Brief Definitive Report

TUMOR NECROSIS FACTOR α IN CEREBROSPINAL FLUID DURING BACTERIAL, BUT NOT VIRAL, MENINGITIS

Evaluation in Murine Model Infections and in Patients

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The pathophysiology of both viral and bacterial meningitis is still poorly understood; brain damage after infection of meninges and of the central nervous system may be mediated directly by the infectious agent or may be the effect of the inflammatory host response to infection. There is accumulating evidence that macrophage-derived cachectin is implicated in many tissue injuries. Cachectin is identical with TNF-α, which was originally discovered in the sera of endotoxin-treated mice and rabbits after infection with Bacillus Calmette-Guerin by its destructive effect on tumor cells in vitro. When passively immunized with antisera against cachectin/TNF-α, mice were found to be protected against the lethal effect of endotoxin. Furthermore, injection of cachectin/TNF-α into rats causes hemorrhagic necrosis, which is indistinguishable from the pathologic effects seen after endotoxin administration (see reference 1 for review).

In the present study, we assessed the presence of TNF-α in cerebrospinal fluid (CSF) during bacterial or viral meningitis in both mice and humans and found considerable levels of TNF-α early after initiation of bacterial but not at any time during virally induced meningitis.

Materials and Methods

Mice. 6–8-wk-old inbred (C57Bl/6 (H-2^b) and outbred ICR and ICR *nu/nu* mice of either sex were obtained from the breeding colony of the Institut für Zuchthygiene, Tierspital, Zürich, Switzerland.

Viruses. Lymphocytic choriomeningitis virus (LVMV) Armstrong was originally obtained from Dr. M. Buchmeier, Scripps Clinic and Research Foundation, La Jolla, CA. Virus dilutions were made in MEM containing 2% of heat-inactivated FCS.

Culturing and Enumeration of Bacteria. A seed of *Listeria monocytogenes* was originally received from Dr. R. V. Blanden, Australian National University, Canberra (2), and was maintained in a virulent state by passage in mice. A frozen stock was used to prepare a fresh 12–16-h culture in Trypticase soy broth (BBL Microbiology Systems, Cockeysville, MD) for each experiment and the infectious dose was assessed retrospectively by plating each inoculum. Viable bacterial counts were determined in individual spleens and livers as described by Mackaness (3).

Infection and Harvesting of Blood and Cerebrospinal Fluid. Animals were inoculated intracerebrally (i.c.) or subcutaneously (s.c.) with either ~600 CFU of Listeria or 100

plaque-forming units (PFU) of LCMV Armstrong. On days indicated, mice were ether anaesthesized and bled out; the serum was obtained after centrifugation of blood in Microtainers[B] (Becton Dickinson & Co., Rutherford, NY). CSF was collected as described by Carp et al. (4); CSF samples of 3–10 animals were pooled. After centrifugation, cells in the pellets were counted and supernatants were used for TNF titration.

TNF Assay. TNF-α was quantitated in a bioassay using the TNF-sensitive L-M cells (5). Data are given as percent survival rate or as units of TNF-α, 1 U/ml being defined as the concentration that results in lysis of 50% of the L-M cells. The detection limit of TNF-α in CSF was ~0.3 U/ml. For characterization of the cytotoxic activity in CSF, the samples were incubated either with an excess of rabbit anti-recombinant murine or anti-recombinant human TNF-α antiserum (generated by Genentech Inc., South San Francisco, CA and kindly provided by Dr. G. R. Adolf, Ernst-Boehringer-Institut für Arzneimittelforschung, Vienna, Austria) or with control rabbit serum. After 2 h at 37°C, residual cytotoxicity was determined by adding the test samples to L-M cells.

Human CSF and Serum Samples. CSF samples were tested for TNF-α activity from (*a*) seven patients (four male, three female, 14–65 yr old) with meningococcal (three patients) or pneumococcal (four patients) meningitis; (*b*) seven patients with aseptic meningitis caused by echo virus (two patients), coxsackie virus (three patients), or mumps virus (two patients); (*c*) seven patients with confirmed diagnosis of multiple sclerosis; and (*d*) five patients with other neurological diseases (OND) such as motoneuron disease (one patient), peripheral neuropathy (one patient), and chronic cephalea (three patients). The samples were collected from patients treated in the Department of Infectious Diseases, University Hospital, Linköping and the Department of Neurology, Karolinska Institute, University Hospital, Huddinge, Sweden.

Results and Discussion

Mice i.c. infected with Listeria exhibited the first signs of disease 8–12 h after inoculation of the bacterium and died within the first 4 d after infection irrespective of whether the injected dose was 6 (mean day of death 3.8 ± 0.4) or 6,000 CFU (mean day of death 3.2 ± 0.4). The clinical course of disease was independent of the presence of mature T cells since no differences with respect to susceptibility and time point of onset of disease could be observed between inbred C57Bl/6 (H-2^b) mice and outbred ICR +/+ and *nu/nu* mice (H-2^q) (Table I). Infection of C57Bl/6 mice injected i.c. with 600 CFU resulted in ~15 times higher Listeria titers in their spleen after 24 h when compared with animals infected s.c. with the same Listeria dose. The difference in bacterial titers in the spleens of the same strain of mice infected i.c. vs. s.c. was ~4 logs (i.e., 6.3 logs for i.c. injection vs. 2.2 logs for s.c. inoculation) 48 h after infection; titers were only slightly higher after 72 h (Table I). The number of cells found in the CSF was drastically augmented ~250 times 48 h after i.c. infection when compared with the s.c. inoculated or control mice. As early as 3 h after i.c. infection, the number of cells in the CSF started to be increased. Histological analysis of the brains 48 h after inoculation of Listeria revealed severe choriomeningitis.

As described in many studies (6, 7), ICR mice i.c. inoculated with LCMV showed onset of disease 5–6 d after infection and died 6–8 h later. For C57Bl/6 mice, first signs of the fatal neurological disease could be observed 12–24 h later. ICR *nu/nu* mice infected i.c. with LCMV did not develop disease. In histological sections of the brain, first signs of meningitis could be seen in the perivascular space 5 d after infection and on day 6, severe meningitis was observed.

TABLE I

Kinetics of Listeria in Liver and Spleen of Different Mouse Strains, Susceptibility to Listerial Meningitis, and TNF-α Production in the CSF

Mouse strain	Route of infection	Log_{10} CFU of listeria per organ						% Mortality* (time to death)	TNF-α‡	
		24 h		48 h		72 h			CSF	Serum
		Liver	Spleen	Liver	Spleen	Liver	Spleen			
									U/ml	
C57Bl/6	s.c.	<1.6	<1.3	2.6	2.2	3.5	3.7	0	0	0
C57Bl/6	i.c.	2.8	3.1	5.9	6.3	7.2	6.9	100 (3.3)	4,000	0
ICR +/+	i.c.	2.3	3.4	5.4	6.5	7.5	7.1	100 (3.2)	16,670	0
ICR *nu/nu*	i.c.	2.3	2.9	5.1	6.2	7.2	7.9	100 (3.7)	10,000	0

Animals were infected i.c. or s.c. with ~600 CFU of Listeria in 30 μl. Values shown for Listeria titers are means of five to six animals; SEM <0.4.

* Groups of five mice.

‡ TNF-α was measured at 48 h after infection and activities are indicated as U/ml, one unit being defined as the concentration resulting in 50% lysis of the L-M cells in the bioassay (see Material and Methods).

TNF titers in the CSF differed vastly between the two meningitis models in mice; TNF-α could be detected in the CSF of mice (ICR +/+, ICR *nu/nu,* and C57Bl/6) inoculatd i.c. with Listeria as early as 3 h after infection with a further increase of TNF-α levels over the next 24–48 h (Fig. 1*A;* Table I). On the basis of dilution curves performed with TNF-α and with CSF from ICR mice taken 48 h after infection, the concentration of TNF-α in CSF samples was calculated to be ~9,800 U/ml. After i.c. inoculation of Listeria, TNF-α could only be detected in the CSF but not in the serum of both ICR and C57Bl/6 mice (Fig. 1*A,* Table I). The s.c. route of infection does not lead to clinical disease, and the Listeria titers in spleen and liver were much lower than in mice infected i.c. (see above); TNF-α was also not found in their serum (Fig. 1*A*) nor in CSF

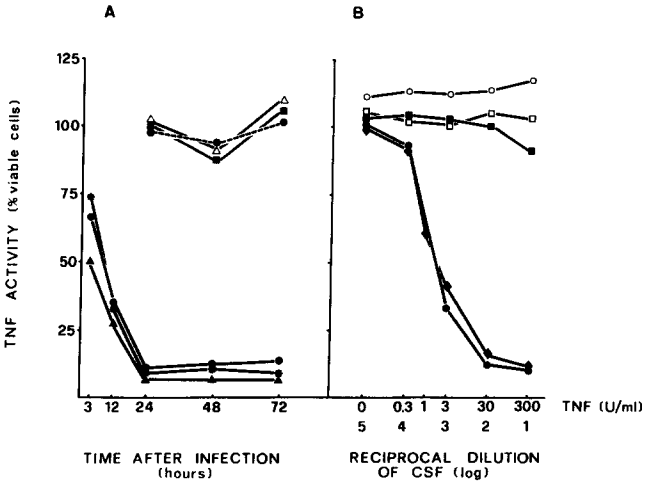

FIGURE 1. Presence of TNF-α in CSF of mice during Listerial, but not LCMV meningitis. (*A*) At times indicated, TNF-α was determined in CSF (—) or serum (---) of mice inoculated with either Listeria s.c. (C57Bl/6, △) or i.c. (C57Bl/6, ▲; ICR +/+, ●; ICR *nu/nu,**) or with LCMV i.c. (ICR +/+, ■). The samples were assayed at final concentrations of 5% for CSF and 10% for serum. Data are expressed as survival rate (% of viable cells) of L-M cells in the presence of test samples or serum-free salt solution as control. (*B*) Dose response curves were performed with CSF samples from mice after i.c. infection with either Listeria (ICR+/+, day 2 ●) or LCMV (ICR +/+, day 6, ■; C57Bl/6, day 7, □). CSF from Listeria-infected ICR +/+ mice was preincubated with a neutralizing polyclonal rabbit anti-murine TNF-α antiserum (final dilution 1:50) for 2 h (○). The standard curve (♦) was performed with human TNF-α.

TABLE II

Differential Cell Counts and Concentration of TNF-α of Mice with Acute Meningitis

Mouse strain	Pathogen	Time after infection	CSF			
			Leukocytes per μl	Polymorphonuclear cells*	Mononuclear cells*	TNF‡
		d	log_{10},	%	%	*U/ml*
ICR +/+	Listeria	2	3.6 ± 0.5	92.5 ± 4.6	7.5 ± 4.6	>2,000
ICR +/+	LCMV	6	4.1 ± 0.4	56.1 ± 10.1	43.9 ± 10.1	<0.3
C57Bl/6	LCMV	7	3.8 ± 0.4	55.1 ± 0.2	44.9 ± 0.2	<0.3

Mice were i.c. infected with either 600 CFU of Listeria or 100 PFU of LCMV. CSF were taken 48 h after inoculation.

* Fixed CSF-derived cells were stained with May-Grünwald/Giemsa; 1,000 cells per sample were counted. Values indicated represent mean ± SD of two independent experiments.

‡ See legend Table I.

(Table I). To evaluate whether the cytotoxic activity seen against L-M cells was mediated by lymphotoxin (TNF-β) or TNF-α, the CSF samples were pretreated with anti-TNF-α antiserum before testing. As shown in Figure 1*B*, the cytotoxic activity against L-M cells can be neutralized completely with the TNF-α-specific antiserum but not with control serum. In contrast to what was found in Listeria infected mice, measurable titers of TNF-α could not be detected in any of the CSF from mice infected i.c. with LCMV when measured from day 1 to the day of onset of disease and death (i.e., day 6 for ICR and day 7 for C57Bl/6 mice; Fig. 1, Table II).

The results obtained in experimental meningitis in mice are paralleled by detection of TNF-α in patients with bacterial meningitis. Of the seven CSF from patients with bacterial meningitis, three contained measurable titers of TNF-α (i.e., 130 U/ml, 200 U/ml, and 400 U/ml). Based on binding studies using anti-recombinant human TNF-α antibodies, the activity in CSF could be attributed to TNF-α. None of the CSF samples from patients with aseptic meningitis caused by virus (echo, coxsackie, and mumps virus), from patients with multiple sclerosis, or from patients with other neurologic diseases was positive for TNF. Of the three patients with TNF in the CSF, two had pneumococcal and one had meningococcal meningitis. When compared with the four TNF-α negative meningitis patients, the three positive cases do not differ with respect to numbers of white blood cells in CSF or to CSF/serum albumin ratio; they do differ, however, with respect to the time point during the course of infection at which the samples were taken. TNF-α positive CSF were taken from the patients within the first 24 h of admission to the hospital, the negative CSF samples were obtained at later time points (i.e., 3–19 d after hospitalization). Recently serum of patients with diagnosed meningococcal meningitis were examined for TNF-α; of the 41 samples tested, only four (10%) contained TNF-α at the time point of hospitalization (8). In our experiments, the intracerebral inoculation of Listeria in mice was followed by TNF-α production in CSF and growth of Listeria also outside of the brain, e.g., in the liver and spleen. However, TNF-α could not be detected in the serum. This observation could either be explained by the difference of the half life of TNF-α in serum vs. CSF or by faster reticuloendothelial

clearance mechanisms of systemic bacteriaemia when compared with elimination in the central nervous system (CNS). Since TNF-α, which can be produced by activated monocytes/macrophages (1), was detected only in CSF of infected mice, the factor is likely to be synthesized within the CNS be it either by recruited blood-derived monocytes or by macrophages of the meninges. In addition, microglial cells, the macrophage-like cells of the brain, may contribute to TNF-α production in Listeria infection, a possibility suggested by the finding that secretion of the factor by activated microglial cells has been demonstrated in vitro (5). LCMV-induced CNS disease is triggered by cytotoxic T cells (9) with both T cells and monocytes/macrophages being recruited into the meninges and CSF (10). The absence of TNF-α in the CSF of the mice suffering from LCMV disease suggests that recruited monocytes/macrophages do not play a significant role in tissue and cell destruction and in development of disease (11, 12), since despite the presence of both macrophages and activated virus-specific $CD8^+$ T cells (11), in the LCMV model there is no interaction between T cells and macrophages, apparently resulting in TNF-α production detectable in CSF.

Monocytes/macrophages play a crucial role in host response to bacterial infection. When certain inducible functions of monocytes/macrophages such as cachectin/TNF-α are expressed inappropriately, the cells may contribute to development of tissue injury; e.g., intravenous injection of cachectin/TNF-α into rats mimics the clinical symptoms and tissue lesions including severe vascular damage seen after endotoxin administration (1). Secretion of TNF-α into the CSF could be important in initiating damage of the blood brain barrier and the tissue injury observed in bacterial meningitis (13). Thus cachectin/TNF-α may exert direct cytotoxic effects and/or operate by acting on polymorphonuclear cells, the main inflammatory cells detected in CSF during Listerial meningitis; cachectin/TNF-α has been found to be chemotactic for polymorphonuclear cells and to cause superoxide anion release by granulocytes (14, 15). Further studies are needed to assess the effects of cachectin/TNF-α within the CSF and to establish whether the examination of CSF for cachectin/TNF-α may be helpful in differentiating between bacterial and viral meningitis in human patients with acute disease.

Summary

To evaluate the potential role of cachectin/TNF-α in the pathogenesis of bacterial and viral meningitis, concentrations and kinetics of TNF-α were determined in cerebrospinal fluid (CSF). After intracerebral, but not systemic, infection with *Listeria monocytogenes* in mice, TNF-α was detected as early as 3 h after infection reaching maximum titers after 24 h. However, TNF-α was not found in serum during the course of Listeria infection. In contrast to bacterial meningitis, no TNF-α was detected at any time in CSF of mice suffering from severe lymphocytic choriomeningitis induced by intracerebral infection with lymphocytic choriomeningitis virus. This difference is striking since both model infections led to a massive infiltration of polymorphonuclear and mononuclear leukocytes into the meninges and CSF. The results found for the two model infections were paralleled by findings in humans; CSF from three out of three

patients with bacterial meningitis examined during the first day of hospitalization showed significant levels of TNF-α; none of the CSF obtained later than 3 d after hospitalization was positive. In addition, similarly to what was found in mice with viral meningitis, zero out of seven patients with viral meningitis had detectable TNF-α in CSF.

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References

1. Cerami, A. and B. Beutler. 1988. The role of cachectin/TNF in endotoxic shock and cachexia. *Immunol. Today.* 9:28.
2. Blanden, R. V., and R. E. Langman. 1972. Cell mediated immunity to bacterial infection in the mouse. Thymus derived cells as effectors of acquired resistance to Listeria monocytogenes. *Scand. J. Immunol.* 1:379.
3. Mackaness, G. B. M. 1962. Cellular resistance to infection. *J. Exp. Med.* 116:381.
4. Carp, R. I., A. T. Davidson, and P. A. Merz. 1971. A method for obtaining cerebrospinal fluid from mice. *Res. Vet. Sci.* 12:499.
5. Frei, K., C. Siepl, P. Groscurth, S. Bodmer, C. Schwerdel, and A. Fontana. 1987. Antigen presentation and tumor cytotoxicity by interferon-γ-treated microglial cells. *Eur. J. Immunol.* 17:1271.
6. Lehmann-Grube, F. 1982. Lymphocytic choriomeningitis virus. *In* The Mouse in Biomedical Research. H. L. Forster, J. D. Small, and J. G. Fox, editors, Academic Press, New York, NY. 231–266.
7. Doherty, P. C., and R. M. Zinkernagel. 1974. T-cell mediated immunopathology in viral infection. *Transplant. Rev.* 19:89.
8. Waage, A., A. Halstensen, and T. Espevik. 1987. Association between tumor necrosis factor in serum and fatal outcome in patients with meningococcal disease. *Lancet.* i:355.
9. Leist, T. P., S. P. Cobbold, H. Waldmann, M. Aguet, and R. M. Zinkernagel. 1987. Functional analysis of T lymphocyte subsets in antiviral host defense. *J. Immunol.* 138:2278.
10. Cederig, R., J. E. Allan, Z. Tabi, F. Lynch, and P. C. Doherty. 1987. Phenotypic analysis of the inflammatory exudate in murine lymphocytic choriomeningitis. *J. Exp. Med.* 165:1539.
11. Thomsen, A. R., and M. Volker. 1983. Studies on the role of mononuclear phagocytes in resistance to acute lymphocytic choriomeningitis virus infection. *Scand. J. Immunol.* 18:271.
12. Woods, S. J., M.-F. Sarom, and C. J. Pfau. 1987. Meningeal macrophages reflect lymphocytic choriomeningitis virus pathogenic phenotypes. *Scand. J. Immunol.* 26:97.
13. Quagliarello, V. J., W. J. Long, and W. M. Scheld. 1986. Morphologic alterations of the blood-brain barrier with experimental meningitis in rat. *J. Clin. Invest.* 77:1084.
14. Ming, W. J., L. Bersani, and A. Mantovani. 1987. Tumor necrosis factor is chemotactic for monocytes and polymorphonuclear leukocytes. *J. Immunol.* 138:1469.
15. Larrick, J. W., D. Graham, K. Toy, L. S. Lin, G. Senyk, and B. M. Fendly, 1987. Recombinant tumor necrosis factor causes activation of human granulocytes. *Blood.* 69:640.