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Elevated levels of the 26K human hybridoma growth factor (interleukin 6) in cerebrospinal fluid of patients with acute infection of the central nervous system

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SUMMARY

We reported recently that a human protein, previously described as IL-1 inducible 26K factor (26K) or interferon-β_2, was a potent growth factor for B cell hybridomas *in vitro*. Subsequently, it appeared that this protein was also identical with the lymphokine B cell stimulatory factor 2. Here we report that the levels of 26K are considerably increased during the early stages of acute infections of the central nervous system. This elevation in 26K titres was not observed in either chronic infections or in non-infectious diseases.

Keywords 26 kD protein hybridoma growth factor interleukin 6 infection meningitis central nervous system

INTRODUCTION

We have recently developed a set of murine B cell hybridomas and plasmacytomas whose growth and survival *in vitro* are strictly dependent on a new murine lymphokine named interleukin-HP1 (Van Snick *et al.*, 1986; 1987). Subsequently, we discovered that a previously cloned 26 kD human protein (26K) alternatively called interferon-β_2 (Haegeman *et al.*, 1986; Zilberstein *et al.*, 1986), also supported the growth of these cells (Van Damme *et al.*, 1987a; Poupart *et al.*, 1987). Besides its growth factor activity for hybridomas, the 26K factor also acts on lymphoblastoid B cell lines to increase their rate of immunoglobulin synthesis. This property led to its identification as B cell stimulatory factor 2 by the group of Kishimoto (Hirano *et al.*, 1986). The 26K factor is produced by many different cell types including monocytes (Aarden *et al.*, 1985; 1987), activated T cells (Hirano *et al.*, 1986) and fibroblasts exposed to interleukin 1 and poly-rl:rC (Van Damme *et al.*, 1987a, b).

To evaluate the biological significance of this new lymphokine, we initiated a study of the factors that affect its production *in vivo*. The central nervous system was selected for this study because of its self-contained character, which makes it more sensitive for the detection of lymphokine responses. Here we report that the levels of 26K are considerably increased in cerebrospinal fluid during the early stages of many infectious diseases.

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MATERIALS AND METHODS

Patients

Two hundred and eighteen cerebrospinal fluid (CSF) samples from patients with various neurological disorders were obtained by spinal puncture and frozen at −20°C. Samples from patients with meningitis were taken within 3 days of onset of the disease. The 18 bacterial meningitis samples were collected from 16 patients infected with the following microorganisms: *Streptococcus pneumoniae* ($n=3$), *Neisseria meningitidis* ($n=3$), *Listeria monocytogenes* ($n=1$), *Staphylococcus aureus* ($n=1$), *Streptococcus β-haemolyticus* ($n=1$), *Serratia marcescens* ($n=1$), *Peptococcus* ($n=1$), none detected ($n=5$). The 26 patients suffering from chronic headache were free of objective signs of neurological disorders and had normal CSFs with respect to protein content (< 0·45 g/l), cell count (< 5/μl) and agar gel electrophoresis.

Hybridoma growth factor (HGF) assay

Interleukin-HP1-dependent mouse hybridoma 7 TD1 was cultured in flat-bottomed microtitre plates (2000 cells/well) in the presence of serial dilutions of CSF or serum decomplemented by heating at 56°C for 20 min. After 4 days of culture, the number of surviving cells was determined by a colorimetric assay for hexosaminidase, as previously described (Van Snick *et al.*, 1986). HGF activity was expressed in U/ml defined as the dilution giving half-maximal proliferation of 7 TD1 cells. One unit corresponds to circa 5 pg of 26K protein. 7TD1 cells do not

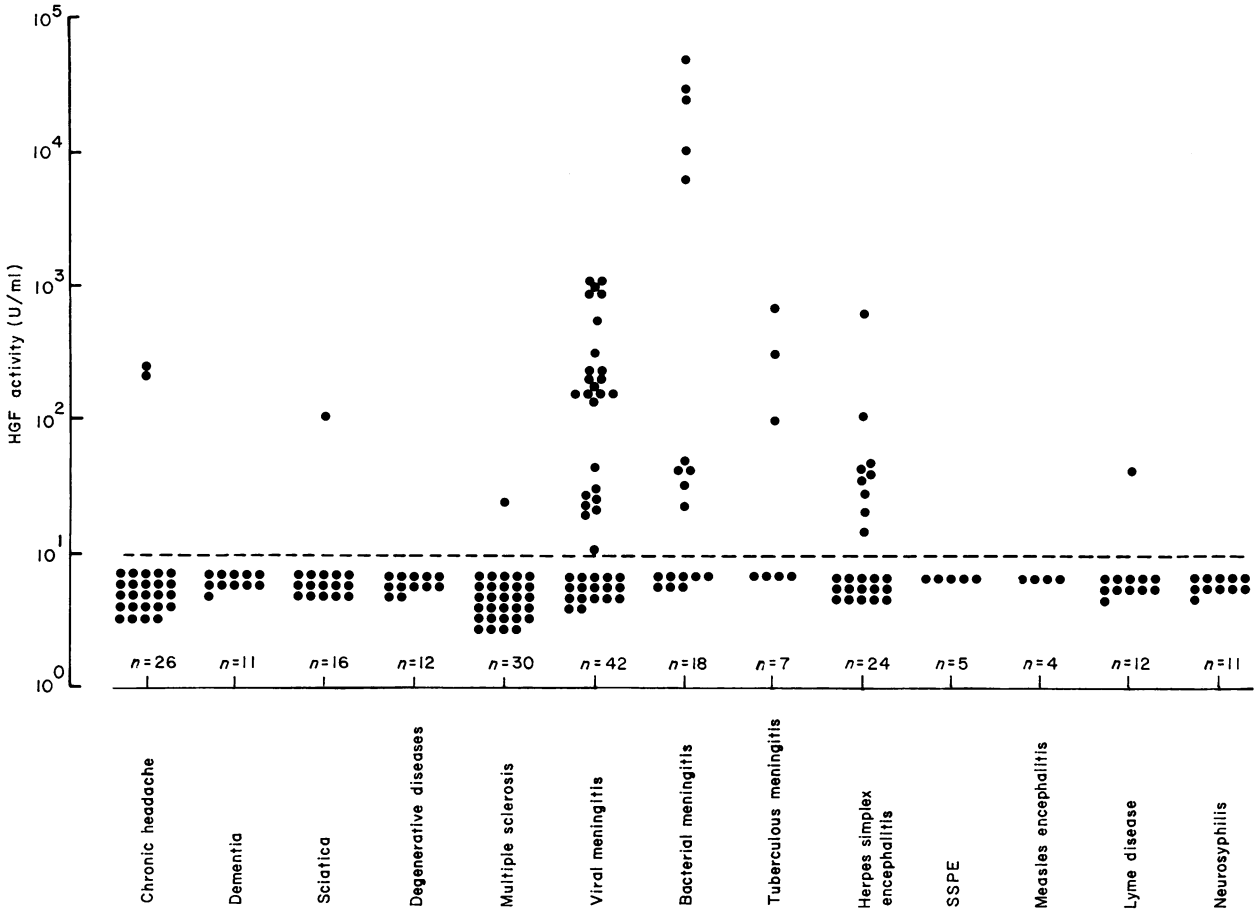

Fig. 1. HGF activity of CSF in various neurological diseases. The dotted line represents the limit of detection.

respond to either interleukin-1, interleukin-2, interferons α/β nor to any of the known colony-stimulating factors (Van Snick *et al.*, 1986; Van Damme *et al.*, 1987b).

CSF immunoglobulin indices

IgG and albumin were assayed by immunonephelometry (Technicon, Tarrytown, NY). IgA and IgM were measured by particle counting immunoassay (Masson *et al.*, 1981). Immunoglobulin (Ig) indices were calculated as follows:

$$\frac{\text{(CSF Ig/serum Ig)}}{\text{(CSF albumin/serum albumin)}}.$$

RESULTS

HGF activity in CSF

HGF-activity was undetectable (< 10 U/ml) in most CSF samples from patients with chronic headache, dementia, sciatica, clinically definite multiple sclerosis, subacute sclerosing panencephalitis, measles encephalitis, and degenerative diseases such as Parkinson's disease, amyotrophic lateral sclerosis and spinocerebellar degenerations (Fig. 1). In contrast, HGF activity was considerably elevated in the CSF of about half the patients with Herpes simplex encephalitis and with viral, bacterial and tuberculous meningitis. For bacterial meningitis, we were unable to correlate the HGF activity with a specific causative organism. Cerebrospinal fluid from patients with Lyme disease or neurosyphilis had no HGF activity.

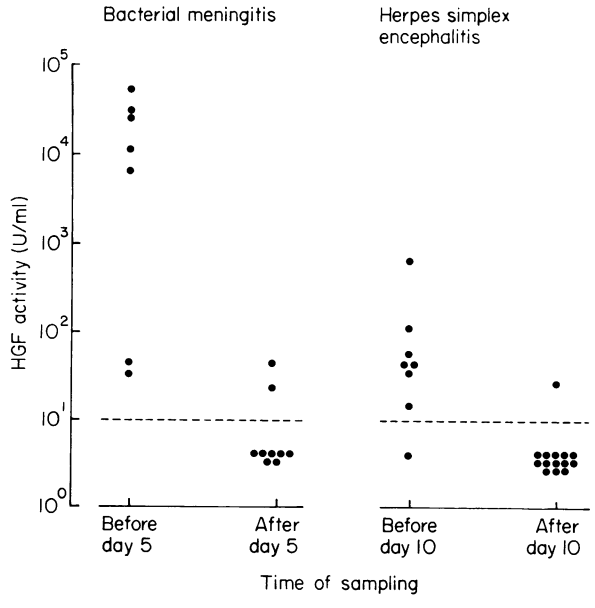

Fig. 2. HGF titres in CSF as a function of time.

Table 1. Effect of anti-26K antiserum on HGF activity in CSF samples

Patient	Disease	HGF activity (U/ml) Without anti-26K	With anti-26K
1	Herpes simplex encephalitis	650	<64
2	Tuberculous meningitis	740	<64
3	Viral meningitis	1050	<64
4	Viral meningitis	1020	<64
5	Viral meningitis	900	<64
6	Bacterial meningitis	26300	<320
7	Bacterial meningitis	51800	<320

Appropriately diluted CSF samples (10 μl) were incubated with an equal volume of a 1:500 dilution of goat antiserum raised against partially purified human fibroblast-derived 26K protein as previously described (Van Damme *et al.*, 1987b). Controls were similarly incubated without antiserum. After 1 h at room temperature, the reaction mixes were titrated for HGF activity.

Table 2. Correlation between 26K activity and other CSF variables

	Viral meningitis ($n=32$*)	Bacterial meningitis ($n=8$*)
Cell count	$r=0{\cdot}35$ ($P=0{\cdot}05$)	$r=0{\cdot}99$ ($P<0{\cdot}001$)
Protein level	$r=0{\cdot}38$ ($P<0{\cdot}05$)	$r=0{\cdot}97$ ($P<0{\cdot}001$)
IgG index	$r=-0{\cdot}02$ (NS)	$r=0{\cdot}52$ (NS)
IgA index	$r=-0{\cdot}13$ (NS)	$r=-0{\cdot}08$ (NS)
IgM index	$r=-0{\cdot}08$ (NS)	$r=0{\cdot}02$ (NS)

NS, not significant.

Probability levels were calculated by Student's *t*-test.

* Samples were included in this study only when data were available for each of the five indicated CSF variables.

Kinetics of HGF production

For patients with Herpes simplex encephalitis and bacterial meningitis we evaluated the correlation between HGF titres and disease stage. The results shown in Fig. 2 clearly demonstrate that high HGF titres were only found during the first days of the disease.

Inhibition of HGF activity with antibodies against 26K

To examine the relation of 26K with the HGF found in CSFs of a representative set of patients, we tested the ability of a goat antiserum reactive with the 26K protein to inhibit the HGF activity of these samples. As shown in Table 1, the HGF activity of all tested CSF samples was completely abolished by pre-incubation with this antiserum, demonstrating a serological identity between this activity and 26K.

Serum 26K activity

To investigate the origin of the 26K activity found in CSFs of our patients, we also measured HGF activity in their serum. Patients with 26K titres greater than 100 U/ml in CSF were selected for this study. Of 16 serum samples tested, none contained detectable 26K activity (< 10 U/ml). This was not due to the presence of a serum inhibitor of 26K as indicated by the fact that purified 26 K fully retained its activity in the presence of human serum (data not shown).

Correlation of HGF titres with Ig indices and other CSF parameters

Both cell counts and protein levels, which are good indicators of inflammation, were positively correlated with 26K activity in viral and bacterial meningitis (Table 2). By contrast, no correlation was observed between 26K activity and Ig indices.

DISCUSSION

26K is a newly described human lymphokine produced by cells as different as T cells, monocytes and fibroblasts, which enhances immunoglobulin production by EBV-transformed B cells (Hirano *et al.*, 1986) and supports the *in vitro* proliferation and survival of interleukin-HP1-dependent murine B cell hybridomas and plasmacytomas (Van Damme *et al.*, 1987a).

Analysis of 26K activity in a wide variety of diseases of the central nervous system disclosed a striking association between infection and elevated 26K titres in CSF. The data reported here indeed demonstrated a considerable increase in 26K activity in about half the CSF samples of patients suffering from acute viral, bacterial or tuberculous infections. By contrast, 26K activity was rarely detected in subacute and chronic infections as well as in non-infectious diseases such as chronic headache, dementia, sciatica, degenerative diseases and multiple sclerosis.

Comparison of HGF titres in bacterial versus viral diseases disclosed the existence of a unique group of patients with very high HGF titres among those suffering from bacterial infections. If this quantitative difference between bacterial and viral infections was confirmed with a large group of patients, determination of HGF levels in CSF could be a valuable aid to differentiate bacterial and viral infections. However, this would require the development of a more rapid assay than the hybridoma growth factor assay used in the present work.

The 26K protein detected in the CSF after infection was clearly of local origin since patients with high 26K titres in CSF had no detectable 26K activity in serum. In addition, this absence of 26K in the serum at a time when its titres in CSF exceeded several thousand U/ml highlights the advantage of a closed system such as the CNS for the study of the physiology of cytokines.

Analysis of 26K titres as a function of time showed a very early and transient peak of activity after infection. For example, in the group of patients with Herpes simplex encephalitis, positive samples were found in seven out of eight cases when CSF was collected within 10 days from the onset of the disease, whereas only one of 14 samples taken at a later stage were positive in our assay. Considering this transient character of the 26K response, a great deal of the negative results observed in our original screening of infected patients can probably be attributed to delayed sampling.

As mentioned above, 26K enhances immunoglobulin secretion by several EBV-B cell lines (Hirano *et al.*, 1986). However, the lack of correlation between immunoglobulin and 26K levels observed in our samples suggests that the molecule does not play a major role in the control of immunoglobulin secretion *in vivo* or, more likely, that, in the diseases examined here, its effects are obscured by other factors. One should therefore not exclude the possibility that the activity of 26K may in fact not be restricted to the B cell lineage and that, especially in the central nervous system, other cells may be responsive to its growth and differentiation inducing activities.

While the actual physiological target and function of 26K remain presently unclear, the data presented here demonstrate that the production of this molecule can be dramatically and promptly amplified during infection in a way typical of acute phase reactants.

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