Interferon in serum and cerebrospinal fluid in subacute sclerosing panencephalitis

To the editor: Antivirals and other therapeutic measures have been tried in cases of subacute sclerosing panencephalitis (SSPE) with little success.[1] Anticipating the use of interferon in the treatment of this disease, we wanted to know whether interferon was already present in the cerebrospinal fluid (CSF) of these patients. In the few patients with SSPE who have been reported to respond to transfer factor therapy, interferon has become detectable in CSF or serum or both;[2,3] in only one was it detectable prior to therapy, and the titre was low. Therefore, we tested 83 samples of CSF and 20 samples of serum from 72 patients with various diseases of the central and peripheral nervous system for the presence of interferon by a microassay adapted from the procedure of Dahl and Degré.[4]

Microassay

Each cup of a microplate was seeded with 0.1 ml of a human embryo fibroblast cell suspension (IMHP*) containing 400-000 cells per millilitre. The cells were suspended in an equal mixture of minimum essential medium (MEM) plus Earle's salts and 199 plus Hanks salts medium containing 10% fetal calf serum. Twofold dilutions of interferon or of the CSF or serum sample to be tested were added, 0.1 ml per cup. The plate was incubated at 37°C in a CO_2 incubator for 24 hours. The next day confluence of the monolayer was checked and the medium removed by inverting the plate over a sterile piece of gauze.

Added to each cup were 0.2 ml of MEM plus Earle's salts without serum and 0.025 ml of challenging Sindbis virus containing 1000 tissue culture infective doses ($TCID_{50}$s). The plates were then examined after 48 hours' incubation in a CO_2 incubator at 37°C. The titre was estimated as the dilution that inhibited the cytopathic effect of the virus in 50% of the four cups used per dilution.

A cell control and a virus control were included in the test; the cell control received 0.025 ml of medium instead of virus, and the virus control, 0.1 ml of medium instead of interferon. Back titration of the virus and assay of a local standard interferon as a control were also performed with each test. The World Health Organization interferon standard 69/19 was tested by our microassay; in this test 1 international unit is equivalent to 2 of our units.

The microassay was compared with the plaque reduction assay and the infectivity inhibition macroassay and found to be the most sensitive of the three in our hands. The plaque reduction assay was twice as sensitive as the macroassay, and the microassay four to six times as sensitive as the macroassay.

Of the 72 patients 8 had SSPE, the diagnosis being firmly established by accepted criteria; their clinical and laboratory features have been reported.[5] The other patients, serving as controls, had various neurologic diseases, including herpes simplex encephalitis; enteroviral, mumps and other types of aseptic meningitis; leukemic meningitis; and Guillain-Barré syndrome.

Interferon was detectable in unconcentrated CSF samples from three of the eight patients with SSPE and from only 4 of the 64 controls. Using two of the larger samples that had interferon titres of 40 and 80 U we ascertained that the interferon was stable to acid and was destroyed by trypsin, like the control interferon preparation, and that the samples did not contain antibody to the challenging virus. The highest titre, 100 U/0.1 ml (1000 U/ml), was found in the CSF sample from a patient with rheumatoid spondylitis. Excluding this patient, the mean titre of interferon was higher in patients with SSPE than in controls. Serial CSF samples were available from four patients with SSPE and four controls (Table I). In one patient with SSPE interferon was present in the CSF but not in serum collected at the same time, and in another patient with SSPE the reverse was true.

These findings agree with recent observations showing that administered interferon does not pass freely from blood to CSF and vice versa, and suggest that CSF interferon may be produced in situ.[6,7] The variation in CSF interferon titre in the follow-up of patients with SSPE, from less than 4 to 40 U/0.1 ml, suggests intermittent production of interferon, possibly as a result of intermittent expression of the viral genome. We could find no correlation, however, between the titre of interferon and parameters such as CSF lymphocyte count or protein content and CSF measles antibody titre.

These results suggest that interferon will probably not be useful in the therapy of SSPE except if the CSF or serum interferon titres in SSPE do not reflect the concentration of interferon in brain tissue, or if the specific activity of SSPE interferon for an SSPE strain of measles virus differs from that for the challenging virus in our bioassay. This is unlikely but possibly worth investigating.

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Table I—Cerebrospinal fluid (CSF) and serum interferon titres (U/0.1 ml) in patients with subacute sclerosing panencephalitis (SSPE) and in controls

Patient no.	Diagnosis	Sample tested	Mean titre*
1	SSPE	CSF	20 (one, < 4)
		Serum	<4
2	SSPE	CSF	<4 (one, < 4)
		Serum	80 (four, 20)
3	SSPE	CSF	40 (one, < 4)
4	SSPE	CSF	20 (one, < 4)
5	Rheumatoid spondylitis	CSF	100
6	Leukemic meningitis	CSF	10
7	Febrile convulsion	CSF	10
8	Bronchiolitis with meningismus	CSF	10

*Since the first dilution of serum or CSF tested was ½, the lowest titre of interferon detectable by the microassay was approximately 4 international units (IU). The assay could detect a minimum of 2 IU in undiluted serum or CSF.

References

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