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Tumour necrosis factor-α in infectious meningitis

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SUMMARY During a one year period tumour necrosis factor-α (TNF-α) was prospectively determined in the cerebrospinal fluid of 49 patients with infectious meningitis. TNF-α was found in the cerebrospinal fluid of 15 of 18 patients with bacterial meningitis. In 11 patients who had cerebrospinal fluid positive for TNF-α it was detected in only one serum (in low concentration). There was no significant correlation between the concentration of TNF-α in cerebrospinal fluid and the patient's age, duration of illness and fever, body temperature, and serum C reactive protein. However, cerebrospinal fluid protein concentrations of ≥2 g/l and leucocyte values of $\geq 2{\cdot}5 \times 10^9$/l were more often associated with high TNF-α concentrations (≥500 pg/ml). In contrast with bacterial meningitis, none of the 31 samples of cerebrospinal fluid from patients with viral meningitis was positive for TNF-α. Thus this investigation supports the conclusion, drawn from animal studies on TNF-α in the cerebrospinal fluid, that the presence of TNF-α is indicative of bacterial meningitis. Absence of TNF-α cerebrospinal fluid, however, was found here not to exclude a bacterial aetiology of the infection.

Tumour necrosis factor-α (TNF-α) was defined originally as a substance that is found in serum of animals sensitised to the *Mycobacterium bovis* strain BCG or *Corynebacterium parvum,* challenged later with endotoxin, and that causes the necrosis of some tumours when passively transferred to tumour bearing animals.[1] TNF-α is a macrophage derived cytokine that not only has antitumour activity but also functions as a central mediator of the inflammatory response. It has been shown to be a critical factor involved in the onset of septic shock.[2] In addition, TNF-α is identical to cachectin, which suppresses the activity of lipoprotein lipase and thus causes hypertriglyceridaemia and cachexia; both are associated with chronic infections.[1]

New information about the role of TNF-α in infections of the central nervous system has emerged recently from animal studies. TNF-α was detected in the cerebrospinal fluid but not in serum of mice infected with *Listeria monocytogenes.*[3] However, acute lymphocytic choriomeningitis virus induced meningitis was not paralleled by production of TNF-α in the central nervous system. In these studies TNF-α was also identified in three of seven patients with bacterial meningitis but in none of seven patients with aseptic meningitis.[3] To substantiate these preliminary findings we prospectively determined TNF-α in the cerebrospinal fluid of children with infectious meningitis. Additionally, available sera from children with bacterial meningitis drawn in parallel with cerebrospinal fluid were retrospectively analysed for TNF-α. The findings were correlated with clinical, microbiological, and routine laboratory data.

Patients and methods

PATIENTS

In the University Children's Hospital of Zurich 205 children with suspected meningitis were seen from 1 October 1987 to 30 September 1988 from a total of 5000 inpatients and 19 400 outpatients. Clinical and laboratory data confirmed the diagnosis of meningitis in 56 patients. History and clinical data were gathered by chart review, and special attention was paid to duration of symptoms and antibiotic treatment in the days before admission. Seven of the 56 patients with meningitis were not investigated for TNF-α as they were admitted to intensive care; two of these patients died. Therefore 49 patients were included in the study.

METHODS

Cerebrospinal fluid was obtained by atraumatic lumbar puncture for direct inoculation of bacterial culture media (chocolate agar and thioglycolate with

saccharose). Additional cerebrospinal fluid was collected in three sterile tubes. One tube with approximately 1 ml of cerebrospinal fluid was centrifuged immediately at 3000 rpm for 15 minutes. Half the volume of the supernatant was coded and frozen at −20°C until used for determination of TNF-α, the other half volume was used for detection of bacterial antigen using a commercially available latex slide agglutination test (Becton Dickinson). The pellet served for preparation of Gram stained slides. Within one hour after the samples were obtained, the second and the third tube were processed in the clinical laboratory for determination of the number of leucocytes and leucocyte differential count, and for glucose and protein concentrations. These determinations were done by standard methods.

Blood was drawn for bacterial cultures (at least in all patients with turbid cerebrospinal fluid), differential cell count, platelets, and determination of serum C reactive protein and blood glucose concentrations. The specimens were processed within one hour after sampling by standard methods. Whenever available stored sera from children with both proved bacterial meningitis and TNF-α positive cerebrospinal fluid were retrospectively used for TNF-α determination.

ASSAYS FOR TNF-α

TNF-α was measured by two different methods. First, the classical bioassay with TNF-α sensitive L-M cells was used.[3] The results are given in units of TNF-α per ml (U/ml), one unit was defined as the amount resulting in lysis of 50% of the L-M cells. When testing human recombinant TNF-α the detection limit was found to be about 0·3 U/ml. For characterisation of the cytotoxic activity in cerebrospinal fluid, the samples were incubated with an excess of antirecombinant human TNF-α antiserum or with control rabbit serum. After incubation for three hours at 37°C residual cytotoxic activity was determined by adding the test samples to the L-M cells. In the second assay, TNF-α was quantitated by enzyme linked immunoadsorbent assay (ELISA) as described previously.[4] Briefly, an affinity purified rabbit antibody was used as capture antibody and a mouse monoclonal antibody labelled with horseradish peroxidase was used as the second antibody in a sandwich ELISA. The results are expressed in pg/ml and the detection limit was 32 pg/ml.

Results

PATIENTS

Forty nine children (30 boys and 19 girls) were enrolled in the study. Bacterial meningitis was found in 10 boys with a mean age of 3·5 years (range 1·3–8·8) and in eight girls with a mean age of 4·9 years (range 0·2–16·7). The diagnosis of bacterial meningitis was based on bacterial culture or bacterial antigen detection in cerebrospinal fluid. The agents grown from cerebrospinal fluid were *Haemophilus influenzae* type b (10/18), *Neisseria meningitidis* type C (3/18), *N meningitidis* type B (3/18), and *N subflava* (1/18). Furthermore, in one child who had been pretreated with antibiotics and who had sterile cerebrospinal fluid cultures *N meningitidis* type C/W135 antigen was detected in the cerebrospinal fluid. Viral meningitis was diagnosed in 20 boys with a mean age of 7·7 years (range 0·8–13·9) and in girls with a mean age 7·5 years (range 0·1–12·4). The diagnosis of viral meningitis was based on absence of bacterial growth in culture, absence of bacterial antigen in cerebrospinal fluid, and no evidence for bacteria in a Gram stain of the cerebrospinal fluid.

TNF-α IN CEREBROSPINAL FLUID

TNF-α in cerebrospinal fluid was detected in 15

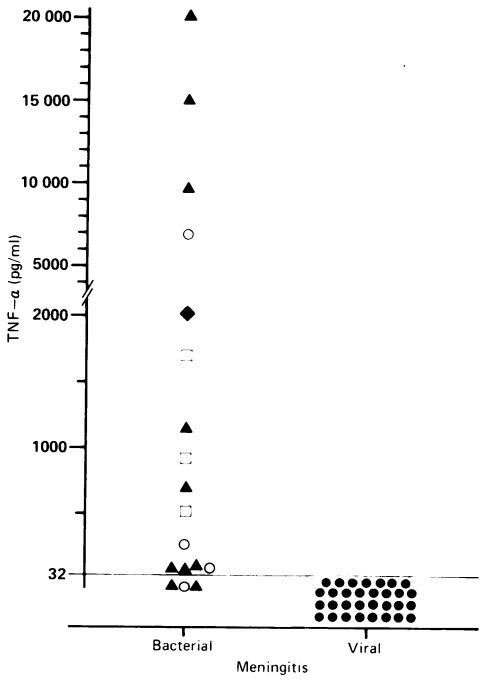

Fig 1 *Concentrations of TNF-α in cerebrospinal fluid of 18 children with bacterial and 31 children with viral meningitis. ▲ Haemophilus influenzae type b; ○ Neisseria meningitidis type C; □ N meningitidis type B; and ◆ N subflava.*

(83%) of the 18 children with bacterial meningitis; in 12 (66%) of these cerebrospinal fluid samples TNF-α was identified by both bioassay and ELISA, and in three further samples of cerebrospinal fluid only by ELISA. The concentrations of TNF-α in cerebrospinal fluid ranged from 30 to 2160 U/ml (mean 586) when measured by bioassay and from 57 to 20 235 pg/ml (mean 3991) when determined by ELISA. No TNF-α could be detected in the cerebrospinal fluid of the 31 children with viral meningitis as assessed by the ELISA technique (fig 1). There was no correlation between the concentration of TNF-α in the cerebrospinal fluid and the causative bacterium.

COMPARISON OF PATIENTS WITH AND WITHOUT TNF-α IN CEREBROSPINAL FLUID

History and clinical and laboratory data of the 49 patients studied are summarised in table 1. With respect to the recorded data patients with bacterial meningitis and TNF-α positive cerebrospinal fluid did not differ from those with TNF-α negative cerebrospinal fluid except for the mean leucocyte count (3·7 compared with $6·1\times10^9$/l). Compared with patients with TNF-α positive cerebrospinal fluid, the three children with bacterial meningitis and absence of TNF-α in their cerebrospinal fluid showed a longer duration of illness and fever with mean values of 48 compared with 23·3 hours and 48 compared with 22·2 hours, respectively. This, however, was due to the only child who had been pretreated with antibiotics whose illness lasted 96 hours. In both other children illness and fever had started 24 hours before admission and *H influenzae* type b was found to be the causative agent. One of these two latter children with TNF-α negative cerebrospinal fluid had the highest leucocyte count of the whole cohort studied. None of the 18 children with bacterial meningitis presented with or developed symptoms of septic shock. All showed a clinically satisfying response to parenteral treatment with ceftriaxone (60–100 mg/kg/body weight/day for seven days). There was no adverse outcome or death.

Children with viral meningitis had a longer mean duration of illness and fever, a lower mean concentration of serum C reactive protein, lower mean leucocyte count in blood and cerebrospinal fluid, and lower mean protein concentration in cerebrospinal fluid than patients with bacterial meningitis.

COMPARISON OF TNF-α CONCENTRATIONS IN CEREBROSPINAL FLUID AND SERUM

Eleven stored serum samples drawn in parallel to cerebrospinal fluid from children with detectable TNF-α in their cerebrospinal fluid were available for TNF-α determination. All these sera but one were negative for TNF-α (table 2). The amount of TNF-α in this positive serum was below 10% of the concentration in the corresponding cerebrospinal fluid. This child with detectable TNF-α in his serum had a six hour history of illness and *H influenzae* type b grew from cerebrospinal fluid and blood culture. Compared with him the other 10 children with detectable TNF-α in their cerebrospinal fluid, but no TNF-α in the serum, had a history of 12 to 48 hours (mean 26·8). In six of these 10 children blood cultures were positive (*H influenzae* type b: 2/6, *N*

Table 1 *Details of children with meningitis (n=49). Results are mean (range)*

	Bacterial meningitis				*Viral meningitis*	
	TNF-α positive (n=15)		*TNF-α negative (n=3)*		*TNF-α negative (n=31)*	
Clinical features on admission:						
Age (years)	4·4	(0·2–16·7)	3·1	(1·0–5·8)	7·6	(0·1–13·9)
Duration of illness (hours)	23·3	(3–48)	48	(24–96)	45	(4–144)
Duration of fever (hours)	22·2	(3–48)	48	(24–96)	42	(0–144)
Temperature (°C)	39·3	(38·6–40·0)	39·3	(38·6–41·0)	38·7	(37·8–40·8)
Blood:						
Serum C reactive protein (mg/l)	154	(29–369)	159	(35–98)	18	(<1–79)
Leucocyte ($\times10^9$/l)	15·4	(2·4–33·1)	18·4	(9·3–24·9)	11·45	(4·3–22·1)
Neutrophils, band forms (%)	56	(34·5–74)	50·6	(34·5–70·5)	36·8	(9·5–61)
Neutrophils, segmented forms (%)	20	(4·5–38·5)	24·6	(11·5–33·5)	34·8	(19·5–68)
Cerebrospinal fluid:						
Leucocytes ($\times10^9$/l)	3·7	(0·02–10·2)	6·1	(1·2–15·2)	0·2	(0·02–1·00)
Polymorphonuclear ($\times10^9$/l)	3·4	(0·02–8·9)	5·5	(1·1–14·0)	0·1	(0·003–0·62)
Mononuclear ($\times10^9$/l)	0·3	(0·005–1·4)	0·6	(0·1–1·2)	0·1	(0·001–0·43)
Protein (g/l)	2·3	(0·3–4·35)	1·7	(0·43–3·7)	0·49	(0·05–0·76)

TNF-α determined by ELISA.

meningitidis type B: 2/6, and *N meningitidis* type C: 2/6), whereas in the other four children *H influenzae* type b, *N meningitidis* type B or C, or *N subflava* were grown from cerebrospinal fluid only.

Table 2 *Concentrations of TNF-α (pg/ml) in cerebrospinal fluid and serum of 11 children with bacterial meningitis*

Patient No	*Cerebrospinal fluid*	*Serum*
1	20 235	<32
2	15 100	<32
3	2 070	<32
4	1690	<32
5	1465	127
6	915	<32
7	534	<32
8	253	<32
9	85	<32
10	65	<32
11	57	<32

ASSOCIATION BETWEEN TNF-α CONCENTRATIONS IN CEREBROSPINAL FLUID AND OTHER FEATURES

No clear cut correlation was found between the concentration of TNF-α in cerebrospinal fluid and age, duration of illness and fever, body temperature, leucocyte count in blood and cerebrospinal fluid, concentration of reactive protein in serum and protein in cerebrospinal fluid, respectively (table 1, fig 2). Compared with cerebrospinal fluid with low TNF-α concentrations, however, cerebrospinal fluid with protein ≥2 g/l and leucocytes $\geq 2{\cdot}5\times10^9$/l were more often associated with high TNF-α concentrations of ≥500 pg/ml. This value represents 2·5% of the maximum TNF-α concentration detected in this study: high leucocyte and protein content was detected in three of nine patients with low TNF-α, and in six of nine patients with a high TNF-α concentration.

Discussion

In a recent study of mice with experimental mening-

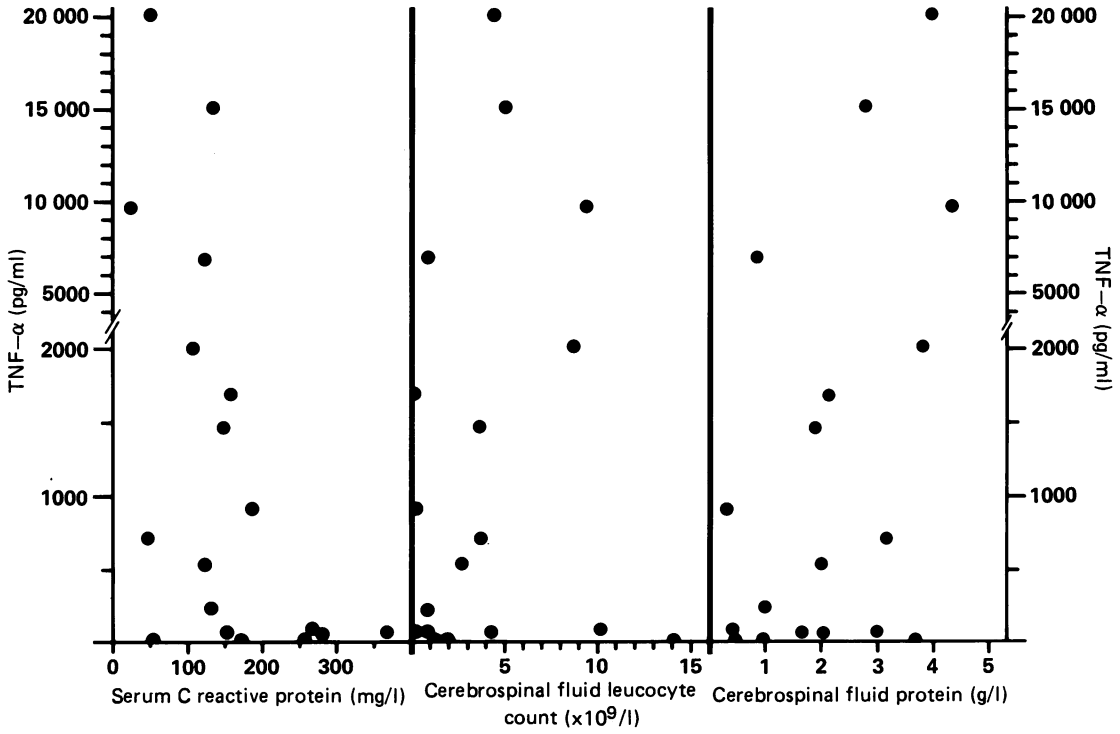

Fig 2 *Association of TNF-α in cerebrospinal fluid with serum C reactive protein, leucocyte count, and protein concentration in 18 children with bacterial meningitis.*

itis TNF-α was detected in cerebrospinal fluid early after initiation of bacterial but not at any time during virally induced disease.[3] In the present study of 49 patients with infectious meningitis TNF-α was also found only in cerebrospinal fluid of patients with bacterial (15/18) but not in subjects with viral (0/31) meningitis. As all but one sera of patients with TNF-α positive cerebrospinal fluid lacked detectable amounts of TNF-α it can be assumed that TNF-α is produced intrathecally. This hypothesis is further substantiated by recent in situ hybridisation studies showing TNF-α transcript positive cells within the inflammatory cell infiltrates in the meninges of mice with *L monocytogenes* induced meningitis (manuscript in preparation).

The lack of detectable TNF-α in the cerebrospinal fluid of three of 18 patients with bacterial meningitis might have been due to the sensitivity of the assays in use. However, in one of the three patients antibiotic pretreatment could have resulted in a reduction of micro-organisms triggering production and secretion of the cytokine. In the second child, who had the most pronounced pleocytosis, TNF-α might have been bound to or degraded by the extremely augmented cells and thus have escaped detection. For the negative results in the third patient we could not find a plausible reason. As a hypothesis, however, individual differences in the sensitivity of macrophages to respond to bacterial infections with production of TNF-α could contribute to the absence of TNF-α in the cerebrospinal fluid of the three patients with bacterial meningitis. Indeed, at least one strain of mice (C3H/HeJ) has been reported to lack TNF-α/cachectin mRNA in macrophages exposed to low amounts of endotoxin. In addition, a post-transcriptional defect prevented the production of the protein.[5]

In a recent study on Gram negative septicaemia with purpura fulminans the serum of 30 of 35 patients was positive for TNF-α, the concentrations being significantly higher in the patients who died than in the survivors.[6] Furthermore, of 79 patients with meningococcal disease TNF-α was detected in 10 of 11 patients who died but from only eight of 68 survivors of the disease.[7] Despite septicaemia with Gram negative micro-organisms in six patients, 10 of 11 serum samples tested for TNF-α in our study were negative for the cytokine investigated. None of these 10 patients suffered from septic shock, however, and all showed a clinically satisfying response to antibiotic treatment. Thus our findings would be compatible with the suggestion that the presence of high concentrations of TNF-α in serum could be indicative of severe disease. In fact in animal studies intravenous injection of TNF-α/cachectin into rats causes haemorrhagic necrosis, which is indistinguishable from the pathologic effects seen after endotoxin administration.[8]

Production of TNF-α within the central nervous system could be important in initiating damage of the blood brain barrier and the tissue injury observed in bacterial meningitis.[9] Thus TNF-α may exert direct cytotoxic effects and/or operate by acting on polymorphonuclear cells which respond to TNF-α with increased adherence to endothelial cells, as well as increased an oxidative burst to unopsonised zymosan particles.[10 11] In the present study no clear cut correlation was found between the concentration of TNF-α in cerebrospinal fluid and duration of illness or fever, bacterial agent, leucocyte count or protein content in cerebrospinal fluid, and leucocyte count in blood or amount of serum C reactive protein. Compared with cerebrospinal fluid with low concentration of TNF-α, however, a trend for higher protein concentration (≥2 g/l) as well as leucocyte counts (≥2·5) in cerebrospinal fluid with TNF-α concentrations ≥500 pg/ml was observed. These findings, which would be in agreement with the effects of TNF-α described on endothelial cells and granulocytes, have to be further investigated in future studies comprising larger groups of patients.

The examination of cerebrospinal fluid for TNF-α may be helpful in differentiating between bacterial and viral meningitis in patients with acute disease. Detectable TNF-α in cerebrospinal fluid was indicative for bacterial meningitis and could be used as diagnostic variable before results of culture are available, especially in cases where leucocyte count, Gram stain, or antigen detection in cerebrospinal fluid are not conclusive. The absence of TNF-α in cerebrospinal fluid, however, did not exclude a bacterial aetiology of meningitis.

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