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CLINICAL STUDIES OF INTERFERON*

(II) RECOVERY OF INTERFERON FROM CEREBROSPINAL FLUID OF PATIENTS WITH ASEPTIC MENINGITIS

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ABSTRACTS

As the test materials for obtaining interferon, 22 specimens of cerebrospinal fiuid from patients with aseptic meningitis have been used, of which 17 specimens have been found to contain interferon. A significant correlationship has been proved to exist between the leucocyte concentration in the cerebrospinal fluid of the patients and the titer of the interferon.

It is the aim of this paper to discuss the source of interferon in the cerebrospinal fluid as used in the experimentation.

INTRODUCTION

Several kinds of virus inhibitors have recently been extracted not only from the pharyngeal washings of some patients infected with influenza virus[1], but also from the cerebrospinal fluid of patients suffering from aseptic meningitis[2].

These inhibitors, however, have been attested to be extracted from those substances mentioned above only when the patients are in the acute phase of the illness and not on any convalescent stages. The properties of these inhibitors, however incomplete their characterization may be, could, in so far as they have been determined, be taken as identical with those of an interferon[3~10].

In order to further investigate the production of interferon in the cerebrospinal fluid (abbr. CSF) and serum of patients with aseptic meningitis, the

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author collected specimens of CSF and serum from patients with the illness, and tested them to see if they had any inhibitory effect against Vesticular Stomatistis Virus (VSV). As a result, interferon was found to exist in the specimens from those patients who were in the acute phase of the illness.

MATERIALS AND METHOD

Patients: Specimens of CSF and serum were obtained from those patients living in Nagoya and its vicinity who were diagnosed as suffering primarily from aseptic meningitis. However, the specimens here examined were only those gotten from the patients whose records contained clinical and laboratory data adequate to the purpose of this study. Twenty-two specimens of CSF and twenty-six specimens of serum were obtained and stored at $-20°C$, until they were tested to prove the presence therein of interforon. Of these specimens, however, those which had been contaminated by bacteria or other substances toxic to FL cells in culture were omitted.

Assay of the interferon in CSF and serum: All the fluids and sera were centrifugazed in a Spinco ultracentrifuge at $100{,}000 \times g$ for one hour.

Dilutions (in tissue culture medium) of each of the supernatant fluids were incubated as previously described[11] with FL cells for 18 hours at 37°C, prior to being challenged with approximately 100 tissue culture infective dose ($TCID_{50}$) of VSV. Specimens of CSF and serum were observed to be inhibitory when less than 20% of the cells were affected by the challenge virus, and when more than 80% of the cells in control culture were destroyed[11].

RESULTS

Of the 22 CSF specimens tested, 17 were observed to contain a viral inhibitor. And of the 26 serum specimens tested, 20 were found to contain the same substance as in the former case.

It was observed that only those specimens of CSF and serum that were obtained from patients at an acute phase of aseptic meningitis exhibited viral inhibition.

Relationship of interferon to concentration of leucocytes in CSF: Most of the inhibition-positive CSF's contained more than 100 leucocytes/mm^3. Of the 5 specimens containing less than 100 cells/mm^3, only one was found to be positive.

The correlation between the number of leucocytes and the titer of interferon in CSF is illustrated in Fig. 2 and Table 3. Generally speaking, the more the leucocyte-counts, the higher the titer of interferon in CSF.

CSF protein and interferon: No apparent relationship was observed to exist between the protein concentration of CSF and the presence of interferon in the

cases of aseptic meningitis.

Viral and serological studies: The author did not undertake virus isolation with regard to the CSF's and stools from the patients with aseptic meningitis.

However, he performed some experiments with the paired sera from 26 patients with aseptic meningitis, and ascertained the significant increase of neutralizing CF antibodies in the process of the disease from acute phase to convalescent stage. From these studies, it was observed that six indirect evidences for the viral etiology of aseptic meningitis were likely to be given (Table 1).

Onset of illness and presence of interferon: In reviewing any hospital records, it is often difficult to determine the onset of the illness with accuracy. However, so far as the author examined, the interferon in CSF and serum of patients with aseptic meningitis was observed to exhibit its highest titer immediately after the onset of the illness and to decrease gradually till it almost disappeared by the seventh day of the illness.

TABLE 1. N.T. and CF-Antibody Titer of 20 Aseptic Meningitis Patients

Tested virus		acute phase (positive/total)	convalescent stage (positive/total)
Echo-4	NT≧4	0/20	2/20
Echo-6		0/20	0/20
Echo-7		1/20	3/20
Echo-9		0/20	0/20
Mumps	CF≧32	0/20	2/20

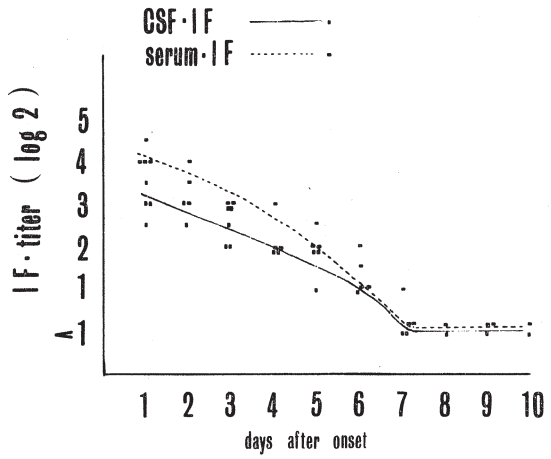

FIG. 1. Interferon titer in CSF and serum of aseptic meningitis patients.

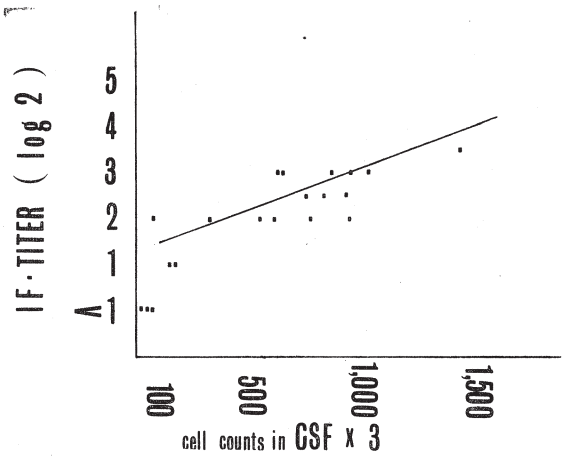

FIG. 2. IF-titer and cell counts in CSF of aseptic meningitis patients.

TABLE 2. Interferon titer in sera and Interferon titer and cell counts in cerebrospinal fluids (CSF) from patients with aseptic meningitis

Case No.	days after onset	IF titer in sera	IF titer in CSF	cell counts ×3
1	1	4	2.5	920
2	1	4	3	860
3	1	4	3	1,020
4	1	4	3.5	1,420
5	2	3	2.5	820
6	2	3.5	—	—
7	2	4.5	3	640
8	3	—	2	940
9	3	3	2	760
10	3	3	2.5	744
11	3	—	3	618
12	3	—	3	940
13	4	2	—	—
14	4	2	2	600
15	4	3	2	560
16	5	2	1	160
17	5	2	2	75
18	5	2.5	2	320
19	6	1	—	—
02	6	1	—	—
21	6	1.5	1	148
22	6	2	—	—
23	7	<1	<1	124
24	7	<1	<1	43
25	7	1	—	—
26	8	<1	<1	68
27	9	<1	<1	27
28	9	<1	—	—
29	10	<1	<1	18

TABLE 3. Characterization of inhibitor in cerebrospinal fluid

Biologic Properties

1. Inhibits multiplication and CPE of Vesicular Stomatitis virus in cultured of FL cells.
2. Does not diminish infectivity of 10 and 100 $TCID_{50}$ of Vesicular Stomrtitis Virus after incubation at 37°C for 2 hrs.
3. Inhibition does not depend on continued presence of inhibitor, since removal of medium containing inhibitor (after 24 hrs incubation with cells) prior to challenge with Vesicular Stomatitis Virus did not abolish the protective effect.

Biochemical and Biophysical Properties

1. Not sedimented by ultracentrifugation at 100,000 g 1 hr.
2. Activity lost after incubation with trypsin 37°C 1 hr.
3. Non-dialyzable through Visking tubing.
4. Activity does not lose somuch by treatment at pH 2.0 24 hrs, 4°C.

The peak of the titer of interferon in CSF was oberved not to exceed that of the interferon in the serum (Fig. 1, Table 2).

Characterization of interferon: In order to ascertain one or more of the biologic and biochemical properties of interferon some representative specimens were tested, though inadequate volume of the material often precluded more than 3 to 4 tests per specimen. For a given test, however, all the specimens yielded results similar to one another. No difference was ascertained to exist between the properties of interferon obtained from patients with aseptic meningitis to be used in this paper (Table 3) and those of the inhibitor previously described[8].

DISCUSSION

Although interferon has been regarded as one of the host's defenses against viral infection[9)12)] there have been few attempts to demonstrate its presence in tissue fluids and secretions of patients suffering from viral diseases.

Recovery of an inhibitor from the pharyngeal washings of patients with influenza[1)] and from the CSF of patients with aseptic meningitis[2)] stimulated the present writer to researching the relationship, if any between the cell-counts and the interferon titer in the cerebrospinal fluid of patients with aseptic meningitis.

The biologic properties of the inhibitor that the writer recovered from the CSF suggested that they were identical with those of interferon. He utilized the term "interferon" in calling the inhibitor that he recovered, because it showed no loss of activity at pH 2.0 (Table 2) quite in comformity with interferon which has been reported to be stable at pH 2.0[2)13)].

The source of interferon is not known yet. Since positive specimens were obtained mostly from patients with viral diseases, one is tempted to relate

viral infection casually with the presence of inhibitor in the CSF. Thus, viral infected parenchymal or stromal cells of the central nervous system may have liberated this factor. In that event, the author may further be allowed to assume that viral infected leucocytes might have been the source of interferon, since several kinds of virus have been isolated from the leucocytic fraction of blood[14)15)], and both human and animal leucocytes have been reported to secrete interferon after infection *in vitro*[16)~20)].

It is also possible to assume that interferon, having been liberated outside of the central nervous system, was passed from the blood to the CSF. If this be the case, the concentration of leucocytes in CSF may have only been an index showing the extent of inflammation, thus to serve to measure the permeability of the blood-brain barrier. As a matter of fact, several experimental studies have hitherto suggested that interferon may be observed to be active and effective at a site distant from that of its liberation[17)22)23)].

Lastly, though without any direct evidence implicating viral infected cells with the source of this inhibitor, a third hypothesis must here be considered, namely that uninfected leucocytes liberate interferon even under certain inflammatory conditions that occur most commonly in a viral infection. In support of this hypothesis the author would like to point out that the titer of interferon in the CSF was found to be lower than that of the interferon in the serum (Fig. 1).

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