

Brief Definitive Report

PRODUCTION OF B CELL STIMULATORY FACTOR-2 AND INTERFERON γ IN THE CENTRAL NERVOUS SYSTEM DURING VIRAL MENINGITIS AND ENCEPHALITIS

Evaluation in a Murine Model Infection and in Patients

**BY KARL FREI,*^{||} THOMAS P. LEIST,[‡] ANTONY MEAGER,[¶] PAOLO GALLO,*^{||}
DAVID LEPPERT,[§] ROLF M. ZINKERNAGEL,[‡] AND ADRIANO FONTANA*^{||}**

*From the *Section of Clinical Immunology, the [‡]Section of Experimental Pathology, the Departments of [§]Neurology and ^{||}Neurosurgery, University Hospital, 8006 Zürich, Switzerland; and the [¶]National Institute for Biological Standards and Control, Hertfordshire, Great Britain*

The production of antibodies within the central nervous system (CNS) is a characteristic finding in primary and postinfectious viral meningoencephalitis. Igs, particularly IgG, are increased in cerebrospinal fluid (CSF), and isoelectrofocusing of CSF shows a series of so-called "oligoclonal" bands of IgG, which can be absorbed by viral antigens (1, 2). Furthermore, inflammatory cells in brain parenchyma and meninges are composed of significant numbers of B lymphocytes (3). The mechanisms leading to activation and differentiation of B cells within the CNS have not been investigated so far. In general, B cell responses result from intercellular communications between B cells, T cells, and monocytes/macrophages. T-B cell cooperation involves distinct T cell-derived helper factors such as IFN- γ and B cell-stimulating factor-2 (BSF-2) (4). BSF-2, which has also been named IL-6, is identical to IFN- β 2, as well as to the 26-kD factor (5). In the present report, we demonstrate that these cytokines are also produced within the CNS in viral meningitis and encephalitis. Synthesis of BSF-2 and IFN- γ in the CNS may trigger generation of antiviral antibodies by B lymphocytes/plasma cells having invaded the CNS in viral diseases.

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, Tier-spital Zürich, Switzerland.

Infection and Harvesting of Blood and Cerebrospinal Fluid. Animals were inoculated intracerebrally with 100 plaque forming units of Lymphocytic choriomeningitis virus (LCMV) Armstrong. On days indicated, CSF and serum was collected from the infected mice as described (6). CSF samples of 3-10 animals were pooled and used for determination of BSF-2 and IFN- γ .

Assay for BSF-2. The assay for BSF-2 was performed using the BSF-2-dependent B cell hybridoma 7TD1 (mouse-mouse). Briefly, 7DT1 cells (kindly provided by Dr. J. van Snick, Ludwig Institute for Cancer Research, Brussels, Belgium) were seeded in 96-well microtiter

This work was supported by grants from the Swiss National Science Foundation (Projects 3.930-0.87 [A. Fontana] and 3.259-0.87 [R. Zinkernagel]), the National Multiple Sclerosis Society, and the European Science Foundation (P. Gallo).

F-plates in Iscove's modified Dulbecco's medium supplemented with 10% FCS, 5×10^{-5} M 2-ME, 1.5 mM L-glutamine, 0.24 mM L-asparagine, 0.55 mM L-arginine, and antibiotics (7). The cells were cultured at a density of 10^3 cells per well in the presence of serial dilutions of CSF or a highly purified standard containing 1,000 U/ml of human BSF-2 (5) (kindly provided by Dr. J. van Damme, Rega Institute for Medical Research, University of Leuven, Belgium). For the final 16 h of the 3-d culture, the cells were pulsed with 1 μ Ci [3 H]thymidine (5 Ci/mmol). 1 U of BSF-2 is defined as the amount of BSF-2 that results in half-maximal thymidine incorporation in the assay. When using highly purified BSF-2, the detection limit of BSF-2 was found to be 0.15 U/ml. When testing sera or CSF, the detection limit of the BSF-2 assay was 10 U/ml due to negative effects of the samples when being tested at concentrations >10%. To characterize the BSF-2-like activity in CSF, a neutralizing goat anti-human BSF-2 antibody was used (5) (a generous gift of Dr. J. van Damme). After a 3-h incubation of the samples with the antibody (final dilution 1:2,000) at 37°C, the residual activity was determined in the assay.

Immunoradiometric Assay for Murine IFN- γ (MuIFN- γ). MuIFN- γ was measured by a solid-phase, two-site "sandwich immunoassay" method (8) using the rat anti-MuIFN- γ mAbs R4-6A2 and AN-18 (kindly provided by Dr. E. Havell, Trudeau Institute, Saranac Lake, NY, and Dr. S. Landolfo, University of Turin, Turin, Italy).

Human Cerebrospinal Fluids. Within the first 48 h of admission to the hospital, CSF samples were collected from 19 patients; 15 had aseptic viral meningitis and 4 had encephalitis due to HSV-1. Of these 19 patients with viral CNS disease, 9 patients were <20 yr old, 8 patients were between 20 and 40, and 2 patients were >40 yr old. Furthermore, CSF were tested from 31 patients with multiple sclerosis and from the following 16 controls, which will be collectively termed "other neurological diseases" (OND): a group of non-inflammatory diseases of the CNS, which was composed of two Alzheimer diseases, two syringomyelia, and one amyotrophic lateral sclerosis. In addition, five patients with tension headache, four with disk syndromes, and two with psychiatric disorders were included.

Results and Discussion

As shown previously, C57Bl/6 mice intracerebrally infected with LCMV showed onset of meningitis 6–7 d after infection and died 6–8 h later (6). In CSF, white blood cell pleocytosis occurred 6 d after infection, reaching 10^4 cells/ μ l on day 7 with 45% mononuclear cells. For measurement of cytokines, serum and CSF were col-

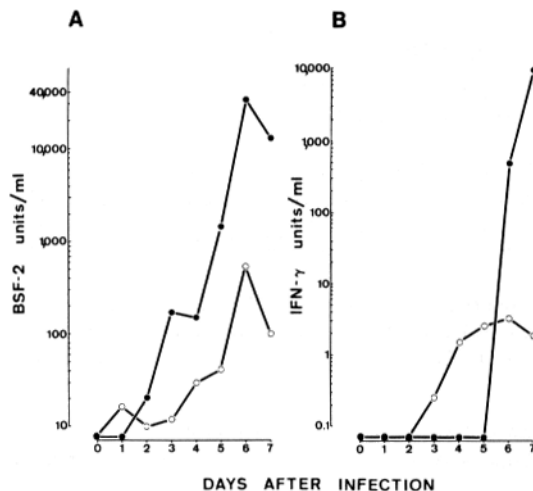


FIGURE 1. Intrathecal synthesis of BSF-2 and IFN- γ during viral infection of the CNS. Serum (O) and CSF (●) of C57Bl/6 mice infected with LCMV were investigated for BSF-2 (A) and IFN- γ (B) at days indicated.

TABLE I
BSF-2 and IFN- γ in Athymic Mice with LCMV Infection

Days after infection	ICR +/+				ICR nu/nu			
	BSF-2		IFN- γ		BSF-2		IFN- γ	
	Serum	CSF	Serum	CSF	Serum	CSF	Serum	CSF
	<i>U/ml</i>				<i>U/ml</i>			
0	<10	<10	<0.01	<0.01	80	<10	<0.01	<0.01
1	120	<10	0.07	<0.01	270	<10	0.15	1.2
3	15	100	0.39	<0.01	900	<10	0.96	1.3
5	530	85,000	1.95	23	250	250	0.42	<0.01
6	1,500	36,000	1.68	1,848	175	200	0.48	<0.01

lected at progressive times during infection. In the course of LCMV disease, BSF-2 was first detected in serum on day 1 and in CSF on day 2 (Fig. 1 A). Maximum BSF-2 levels were reached after rapid increase from day 4 by day 6; the concentration of BSF-2 was \sim 60-fold higher in CSF compared with serum (Fig. 1 A).

In contrast to BSF-2, no IFN- γ was detected in the same CSF samples during the first 4 d after infection (Fig. 1 B). After day 6 of LCMV infection (the time point pleocytosis in the CSF develops), a sharp increase in the levels of IFN- γ was noted. In serum, the amount of IFN- γ gradually increased between day 2 and 5 (Fig. 1 B). However, the levels of IFN- γ were much higher in CSF compared with serum (Fig. 1 B, Table I).

Athymic *nu/nu* mice infected intracerebrally with LCMV failed to develop meningitis (9). When assessed in ICR *nu/nu* mice infected with LCMV, BSF-2 was only moderately increased both in serum and CSF. On day 5, the amount of BSF-2 was \sim 160- and \sim 340-times lower when compared with the corresponding values in CSF of C57Bl/6 and ICR +/+ mice, respectively (Fig. 1 A, Table I). In ICR +/+ mice, maximum levels of IFN- γ were usually observed on day 6 at the time of clinical signs of meningitis. Table I shows that in CSF of athymic LCMV-infected ICR *nu/nu* mice, IFN- γ remained close to baseline levels. These findings in an animal model parallel recent reports of IFN- γ in human CSF during HSV-1 encephalitis (10).

In CSF of patients with infectious CNS diseases, BSF-2 was found in 12 of 15 patients with aseptic viral meningitis and in all of the four patients with encephalitis due to HSV-1 infection. In controls consisting of 16 patients with "other non-inflammatory neurological diseases" and of 31 patients with multiple sclerosis, BSF-2 was detected only in one patient with multiple sclerosis (Fig. 2). Antibodies against human BSF-2 completely neutralized the activity detected in viral meningitis and HSV-1 encephalitis (data not shown), indicating that the hybridoma growth-promoting effect in these CSF is mediated by BSF-2.

The cellular source of BSF-2 in CSF is not known. Although originally described as a T cell-derived factor (4), fibroblasts, monocytes, and endothelial cells have also been found to secrete BSF-2 (7, 11, 12). In a previous study on cachectin/TNF- α (a product of activated macrophages) it was not possible to identify cachectin/TNF- α in CSF of mice with LCMV-induced meningitis (6). In contrast, however, cachectin/TNF- α was present in CSF of patients with bacterial meningitis or of mice infected intracerebrally with *Listeria monocytogenes* (6). In viral meningitis, the

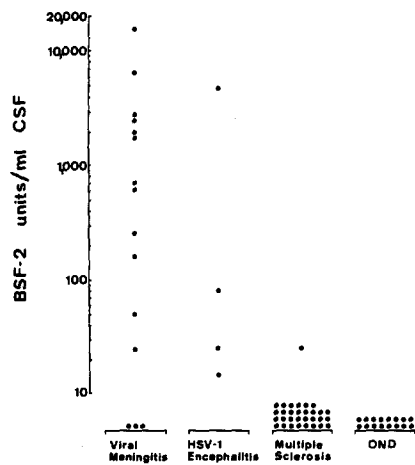


FIGURE 2. BSF-2 in CSF of patients with aseptic viral meningitis and HSV-1-induced encephalitis.

discrepancy between high level of BSF-2 and absence of cachectin/TNF- α in CSF does not support the possibility of BSF-2 being produced independent of T cells by monocytes/macrophages in CNS. Because of concomitant production of BSF-2 and IFN- γ , a T cell-derived lymphokine, one may assume that BSF-2 production within the CNS may depend on local infiltrating T cells. This is further supported by the observation of only minimal synthesis of both IFN- γ and BSF-2 in CNS of LCMV-infected athymic ICR *nu/nu* mice. Production of IFN- γ has been shown to depend on activation of mature T cells, which are deficient in nude mice.

The findings reported in this study may help to explain the synthesis of antiviral antibodies in the CNS during viral infections (1-3). IFN- γ has been shown to synergize with IL-1 and IL-2 in antigen-specific antibody production by B lymphocytes (4). BSF-2 supports the growth of B cell hybridomas and plasmocytomas (7) and induces the final differentiation to high-rate Ig secretion of preactivated B cells (13). Accordingly, BSF-2 detected in CSF may contribute to differentiation and secretion of antiviral antibodies by preactivated virus-specific B cells having invaded the CNS in the course of viral infection.

Summary

Synthesis of B cell-stimulating factor-2 (BSF-2) and IFN- γ was shown in cerebrospinal fluids (CSF) collected from mice with experimental viral meningitis. In the CSF, the level of BSF-2 started to increase 24 h after intracerebral infection with lymphocytic choriomeningitis virus (LCMV) with rapid increase after day 4. IFN- γ was not detected in the CSF before day 5 or 6 after infection, but increased sharply thereafter. In athymic nude mice, LCMV infection did not result in meningitis, and both BSF-2 and IFN- γ levels were only slightly and transiently elevated. These findings suggest that activated mature T cells are required for development of disease and production of both BSF-2 and IFN- γ . As observed in mice, BSF-2 was also detected in 16 out of 19 CSF samples collected from patients with acute viral infections of the central nervous system (CNS). Intrathecal production of BSF-2 and IFN- γ

may be instrumental in local production of antiviral antibodies by B lymphocytes/plasma cells invading the CNS during viral CNS disease.

The authors would like to thank Mrs. C. Schwerdel for excellent technical assistance.

Received for publication 22 March 1988 and in revised form 26 April 1988.

Note added in proof: In a recent report by Houssiau et al. (14), they have also demonstrated elevated levels of BSF-2 in CSF of patients with viral brain diseases.

References

1. Vandvik, B., B. Sköldenberg, M. Forsgren, G. Stiernstedt, S. Jeansson, and E. Norrby. 1985. Long-term persistence of intrathecal virus-specific antibody responses after herpes simplex virus encephalitis. *J. Neurol.* 231:307.
2. Forsberg, P., A. Henriksson, H. Link, and S. Öhman. 1984. Reference values for CSF-IgM, CsF-IgM/S-IgM ratio and IgM index, and its application to patients with multiple sclerosis and aseptic meningoencephalitis. *Scand. J. Clin. Lab. Invest.* 44:7.
3. Moench, T. R., and D. E. Griffin. 1984. Immunocytochemical identification and quantitation of the mononuclear cells in the cerebrospinal fluid, meninges, and brain during acute viral meningoencephalitis. *J. Exp. Med.* 159:77.
4. Kishimoto, T. 1987. B cell stimulatory factors (BSFs): molecular structure, biological function, and regulation of expression. *J. Clin. Immunol.* 7:343.
5. Van Damme, J., M. De Ley, J. Van Snick, C. A. Dinarello, and A. Billiau. 1987. The role of interferon β 1 and the 26-kDa protein (interferon β 2) as mediators of the antiviral effect of interleukin 1 and tumor necrosis factor. *J. Immunol.* 139:1867.
6. Leist, T. P., K. Frei, S. Kam-Hansen, R. M. Zinkernagel, and A. Fontana. 1988. Tumor necrosis factor α in cerebrospinal fluid during bacterial, but not viral, meningitis. *J. Exp. Med.* 167:1743.
7. Van Snick, J., S. Cayphas, A. Vink, C. Uyttenhove, P. G. Coulie, M. R. Rubira, and R. J. Simpson. 1986. Purification and NH₂-terminal amino acid sequence of a T-cell-derived lymphokine with growth factor activity for B-cell hybridoma. *Proc. Natl. Acad. Sci. USA.* 83:9679.
8. Chang, T. W., S. McKinney, V. Liu, P. C. Kung, J. Vilcek, and J. Le. 1984. Use of monoclonal antibodies as sensitive and specific probes for biologically active human γ -interferon. *Proc. Natl. Acad. Sci. USA.* 81:5219.
9. Doherty, P. C., and R. M. Zinkernagel. 1974. T-cell mediated immunopathology in viral infection. *Transplant. Rev.* 19:89.
10. Lebon, P., B. Boutin, O. Dulac, G. Ponsot, and M. Arthuis. 1988. Interferon γ in acute and subacute encephalitis. *Br. Med. J.* 296:9.
11. Aarden, L. A., E. R. De Groot, O. L. Schaap, and P. M. Lansdorp. 1987. Production of hybridoma growth factor by human monocytes. *Eur. J. Immunol.* 17:1411.
12. Van Damme, J., S. Cayphas, J. Van Snick, R. Conings, W. Put, J.-P. Lenaerts, R. J. Simpson, and A. Billiau. 1987. Purification and characterization of human fibroblast-derived hybridoma growth factor identical to T-cell-derived B-cell stimulatory factor-2 (interleukin-6). *Eur. J. Biochem.* 168:543.
13. Muraguchi, A., T. Hirano, B. Tang, T. Matsuda, Y. Horii, K. Nakajima, and T. Kishimoto. 1988. The essential role of B cell stimulatory factor 2 (BSF-2/IL-6) for the terminal differentiation of B cells. *J. Exp. Med.* 167:332.
14. Houssiau, F. A., K. Bukasa, C. J. M. Sindic, J. Van Damme, and J. Van Snick. 1988. Elevated levels of the 26K human hybridoma growth factor (interleukin 6) in cerebrospinal fluid of patients with acute infection of the central nervous system. *Clin. Exp. Immunol.* 71:320.