Intrathecal Production of Interleukin-12 and Gamma Interferon in Patients with Bacterial Meningitis

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To assess the role of interleukin-12 (IL-12) and gamma interferon (IFN-γ) in children with bacterial meningitis, bioactive IL-12 (p70) and the inactive subunit p40 and IFN-γ were measured in serum and cerebrospinal fluid (CSF) from 35 children with bacterial meningitis and 10 control subjects. The production of IFN-γ is induced by IL-12 with tumor necrosis factor alpha (TNF-α) as a costimulator and inhibited by IL-10. CSF concentrations of IL-12 p40 as well as those of IFN-γ were markedly elevated, whereas IL-12 p70 was hardly detectable. Detectable CSF levels of IFN-γ correlated positively with IL-12 p40 ($r = 0.40$, $P = 0.02$) and TNF-α ($r = 0.46$, $P = 0.04$) but not with IL-6, IL-8, or IL-10. In contrast to CSF levels of TNF-α, IL-12, and IL-10, those of IFN-γ were significantly higher in patients with pneumococcal meningitis than in children with meningitis caused by *Haemophilus influenzae* and *Neisseria meningitidis*, presumably because of a high CSF TNF-α/IL-10 ratio in the former. We suggest that IL-12- and TNF-α-induced IFN-γ production may contribute to the natural immunity against microorganisms in the CSF compartment during the acute phase of bacterial meningitis.

Proinflammatory cytokines (tumor necrosis factor alpha [TNF-α], interleukin-6 [IL-6], IL-8, and gamma interferon [IFN-γ]) are involved in the pathophysiology of bacterial meningitis (18). Recently, a novel cytokine with proinflammatory effects has been described (10, 17, 26, 31). This cytokine, IL-12, augments the host defense against bacteria, bacterial products, and parasites. Bioactive IL-12 is a heterodimeric protein of 70 kDa (p70) which consists of 40-kDa (p40) and 35-kDa (p35) subunits linked by a disulfide bond (17). IL-12 is produced predominantly by macrophages/monocytes but also by B cells (9) and polymorphonuclear leukocytes (4). It has been shown to enhance the cytolytic activity of a number of effector cells, including T cells, natural killer (NK) cells, lymphokine-activated killer cells, and macrophages. Furthermore, IL-12 also increases the proliferation of activated NK and T cells and stimulates the production of cytokines, such as IFN-γ, granulocyte-macrophage colony-stimulating factor, and TNF-α (2, 5, 27). The release of IFN-γ is costimulated by TNF-α and IL-1β and inhibited by IL-10 (8, 28).

It has been proposed that IL-12 is involved in the early development of an immune response against infectious agents. Production of IL-12 contributes to the control of the host response against infections with intracellular organisms such as *Listeria monocytogenes* (14), *Toxoplasma gondii* (11), and *Leishmania major* (1). IL-12-mediated protection may be invoked through its ability to stimulate IFN-γ, which inhibits the parasite (23). Furthermore, IL-12 is also a key cytokine mod-

ulating the endotoxin-induced inflammatory process in mice (13). Metzger et al. have suggested that IL-12 augments the natural immune response due to innate or inflammatory components of the host defense system prior to the development of the humoral or cellular immune response (19). This initial response may be important in bacterial meningitis since cerebrospinal fluid (CSF) defense mechanisms against infection are limited.

We questioned whether IL-12 is involved in the early phase of the inflammatory response in the CSF of children with bacterial meningitis. For this purpose, we studied the presence of IL-12 and its relation to other proinflammatory (TNF-α, IL-6, IL-8, and IFN-γ) as well as anti-inflammatory (IL-10) cytokines in serum and CSF of 35 children with bacterial meningitis.

MATERIALS AND METHODS

Patients and controls. Patients between the ages of 3 months and 18 years who were diagnosed with bacterial meningitis between August 1992 and September 1994 were eligible for inclusion. The patients were admitted to the Departments of Pediatrics of the Sophia Children's Hospital and Zuiderziekenhuis, both in Rotterdam, Reinier de Graaf Gasthuis in Delft, and Juliana Children's Hospital in The Hague. Bacterial meningitis was defined as the presence of a positive bacterial culture from CSF or the presence of a positive blood culture in combination with clinical evidence of meningitis and a CSF leukocyte (WBC) count above 10×10^6/liter. Patients with prior antibiotic treatment were excluded. Paired control samples of serum and CSF were obtained from 10 pediatric cancer patients who were in remission. The lumbar punctures in these children were done for diagnostic reasons. CSF samples from patients and controls were examined for WBC count and levels of glucose and protein.

The Medical Ethics Committee of each of the participating centers approved the study protocol. Written informed consent was obtained from the children's parents or legal representatives.

Collection of samples. Samples of blood and CSF were obtained on admission prior to the initiation of antibiotic treatment. Blood was collected into sterilized siliconized Vacutainer glass tubes (Becton Dickinson, Meylan Cedex, France) and allowed to clot at room temperature. CSF samples were collected into

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TABLE 1. Clinical and laboratory characteristics of 35 children with bacterial meningitis and 10 control subjects

Group	Median age (yr) (range)	Gender (no.)		Microorganism (no.)				Median value in CSF (range)		
		Male	Female	*H. influenzae*	*N. meningitidis*	*S. pneumoniae*	Others	WBC count (mm^{-3})	Glucose (mmol/liter)	Protein (g/liter)
Patients (*n* = 35)	1.8 (0.3–13.0)	15	20	15	13	6	1	3,330 (15–34,000)	1.7 (<0.1–5.3)	1.5 (0.2–8.0)
Controls (*n* = 10)	9.1 (4.1–17.9)	5	5					1 (0–2)	3.0 (2.7–3.5)	0.3 (0.3–0.6)

pyrogen-free polystyrene tubes (Falcon; Becton Dickinson, Franklin Lakes, N.J.). The samples were centrifuged at 2,800 × *g* at 4°C for 10 min. The supernatants were stored at −70°C until used for different assays. Samples were never thawed and refrozen before assay.

Laboratory studies. Cultures of blood and CSF were processed according to standard procedures (15). The CSF WBC count and the glucose and protein concentrations were determined by routine laboratory procedures in each of the participating hospitals.

(i) IL-12 p40. IL-12 p40 antigen was measured with an enzyme-linked immunosorbent assay (ELISA) as described previously (29). Results were related to a dose-response curve of recombinant IL-12 p40 and expressed as picograms per milliliter. The lower limit of sensitivity was 50 pg/ml.

(ii) IL-12 p70. IL-12 p70 antigen was measured by using an ELISA with monoclonal antibody (MAb) 20C2, which preferentially reacts with IL-12 p70, and MAb C8.6, which reacts with IL-12 p40 and p70 antigens equally well (9). Concentrations of p40 antigen of up to 20 ng per ml were not detected by this ELISA. Observations in septic baboons showed a good correlation (*r* = 0.87) between results obtained with this ELISA and a bioassay for IL-12 (16). Recombinant human IL-12 p70 was used as a standard. The lower limit of sensitivity of this assay was 2.5 pg/ml.

(iii) IFN-γ. IFN-γ was measured by using an ELISA obtained from the Department of Immune Reagents, Central Laboratory of The Netherlands Red Cross Blood Transfusion Service, according to the manufacturer's instructions. The lower limit of sensitivity of this assay was 10 pg/ml.

(iv) TNF-α, IL-6, IL-8, and IL-10. Serum and CSF levels of TNF-α, IL-6, IL-8, and IL-10 were measured 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; and IL-10, 11 pg/ml.

Statistical analysis. Differences between groups in continuous variables were tested for significance with the Mann-Whitney test. Differences in frequencies of findings between groups were analyzed by Fisher's exact test. Pairwise comparisons regarding the percentage of CSF levels above the detection limit were assessed with McNemar's test. The Spearman correlation coefficient (*r*) was used to evaluate the relation between specific variables. Two-tailed *P* values of ≤0.05 were considered significant.

RESULTS

Patients and controls. The clinical and laboratory characteristics of patients and control subjects are shown in Table 1. Thirty-five patients with a proven bacterial meningitis were enrolled. CSF samples from the controls were sterile and showed normal characteristics. Chemotherapy had been discontinued in all of the control subjects for at least 3 months. None of the control subjects underwent craniospinal irradiation or bone marrow transplantation. The controls were still in remission at the next follow-up visit. The median age (range)

of the children in the control group was significantly higher than that of the patients (9.1 years [4.1 to 17.9 years] versus 1.8 years [0.3 to 13.0 years]; *P* < 0.001).

IL-12 and IFN-γ in serum and CSF samples on admission. The biologically active form of IL-12 p70 was detected in CSF in 2 (6%) of the 35 patients on admission, while the subunit p40 of IL-12 was detectable in 28 (80%) of the 35 patients with bacterial meningitis. CSF levels of IL-12 p40 and p70 were below the detection limit in all 10 control subjects. The percentage of detectable CSF levels of IL-12 p40 in the patients was significantly higher than in control subjects (*P* < 0.001). In contrast, CSF levels of IL-12 p70 were not different between patients and control subjects.

CSF levels of IFN-γ were above the detection limit in 20 (57%) of the 35 patients with bacterial meningitis, whereas none of the control subjects had detectable IFN-γ levels (*P* < 0.001).

Pathogen-dependent differences in CSF levels of cytokines. The pathogen-dependent CSF levels of TNF-α, IL-12, IFN-γ, and IL-10 and the TNF-α/IL-10 ratios are shown in Table 2. Children with meningitis caused by *Haemophilus influenzae* or *Streptococcus pneumoniae* had comparably high CSF levels of IL-12 p40 (median, 503 pg/ml versus 509 pg/ml; *P* = 0.50). CSF levels of IL-12 p40 were lowest in patients with meningococcal meningitis (median, 86 pg/ml). Detectable CSF levels of IFN-γ were observed significantly more frequently in patients with pneumococcal meningitis than in children with meningitis due to *H. influenzae* and *Neisseria meningitidis*. Pathogen-dependent differences in CSF levels of TNF-α and IL-10 were not observed. The CSF ratio of TNF-α to IL-10 was significantly higher in patients with pneumococcal meningitis than in children with meningitis caused by *H. influenzae* and *N. meningitidis*. Comparisons between the TNF-α/IL-10 ratio of *H. influenzae* against those of the other two pathogens and of the TNF-α/IL-10 ratio of *N. meningitidis* against those of the other two pathogens did not yield significant results.

Serum versus CSF levels of IL-12 and IFN-γ on admission. Paired samples of CSF and serum were obtained from 20 patients with bacterial meningitis. The median (range) serum levels of IL-12 p40 were comparable to those of control subjects (165 pg/ml [72 to 1042 pg/ml] versus 109 pg/ml [59 to 299

TABLE 2. CSF levels of TNF-α, IL-12, IFN-γ, and IL-10 and the ratios between TNF-α and IL-10 in patients with pneumococcal meningitis and those with meningitis to *H. influenzae* and *N. meningitidis*

Organism(s)	Level[a]					
	TNF-α (pg/ml)	IL-12 p70 (%)	IL-12 p40 (pg/ml)	IFN-γ (%)	IL-10 (pg/ml)	Ratio, TNF-α/IL-10
H. influenzae and *N. meningitidis* (*n* = 28)	414 (<15–10,600)	2 (7)	358 (<50–11,936)	13 (46)	654 (<10–4,000)	0.9 (0.2–117.5)
S. pneumonia (*n* = 6)	2,324 (355–4,631)	0 (0)	509 (<50–5,390)	6 (100)	736 (109–1,694)	2.1 (1.5–30.3)
P	0.08[b]	1.0[c]	0.55[b]	0.02[c]	0.98[b]	0.04[b]

[a] Median (range) or number of cases above threshold (%).
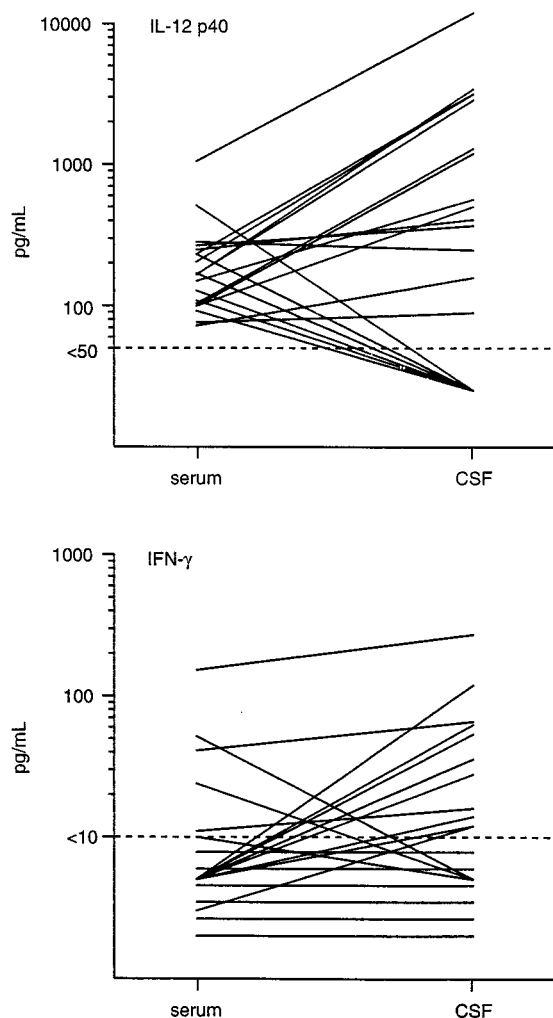[b] Mann-Whitney test.
[c] Fisher exact test.

FIG. 1. Serum and CSF concentrations of IL-12 p40 and IFN-γ on admission in 20 patients with bacterial meningitis. The broken line indicates the detection limit.

pg/ml]; $P = 0.52$). The proportions of detectable serum levels of IL-12 p70 and IFN-γ were also comparable between patients and control subjects (for IL-12 p70, 2 of 20 [10%] versus 0 of 10 [0%] [$P = 0.54$]; for IFN-γ, 6 of 20 [30%] versus 1 of 10 [10%] [$P = 0.37$]).

Detectable concentrations of IL-12 p40 in CSF on admission occurred more often than in corresponding serum samples ($P = 0.03$, McNemar test), whereas those of IL-12 p70 and IFN-γ in serum and CSF were not significantly different (Fig. 1).

Correlation between IL-12 and IFN-γ, TNF-α, IL-6, IL-8, and IL-10 and other characteristics of CSF on admission. CSF levels of IL-12 p40 on admission correlated with those of TNF-α ($r = 0.49$, $P = 0.004$), IL-6 ($r = 0.42$, $P = 0.02$), and IL-10 ($r = 0.61$, $P < 0.001$) and detectable CSF levels of IFN-γ ($r = 0.51$, $P = 0.02$) but not with those of IL-8 ($r = 0.18$, $P = 0.16$). Interestingly, detectable CSF concentrations of IFN-γ on admission correlated only significantly with CSF levels of IL-12 p40 ($r = 0.51$, $P = 0.02$) and TNF-α ($r = 0.48$, $P = 0.04$), as shown in Fig. 2. CSF WBC count and glucose and protein concentrations were significantly correlated with CSF levels of IL-12 p40 ($r = 0.40$ and $P = 0.02$, $r = -0.69$ and $P < 0.001$, and

$r = 0.35$ and $P = 0.05$, respectively). In contrast, CSF levels of IFN-γ were not associated with any of these CSF characteristics.

DISCUSSION

IL-12 is produced by phagocytic cells in response to infection and stimulates adaptive immunity by selectively inducing the Th1 cytokine pattern (IL-2 and IFN-γ) (21, 24). Furthermore, it induces production of IFN-γ and TNF-α and activates the cytotoxic activity of NK cells (2, 5, 27). IFN-γ in turn enhances the function of macrophages and polymorphonuclear leukocytes by stimulating nonspecific defense mechanisms such as phagocytosis and secretion of reactive oxygen intermediates (20). Together, these responses contribute to innate host defense systems against invading microorganisms and are usually effective in reducing the load of infection. Our findings suggest that such a host defense mechanism involving IL-12 and IFN-γ operates in the CSF compartments of patients with bacterial meningitis.

In this study, CSF levels of IL-12 p40 in patients with bacterial meningitis were much higher than those of IL-12 p70. This finding is in agreement with the observation that p40 is released in excess to the bioactive protein (p70) (9, 26). Although CSF levels of IL-12 p70 were below the detection limit (<2.5 pg/ml) in most patients, this not necessarily rule out the presence of IL-12 since biologic activity of IL-12 such as enhancement of NK cell cytotoxicity has been reported in the
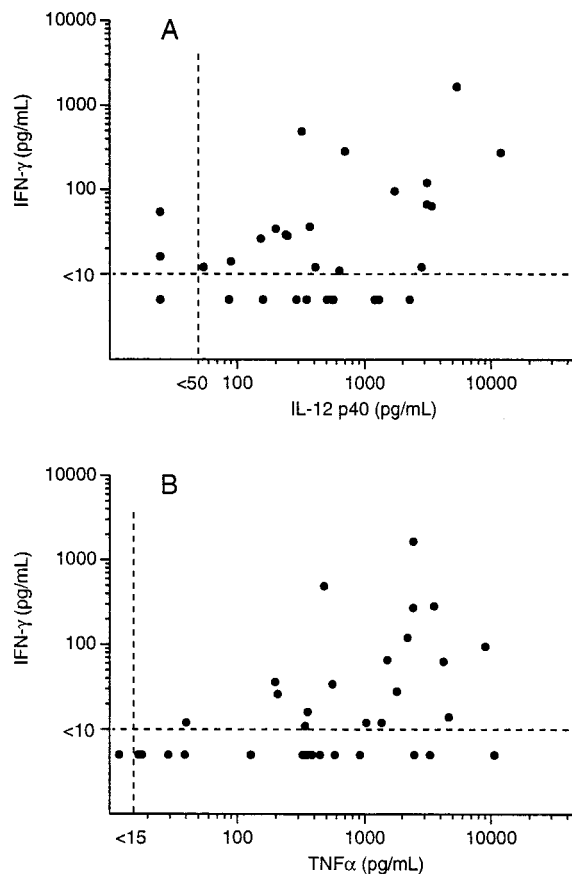

FIG. 2. Correlation between admission CSF levels of IFN-γ and IL-12 p40 (A) and TNF-α (B) in children with bacterial meningitis. Horizontal and vertical lines indicate the detection limits.

presence of concentrations below 1 pM (6, 17). Furthermore, bioactive IL-12 may have escaped detection by binding to specific receptors on cells. CSF levels of IFN-γ were also elevated in our patients with bacterial meningitis, as was previously observed by others (7, 12, 22, 25). The levels of IL-12 p40 were significantly higher in the CSF compartment than in serum, implicating intrathecal production. Our results do not allow conclusions regarding the cellular source of IL-12 in the CSF compartment.

We observed a significantly positive correlation between CSF levels of IL-12 p40 and IFN-γ, TNF-α, IL-6, and IL-10. In vitro studies suggest that these cytokines may not be responsible for the secretion of IL-12 (9). Thus, these correlations likely reflected a common stimulus, i.e., the microorganisms or their products. Experimental data indicate that IL-12 is required for IFN-γ production. This is confirmed by the observation that IL-12 circulates in the serum before the appearance of IFN-γ (13, 32). Furthermore, pretreatment with anti-IL-12 antibodies inhibits production of IFN-γ in response to a challenge by endotoxin. IL-12 is not the only cytokine needed to induce the release of IFN-γ. In the SCID model of listeriosis, TNF-α is an important cofactor for IFN-γ production by NK cells in vivo (3) and in vitro (3, 30). However, TNF-α by itself is unable to induce IFN-γ production. Neutralization of TNF-α with monoclonal antibodies inhibits NK cell IFN-γ production in vitro and inhibits macrophage activation in vivo, resulting in increased systemic bacterial spread (3). Furthermore, the release of IFN-γ is inhibited by IL-10, which prevents production of IL-12 and of the costimulatory cytokines IL-1β and TNF-α by accessory cells (8). These data together explain that CSF levels of IFN-γ in our patients were associated only with IL-12 and a possible costimulator, TNF-α. Thus, both IL-12 and TNF-α were probably responsible for the production of IFN-γ in the subarachnoid spaces of patients with bacterial meningitis.

Interestingly, patients with pneumococcal meningitis had significantly higher CSF levels of IFN-γ in comparison with those with meningitis due to *H. influenzae* and *N. meningitidis*, as was previously observed by others (12). This observation cannot be explained by an increased IL-12 production since CSF levels of IL-12 were not markedly higher in patients with pneumococcal meningitis. However, the high CSF ratio between the costimulator TNF-α and IL-10, which inhibits IL-12-induced IFN-γ production, in the patients with pneumococcal meningitis in comparison to those with meningitis due to other pathogens may provide an explanation for the higher CSF levels of IFN-γ in patients with pneumococcal meningitis.

We conclude that the production of IFN-γ in the CSF compartments of patients with bacterial meningitis is at least partly induced by IL-12, with TNF-α as a costimulator. We suggest that IL-12-induced IFN-γ release may contribute to the local host defense in the subarachnoid space against bacterial meningitis.

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