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

Shouichi Ohga, Tomonobu Aoki, Kenji Okada, Hideki Akeda, Katsuyoshi Fujioka, Akio Ohshima, Toshinori Mori, Ikuko Minamishima, Kohji Ueda

Abstract
To investigate the role of the inflammatory cytokines, the cerebrospinal fluid concentrations of interleukin (IL)-1β, tumour necrosis factor-α (TNF-α), and interferon gamma (IFN-γ) were measured in 11 children with bacterial meningitis and compared with those in 50 children with aseptic meningitis. The cytokine concentrations in the cerebrospinal fluid of patients with bacterial meningitis were higher than those in those children 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.

(Patients and Methods)

Patients
Eleven children with bacterial meningitis and two with mycoplasmic meningoencephalitis who had been treated at our hospital 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 IgG deficiency, respectively. The causal agents were Haemophilus influenzae (n=4), Neisseria meningitidis (n=3), Strep-tococcus 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

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

<table>
<thead>
<tr>
<th>Case No</th>
<th>Age (years)</th>
<th>Sex</th>
<th>Underlying disorder</th>
<th>Causal agent</th>
<th>Use of steroid</th>
<th>IL-1β (pg/ml)</th>
<th>TNF-α (pg/ml)</th>
<th>IFN-γ (U/ml)</th>
<th>Sequelae</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>6.3 F</td>
<td></td>
<td>N meningitidis</td>
<td>–</td>
<td>–</td>
<td>255</td>
<td>85</td>
<td>&lt;0.1</td>
<td>–</td>
</tr>
<tr>
<td>2</td>
<td>7.0 F</td>
<td></td>
<td>C7 deficiency</td>
<td>N meningitidis</td>
<td>–</td>
<td>430</td>
<td>60</td>
<td>&lt;0.1</td>
<td>–</td>
</tr>
<tr>
<td>3</td>
<td>14.0 M</td>
<td></td>
<td>C5 deficiency</td>
<td>N meningitidis</td>
<td>–</td>
<td>120</td>
<td>25</td>
<td>&lt;0.1</td>
<td>–</td>
</tr>
<tr>
<td>4</td>
<td>1.0 F</td>
<td></td>
<td>–</td>
<td>H influenzae</td>
<td>–</td>
<td>185</td>
<td>288</td>
<td>&lt;0.1</td>
<td>–</td>
</tr>
<tr>
<td>5</td>
<td>2.0 M</td>
<td></td>
<td>–</td>
<td>H influenzae</td>
<td>–</td>
<td>80</td>
<td>20</td>
<td>&lt;0.1</td>
<td>–</td>
</tr>
<tr>
<td>6</td>
<td>2.6 M</td>
<td></td>
<td>–</td>
<td>H influenzae</td>
<td>–</td>
<td>405</td>
<td>1700</td>
<td>&lt;0.1</td>
<td>–</td>
</tr>
<tr>
<td>7</td>
<td>1.3 M</td>
<td></td>
<td>–</td>
<td>S pneumoniae</td>
<td>–</td>
<td>5550</td>
<td>10500</td>
<td>2.8</td>
<td>–</td>
</tr>
<tr>
<td>8</td>
<td>15.0 M</td>
<td></td>
<td>Liquorrhoea</td>
<td>S pneumoniae</td>
<td>–</td>
<td>300</td>
<td>265</td>
<td>&lt;0.1</td>
<td>–</td>
</tr>
<tr>
<td>9</td>
<td>1.0 M</td>
<td></td>
<td>–</td>
<td>Unknown</td>
<td>–</td>
<td>80</td>
<td>110</td>
<td>&lt;0.1</td>
<td>–</td>
</tr>
<tr>
<td>10</td>
<td>1.0 F</td>
<td></td>
<td>–</td>
<td>M pneumoniae</td>
<td>–</td>
<td>3000</td>
<td>ND*</td>
<td>3.0</td>
<td>–</td>
</tr>
<tr>
<td>11</td>
<td>14.0 M</td>
<td></td>
<td>–</td>
<td>N pneumoniae</td>
<td>–</td>
<td>ND*</td>
<td>70</td>
<td>&lt;0.1</td>
<td>–</td>
</tr>
<tr>
<td>12</td>
<td>2.1 M</td>
<td></td>
<td>–</td>
<td>H influenzae</td>
<td>+</td>
<td>1610</td>
<td>2650</td>
<td>&lt;0.1</td>
<td>–</td>
</tr>
<tr>
<td>13</td>
<td>1.2 M</td>
<td></td>
<td>IgG2 deficiency</td>
<td>S pneumoniae</td>
<td>+</td>
<td>125</td>
<td>1950</td>
<td>2.1</td>
<td>Deafness</td>
</tr>
</tbody>
</table>

PMR+: Psychomotor retardation, PMR: Psychomotor retardation, ND: Not done.

*ND: not done, PMR: psychomotor retardation, #: no, +, yes.
Each cytokine concentration was determined by immunoradiometric assay. The minimum detectable level of IL-1β, or TNF-α is 5 pg/ml, and that of IFN-γ is 0.1 U/ml.

Accepted 3 September 1993
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-1β, TNF-α, and IFN-γ ASSAYS
IL-1β 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-1β 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-1β, TNF-α, and IFN-γ in Cerebrospinal Fluid of Bacterial Meningitis
The concentrations of IL-1β, TNF-α, and IFN-γ in the cerebrospinal fluid of children with bacterial meningitis or mycoplasmic meningoencephalitis are shown in the table. The IL-1β 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-penicillotic 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-1β was 278 (range 80–5,650) 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-1β 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-1β 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
reach the intrathecal space via the injured blood-brain barrier, but are released in the inflamed meninges.\textsuperscript{12} 16–19 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 (484+) 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.\textsuperscript{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.\textsuperscript{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.\textsuperscript{23} Mustafa et al,\textsuperscript{10} Arditi et al,\textsuperscript{23} and McCracken et al\textsuperscript{24} reported that IL-1β 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-1β/TNF-α lead to the inflammation of vascular endothelium and the activation of coagulation cascades via the formation of arachidonic acid metabolites,\textsuperscript{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.

We are grateful to Dr K. Akazawa of the department of medical information, Kyushu University for performing statistical analysis. This work was supported in part by a grant-in-aid for the encouragement of young scientists from the Ministry of Education, Science and Culture of Japan.