pg/ml, 5 pg/ml, and 0.1 U/ml, respectively; * $p < 0.05$, ** $p < 0.01$, *** $p < 0.005$, **** $p < 0.001$.

reach the intrathecal space via the injured blood-brain barrier, but are released in the inflamed meninges.¹²⁻¹⁹ Surface marker analysis on the cerebrospinal fluid cells at diagnosis of patients 12 and 13 yielded the predominant T cells (>60% of mononuclear cells), in which CD4⁺CD29 (4B4)⁺ cells outnumbered to 33.4% and 20.5%, respectively. This activated population might be the source of inflammatory cytokines. The concentrations of TNF- α in cerebrospinal fluid are supposed to distinguish a bacterial from a viral meningitis.¹⁹⁻²¹ The cytokine analysis in pleocytotic cerebrospinal fluid may be useful in determining the origin of meningitis, aseptic or bacterial.

Waage *et al* indicated that increased serum TNF- α concentrations were associated with fatal outcome in meningococcal septic shock.²² Arditi *et al* described a significant correlation between TNF- α concentrations in the cerebrospinal fluid and the consecutive febrile days or the occurrence of seizures in bacterial meningitis.²³ Mustafa *et al*,¹⁰ Arditi *et al*,²³ and McCracken *et al*²⁴ reported that IL-1 β and TNF- α are produced in the purulent cerebrospinal fluid, and their concentrations may be associated with the initial clinical status and the outcome. They postulated that IL-1 β /TNF- α lead to the inflammation of vascular endothelium and the activation of coagulation cascades via the formation of arachidonic acid metabolites,²⁵⁻²⁸ and the inflammatory processes alter the cerebrospinal fluid dynamics and metabolism to produce neurological damage. Although a few patients with long term sequelae exhibited prominent TNF- α concentrations in the cerebrospinal fluid in our study, a much larger study on patients with remaining sequelae would be required to establish that persistent increases of cytokines is associated with continued disease activity.

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