Recovery of an Interferon-Like Substance from Cerebrospinal Fluid.* (29559)

ION GRESSER† AND KIARASH NAFICY‡ (Introduced by J. F. Enders)
Research Division of Infectious Diseases and Children's Cancer Research Foundation, Children's Hospital Medical Center, Boston, and Department of Medicine, Harvard Medical School, Boston, Mass.

A virus inhibitor was recently recovered from pharyngeal washings of some patients with influenza virus infection(1). The inhibitor was found during the acute phase of illness but not in convalescence. Although incompletely characterized, its properties insofar as determined were comparable with those of an interferon(2).

To explore further the occurrence of interferon-like substances in viral infections of man, specimens of cerebrospinal fluid (CSF) were collected from patients with infectious and non-infectious diseases of the central nervous system and tested for inhibitory effect against Sindbis virus. An inhibitor resembling interferon was detected in a significant proportion of cases with viral infection but only in a small number of those with presumably non-viral disorders.

Methods and materials. Patients. Cerebrospinal fluid was obtained from patients examined in Boston at the Children's Hospital Medical Center, New England Deaconess Hospital, Chelsea Naval Hospital, Peter Bent Brigham Hospital and Beth Israel Hospital. Only specimens from patients whose records contained clinical and laboratory data adequate to the purpose of this study were examined. One hundred and ninety-seven specimens were tested for presence of inhibitor. Since some specimens were either contaminated by bacteria or toxic for cells in culture, only the results of tests of 181 specimens from 152 patients are presented.

Storage of CSF specimens. Sixty-five specimens were obtained in 1961 and 1962 by Dr. D. Carver and one of us (K.N.) during an investigation of aseptic meningitis(3). These were stored at —20°C until tested in 1963. One hundred and sixteen specimens collected from 88 patients in 1963 were kept at 4°C for 0-4 days prior to testing. Comparable results were obtained from frozen stored specimens (1961-2) and those collected in 1963 and are presented together (Table I).

Assay of inhibitor in CSF. All fluids were centrifuged for one hour at 110,000 *g* in a Spinco Model L ultracentrifuge. Dilutions (in tissue culture medium) of each of the supernatant fluids were incubated as previously described(4) with primary cultures of human amnion cells(5) for 18-24 hours at 37°C prior to "challenge" with approximately 100 $TCID_{50}$ of Sindbis virus (laboratory strain AR-339). In a few additional tests approximately 100 $TCID_{50}$ of ECHO 11 virus (laboratory strain Gregory) was employed as the challenging agent. A CSF specimen was considered to be inhibitory when less than about 20% of the cells were affected by the challenge virus and when more than 80% of the cells in control cultures were destroyed(4). Due to small volumes available in many instances, the fluids were usually tested at only one dilution, *i.e.* 1:2, 1:4, 1:8 or 1:16.

Results. Of the 181 CSF specimens tested (from 152 patients), 32 specimens (28 patients) contained a viral inhibitor. Cerebrospinal fluids exhibiting viral inhibition were most frequently obtained from patients with aseptic meningitis (and/or encephalitis) whose clinical manifestations were compatible with a viral aetiology. Thus, CSFs from 23 of 58 patients with this diagnosis were inhibitory (39.6%), while CSFs from only 3 of 25 patients with bacterial meningitis

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† Career Development Award, U.S.P.H.S., Nat. Inst. of Allergy & Infect. Dis.

‡ International post doctoral fellow of Nat. Inst. Health, U.S.P.H.S. Present address: Inst. of Parasitology and Malariology, Teheran Univ. School of Med., Teheran, Iran.

TABLE I. Incidence of Interferon-Like Substance in Cerebrospinal Fluid.

Dilution tested*	Aseptic meningitis (and/or encephalitis) probably viral				Bacterial meningitis				Non-infectious disease of CNS			
	Leucocytes in cerebrospinal fluid, mm³											
	<20	20–100	100–500	>500	<20	20–100	100–500	>500	<20	20–100	100–500	>500
1:2		1/4[2]	1/1	1/1	0/1	1/3[8]	0/3	1/11[4]	0/29	0/2	1/2[7]	0/7‡
1:4	0/3†	2/6	10/17	5/8		0/1		1/4 [5]	1/22[6]	0/1		0/1
1:8	1/2[1]	0/6	1/2	1/2	0/1			0/1	0/3			
1:16	0/1	0/2	0/1	0/2					0/2			
Total	23/58				3/25				2/69			

* All specimens were assayed at only one dilution.
† No. of patients whose CSF contained a viral inhibitor/No. of patients tested.
‡ Patients with acute leukemia and leukemic involvement of CNS.

(1) R.O., a 4½-yr-old male with Guillain-Barré syndrome. The spinal fluid protein was 144 mg % and no cells were present. ECHO virus 22 was isolated from the CSF(3).

(2) J.C., a 55-yr-old male with sudden onset of confusion, right sided facial pain and fever. The spinal fluid contained 65 leucocytes/mm³ (predominantly mononuclear cells), and total protein was 107 mg %. Electroencephalogram and arteriography suggested a focal cerebral lesion, but the final clinical diagnosis was viral encephalitis. Patient died and an autopsy was not performed.

(3) R.S., a 3-yr-old male with *Hemophilus influenzae* meningitis. Nine days after admission to the hospital he developed a subdural effusion. Spinal fluid obtained from lumbar puncture at this time contained 40 mononuclear cells/mm³, total protein of 27.8 mg % and did not demonstrate any viral inhibitor. Cerebrospinal fluid obtained at the same time from the subdural effusion contained 63 mononuclear cells and the total protein was 368 mg %. This specimen did contain an inhibitor.

(4) M.B., a 3-yr-old male with *Hemophilus influenzae* meningitis whose spinal fluid contained inhibitor on the 2nd day of illness but not on the 3rd day. Spinal fluid leucocyte count was 9,000 and 10,000/mm³, respectively. Two days after admission to the hospital the patient developed multiple vesicular lesions on the mouth and fingers of both hands. These were diagnosed clinically as due to herpes simplex virus.

(5) K.L., a 10-yr-old male with congenital agammaglobulinemia, dermatomyositis, treated with cortisone, developed listeria monocytogenes meningitis. Inhibitor recovered on 3 separate occasions from the spinal fluid. Leucocyte count in the spinal fluid varied between 270-1150/mm³, (predominantly mononuclear cells), and the total protein ranged between 148-349 mg %. An unidentified transmissible agent was recovered in cell culture from the liver obtained post mortem(6).

(6) D.B., a 2½-yr-old male with overwhelming Klebsiella pneumonia. His hospital course was complicated, and the diagnosis of encephalitis of undetermined aetiology was considered. The spinal fluid, however, was normal. No cells were present, and total protein was 23 mg %.

(7) C.M., a 5-yr-old female with acute lymphocytic leukemia of 4 years' duration. She was in hematologic remission on cyclophosphamide. Cerebrospinal fluid contained 210 leucocytes/mm³, and total protein was 196 mg %.

(12%) and only 2 of 69 patients with presumed non-infectious diseases of the CNS (2.9%) were positive (Table I).

Relationship of inhibitor to concentration of leucocytes in CSF. Most of the inhibitory CSFs from patients with aseptic meningitis contained more than 100 leucocytes/mm^3 (Table I). Only 4 of 24 specimens (16.6%) with less than 100 cells/mm^3 were positive, as compared with 19 of 34 specimens (55.8%) containing more than 100 cells/mm^3.

The correlation between number of leucocytes in the spinal fluid and presence of inhibitor did not appear to extend to fluids obtained from patients with bacterial meningitis, or non-infectious disease of the CNS. In the former group, of 16 specimens containing more than 500 cells/mm^3, only two (12.5%) were inhibitory, though some specimens contained as many as 10,000 cells/mm^3. Included in the latter group were cerebrospinal fluids from 14 patients with acute leukemia and leukemic involvement of the CNS. Only one specimen (7.1%) was positive (Table I, case 7) though 10 specimens contained more than 100 cells/mm^3.

CSF protein and inhibitor. There was no apparent relationship between protein concentration of the CSF and presence of inhibitor in the cases of aseptic meningitis. Some specimens with inhibitor obtained from patients with non-viral diseases exhibited increased protein, but these were too few to permit demonstration of any correlation.

Viral studies of patients with aseptic meningitis and presence of inhibitor in CSF. In 1961 specimens (throat, blood, fecal and CSF) were obtained for virus isolation utilizing primary cultures of human amnion or rhesus monkey kidney cells(3). Viruses§ were recovered from one or more of these sources from 28 of 56 patients. Inhibitor was found in the CSFs of 7 of 28 of these patients (25%) and in 9 of 28 patients (32.1%) from whom no virus was recovered. Viruses‖ were isolated from the CSF of 4 patients, and only one of these CSFs contained inhibitor. From these limited studies, therefore, there did not appear to be a direct relationship between presence of inhibitor in CSF and recovery of a virus in cell culture.

§ Coxsackie Viruses B4, B5, ECHO Virus 22, Poliovirus I, Adenovirus 2, and several unidentified agents.

‖ ECHO Virus 22 (1) and unidentified agents (3).

TABLE II. Characterization of Inhibitor in Cerebrospinal Fluid.

Biologic Properties
1) Inhibits multiplication and CPE of Sindbis and ECHO 11 viruses in primary cultures of human amnion cells.
2) Does not diminish infectivity of 10 and 100 $TCID_{50}$ of Sindbis virus after incubation at 37°C for 2 hr.
3) Inhibition does not depend on continued presence of inhibitor, since removal of medium containing inhibitor (after 24 hr incubation with cells) prior to challenge with Sindbis virus did not abolish the protective effect.
4) Does not inhibit infectivity of 10 $TCID_{50}$ of Sindbis virus for primary cultures of chick embryo cells.
Biochemical and Biophysical Properties
1) Not sedimented by ultracentrifugation at 110,000 *g* 1 hr.
2) Activity lost after incubation with 100 g crystalline trypsin 37°C 1 hr.
3) Non-dialyzable through Visking tubing.
4) Loss of activity at pH 2.0-2.2 24 hr, 4°C.

Onset of illness and presence of inhibitor. In reviewing the hospital records, it was often difficult to determine with accuracy the onset of illness. A number of patients with aseptic meningitis had minor symptoms preceding their major illness by several days, rendering correlation between presence of inhibitor and a given day of illness unreliable.

Characterization of inhibitor. Representative specimens were tested for one or more of the biologic and biochemical properties of interferon(2) though inadequate volumes of material often precluded more than 3-4 tests per specimen (Table II). For a given test all specimens yielded similar results. No difference was ascertained, insofar as tested, between the properties of the inhibitor obtained from patients with aseptic meningitis and the inhibitor present in the CSF of patients with leukemia (case 7, Table I), bacterial meningitis (case 3) and possible viral encephalitis (case 2).

It was of interest that exposure to a pH of 2.0-2.2 for 24 hours at 4°C markedly

reduced the activity of the inhibitor at the one dilution tested. Since CSFs were tested at only one dilution, it was not possible to determine whether a pH of 2.0-2.2 completely inactivated the inhibitor or only caused a fall in titer.

Discussion. Though interferon(2) has been considered one of the host's defenses against viral infection(7), there have been few attempts to demonstrate its presence in tissue fluids and secretions of patients suffering from viral disease. Recovery of an inhibitor, compatible with interferon, from the pharyngeal washings of patients with viral influenza(1) prompted our search for interferon in the cerebrospinal fluid of patients with suspected viral meningitis. The biologic properties of the inhibitor which we have recovered from the CSF, insofar as determined, suggested that they were identical to those of interferon. We have utilized, however, the term interferon-like, since at pH 2.0-2.2 a loss of activity was observed, whereas interferon is reported to be stable at pH 2(8).

There appeared to be a direct correlation between concentration of leucocytes and presence of inhibitor in the CSF of patients with *aseptic meningitis*. Leucocytosis *per se* did not account for the presence of inhibitor, since most specimens obtained from patients with bacterial meningitis contained a far greater concentration of leucocytes than the CSFs from patients with aseptic meningitis and yet were not inhibitory.

Since the accuracy of the methods employed to determine differential cell counts was at times unreliable we have presented only total leucocyte counts. Analysis of the results in terms of differential cell counts as recorded in the hospital records rather than in terms of total leucocyte concentrations did not contribute significantly to the interpretation of the data. A predominantly mononuclear cell count was encountered in the CSF of patients with aseptic meningitis, whereas the polymorphonuclear leucocyte predominated in cases of bacterial meningitis. It is of interest, however, that the total number of mononuclear cells in some negative CSFs of patients with bacterial meningitis often equalled or exceeded the number of these cells in positive CSFs of patients with aseptic meningitis.

The source of this interferon-like substance in the CSF is unknown. Since most positive specimens were from patients with probable viral disease, it is tempting to relate viral infection causally to the presence of inhibitor in the CSF. Thus, viral infected parenchymal or stromal cells of the central nervous system may have liberated this factor. We may also speculate that viral infected leucocytes may have been the source, since several viruses have been isolated from the leucocytic fraction of the blood(9,10) and human and animal leucocytes released interferon after infection *in vitro*¶(11,12).

It is also possible that the interferon-like substance was liberated outside of the central nervous system and passed to the CSF from the blood. If so, the concentration of leucocytes in the CSF may only have been an index of the extent of inflammation and thus a measure of the permeability of the blood-brain barrier. Several experimental studies have indeed suggested that interferon may be effective at a site distant from its site of liberation(12,17,18).

Lastly, without direct evidence implicating viral infected cells as the source of this inhibitor, a third hypothesis must also be considered, *i.e.* that under certain inflammatory conditions occurring most commonly in viral infection, *uninfected* leucocytes liberate an interferon-like substance. In support of this hypothesis we should point out that the titers

¶ Chang and Weinstein(13) found that "there appeared to be an inverse relationship between the number of cells in the CSF and the frequency with which ECHO 9 virus was recovered from it" and suggested the possibility of interferon as the critical factor. Portnoy, Wehrle and Hanes(14) found no correlation between CSF leucocytic concentration and recovery of ECHO 9 virus, while Warren *et al.*(15) demonstrated a direct relationship between number of cells and frequency of isolation of ECHO 4 virus in rhesus monkey kidney cells. It should be emphasized that interferon is markedly species specific, and "human" interferon may not protect monkey cells in culture(16).

of the interferon-like substance in the CSF were low and surprisingly uniform. If viral infected cells were the source, one might have expected a greater variation in the amount of inhibitor present, since viruses vary considerably in their efficiency as interferon inducers(19).

Whether the interferon-like substance we have detected in the CSF of certain patients exerts antiviral properties *in vivo* remains speculative, although its presence was associated most often with probable viral infection. *A priori,* it seems logical to regard it as one of the factors that may limit spread of viral infection in the CNS.

Summary. One hundred and eighty-one cerebrospinal fluids from 152 patients with infectious and non-infectious diseases of the central nervous system were tested for interferon. Thirty-two specimens from 28 patients contained a viral inhibitory factor similar to interferon, as assayed in primary cultures of human amnion cells "challenged" with Sindbis virus. The cerebrospinal fluids from 23 of 58 patients with probable viral meningitis were inhibitory, while the CSFs from only 3 of 25 patients with bacterial meningitis and 2 of 69 patients with non-infectious diseases of the CNS were positive. There was a correlation between leucocyte concentration in the CSF of patients with aseptic meningitis and presence of the interferon-like substance. The source of this inhibitor in the CSF is discussed.

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