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Endogenous Interferon in the Cerebrospinal Fluid of Herpes Encephalitis Patients (41047)

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Abstract. Low titers of antiviral activity were found in the cerebrospinal fluids (CSF) of herpes encephalitis patients when using a sensitive interferon assay. With this dye-uptake assay on human fibroblasts, trisomic for chromosome 21, 1 IU of human fibroblast or leukocyte interferon per milliliter can unequivocally be determined. Eight CSF samples taken during the first 10 days of illness were antivirally active whereas only 1 of 11 taken later proved positive. With the exception of one serum no interferon was found in the serum samples taken simultaneously with the CSF samples. By antibody neutralization using specific antisera against human fibroblast and leukocyte interferon it was found that the antivirally active substance is identical with or closely related to human leukocyte interferon.

Gresser and co-workers have reported that viruses infecting mice induce the synthesis of interferon, which then contributes to the early host response against this virus infection (1). This was proven by the simultaneous administration of anti-mouse interferon globulin and challenge virus. Particularly, in the case of herpes simplex virus (HSV) infection these authors were able to show that antiinterferon globulin treatment abrogating the interferon effect in vivo, was associated with a marked effect on the evolution of HSV disease (2). It resulted in the acceleration of the disease or death and in a considerable increase of the lethal efficiency of the challenge virus preparation used. Such an experiment can naturally not be carried out on humans in order to prove that endogenous interferon plays an important role in host resistance to HSV infections. One can, however, at least look for endogenous interferon in human patients suffering from HSV infections. Since no data on interferon in such patients were available, we decided to look for interferon in the CSF of HSV encephalitis patients at as early a stage of the illness as possible. This seemed to be reasonable for three reasons: (i) Bostandzhyan and Bikbulatov showed that HSV could induce measurable amounts of interferon in the brain of intracerebrally infected mice (3), (ii) Luby *et al.* reported that in the CSF of St. Louis encephalitis patients interferon was found (4), and (iii) higher interferon levels should be detectable in the CSF than in the serum of HSV encephalitis patients because of the brain–blood barrier which exists for interferon (5, 6). Here we report on low interferon titers found in the CSF of HSV encephalitis patients during the first 10 days of illness.

Materials and Methods. *Patients.* Ten patients (four male, six female, age: 16–68 years) suffering from HSV encephalitis were studied. Each of these patients had been unmistakably diagnosed by virus isolation from the brain and/or a significant increase of anti-HSV antibody titers in the CSF. Viewed retrospectively, the onset of illness could be defined with a variance of 2 days at maximum. Seven of these patients died from encephalitis while three of them survived showing severe, psychic defects.

Antibody determination. Complement fixing (CF) anti-HSV antibody titers were determined with a routine microassay according to a procedure described by Sever (7). Neutralizing antibodies were assayed with a method published by Haag and Schneweis (8).

Interferon determination. Interferon was assayed with the neutral red dye-uptake method originally introduced by Finter (9). We used the human fibroblast strain GM2504 (obtained from the Human Genetic Mutant Cell Repository, Camden, N.J.), trisomic for chromosome 21, and encephalomyocarditis virus as challenge. GM2504 cells were used because Tan *et al.*

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(10) had shown that human fibroblasts which are trisomic for chromosome 21, were much more sensitive to the antiviral activity of human interferons than diploid cells. Confluent cell monolayers in 96-well tissue culture plates were incubated with twofold serial dilutions of CSF or serum samples (lowest dilution being 1:5). After overnight incubation the culture supernatants were drained off and EMC virus (suspended at an appropriate concentration in maintenance medium) was added. After a further 2-day incubation the supernatants were withdrawn and the viable cells stained with neutral red and the dye then extracted from the cells as described elsewhere (11). The optical densities of the samples at 541 nm were automatically measured in the wells with a Titertek Multiskan photometer (Flow Laboratories GmbH, Bonn, FRG). Interferon titers are given in international units of human fibroblast interferon, i.e., corrected to the value of the international human fibroblast interferon standard preparation NIH G-023-902-527 (12).

Characterization of human interferon species. Anti-human fibroblast interferon antiserum from a goat and anti-human leukocyte interferon also obtained from a goat were used to characterize endogenous interferon as human fibroblast (FHIF) or human leukocyte interferon (LHIF). The anti-FHIF antiserum lacked any anti-LHIF antibodies (anti-FHIF antibody titer: 1:20000 against 10 IU FHIF/ml, determined on diploid fibroblasts). The anti-LHIF antibody titer of the anti-LHIF antiserum (anti-LHIF antibody titer: 1:2000 against 10 IU LHIF/ml, determined on U cells) was more than 100-fold higher than the anti-FHIF titer of this serum. Dilutions of antiviral activity containing CSF were incubated with anti-FHIF (final dilution 1:4000) or anti-LHIF antiserum (final dilution 1:200) at 37° for 1 hr. The dilutions of interferon samples chosen contained approximately 4 arbitrary units of antiviral activity. The antibody concentrations used were capable of neutralizing 10–20 units of the homologous HIF completely. In the dilutions used no antibodies against the heterologous HIF species were detectable. Finally, the HIF antibody mixtures were investigated for antiviral activity with the dye-uptake assay on GM2504 cells as described above.

Results. Since a first titration of a few CSF samples from HSV encephalitis patients on normal, diploid fibroblasts yielded only very low or no antiviral activity titers, a more sensitive assay on human fibroblasts, trisomic for chromosome 21, was employed. We found this assay about 10-fold more sensitive to FHIF as well as to LHIF than our routine assays (FHIF: human diploid foreskin fibroblasts, EMC virus; LHIF: established human amniotic cells (=U cells), EMC virus). Thus with this assay 1 IU per milliliter could be detected in CSF samples (Fig. 1). Although for other human interferon assays we have determined that the sensitivity of an assay can considerably differ for FHIF and LHIF (11), dose–response curves and sensitivities in this assay were comparable for both HIF species.

Because of the small amounts of CSF samples available and due to the low interferon titers of these samples, only three

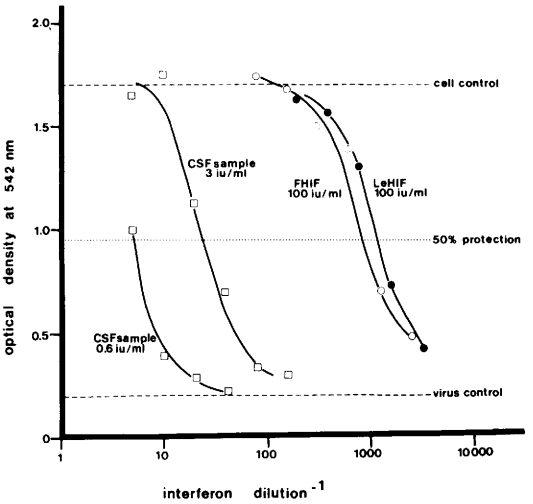

FIG. 1. Determination of antiviral activity in CSF samples. The sensitive dye-uptake assay was carried out as described under Materials and Methods. The reciprocal of that dilution which yields 50% protection gives the antiviral activity in arbitrary units per ml. Internal laboratory standard preparations containing 100 IU/ml of FHIF or LeHIF, respectively, have been included. In the graph in addition to arbitrary units the antiviral activity is also given in international units.

samples could be used for the characterization of the HIF species with a neutralization assay, namely, CSF sample Sch.S./Day 5, and a mixture of CSF samples C.E./Day 6 + St.U./Day 9, and of CSF samples St.U./Day 3 + B.G./Day 7 + I.M./Day 16 (CSF sample codes refer to Table II). As can be seen from Table I, with these samples we were able to show unequivocally that the antiviral activity is neutralized only with anti-LHIF antiserum. Hence the interferon found in the CSF is either identical with or closely resembles LHIF. Despite this fact all interferon titers are given in international units of FHIF, since we routinely use a FHIF standard preparation in this assay and since the interferon titrations were performed before the characterization of the endogenous interferon.

Table II summarizes the results of all CSF samples tested, showing that 9 of 19 samples taken from HSV encephalitis patients contained interferon in the CSF. Two further CSF samples could not unequivocally be proven to be interferon positive. None of 8 CSF samples taken during the first 10 days of illness was interferon negative. On the contrary only 1 CSF sample taken later (Day 16) was definitely positive, namely, that one which had a considerably higher interferon titer on Day 10 than any other interferon-positive sample. Furthermore, the respective serum sample (from the 55-year-old patient I.M./Day 10) was the only one which showed some antiviral activity, whereas all the serum samples were negative. No antiviral activity was found in the CSF of five patients suffering from neurological diseases other than HSV encephalitis (data not shown in Table II). Neutralizing or CF antibody titers against HSV-1 could usually not be detected in the CSF before Day 10 of illness.

Discussion. On investigating CSF samples of HSV encephalitis patients we found that all samples taken during the first 10 days of illness were positive for interferon. Although, with the exception of one sample, all interferon titers were low, they could be reproduced in a highly sensitive assay. From the neutralization with an anti-FHIF and anti-LHIF serum it is evident that the antivirally active molecule is not identical with FHIF but rather with LHIF or at least shares highly cross-reactive antigenic sites with LHIF. One CSF sample contained 10-fold more HIF than the others. Although we cannot explain this exceptional value, it seems to be very reliable because (i) a CSF sample from the same patient taken on Day 16 was the only one at this late stage of illness which

TABLE I. NEUTRALIZATION OF ANTIVIRAL ACTIVITY IN CSF SAMPLES WITH ANTI-FHIF OR ANTI-LHIF ANTISERUM[a]

Sample	EMC virus propagation in cell cultures pretreated with a mixture of the sample plus		
	Medium	anti-FHIF	anti-LHIF
Medium	+	+	+
FHIF standard preparation	–	+	–
LHIF standard preparation	–	–	+
CSF: Sch.S./Day 5	–	–	+
CSF: C.E./Day 6 + St.U./Day 9	–	–	+
CSF: B.G./Day 7 + St.U./Day 3 + I.M./Day 16	–	–	+

[a] Antiviral activity containing CSF samples were preincubated with appropriate dilutions of anti-FHIF and anti-LHIF antisera or medium only as described under Materials and Methods, and investigated for antiviral activity with the interferon assay. In control cultures or in those treated with neutralized interferon cells were completely destroyed (+) by the cytopathogenic EMC virus, while in the presence of antiviral activity they were completely protected (–).

was unequivocally positive; and (ii) the serum from this patient, taken on Day 10, was the only one with antiviral activity. The fact that with the exception of this serum sample no interferon was found in the sera of the other CSF-positive patients indicates that also in these patients the brain–blood barrier for interferon at least partially exists. This is in accord with the data published by De Clercq *et al.* (13). From studies on monkeys we know that the spreading of HIF in the healthy individual is limited by this barrier (5, 6). This could probably also be the reason that one can detect interferon synthesized in the brain at a higher level in the CSF than in the serum. If it were destributed over the entire body, interferon concentrations in the CSF would also decrease to undetectable levels.

Our results are supported by those recently reported by Lebon *et al.* (abstract of a poster presentation at the International Conference on human herpes viruses, Atlanta, March 17–21, 1980), who also found antiviral activity in the CSF of HSV encephalitis patients. However, we do not agree with these authors in that interferon titers determined in the CSF could be used for an early diagnosis of HSV encephalitis. Although our results indicate that interferon can be detected earlier in the CSF than CF or neutralizing anti-HSV antibody titers, we doubt that interferon can be used as a specific parameter for HSV encephalitis because in the CSF it has not only been found in relation to viral infections of the brain other than HSV (4) but also in patients suffering from cerebral diseases of unclarified aetiology (14, 15). It rather might be possible to earlier diagnose HSV encephalitis specifically by anti-HSV antibody determination in the CSF with an highly sensitive enzyme immunoassay.

It has been shown that interferon plays a role in controlling HSV infection in mice (2) and also in controlling HSV recurrency in man (16). Considering the fatal outcome of HSV encephalitis in the 10 patients studied one can not conclude that the low interferon levels found in the CSF play an important role in controlling this disease but it is tempting to speculate whether 100- to

TABLE II. ANTIVIRAL ACTIVITY AND ANTI-HSV ANTIBODY TITERS IN THE CSF OF HSV ENCEPHALITIS PATIENTS

Patient	Days after onset of illness	Antiviral activity[a] (IU/ml)	Anti-HSV type 1 antibody titers	
			Neutralizing	Complement fixing
B.P.	2	2.0	n.d.[b]	–
St.U.	3	2.2	—	–
Sch.S.	5	8.2	—	–
C.E.	6	2.6	—	+ (Undiluted)
St.R.	7	2.2	—	–
B.G.	7	1.9	—	–
St.U.	9	2.2	—	+ (Undiluted)
I.M.	10	29.0	1:2.2	+ (Undiluted)
Sch.S.	13	<1.0	1:75	1:128
B.S.	14	<1.0	—	–
C.E.	16	≤1.0	—	1:4
I.M.	16	2.2	1:5	+ (Undiluted)
B.S.	19	<1.0	1:10	1:8
H.H.	20	≤1.0	1:9.4	1:4
D.E.	23	<1.0	1:32	1:64
H.H.	24	<1.0	1:13	n.d.
Cl.E.	20–30	<1.0	n.d.	1:16
Cl.E.	30–40	<1.0	1:158	1:32
B.G.	48	<1.0	1:4	1:16

[a] Every sample was assayed under code; those yielding negative results were tested at least twice, those showing antiviral activity were tested three times. Geometric means of antiviral activity titers are given in terms of the international FHIF standard.

[b] Not done.

1000-fold higher levels of interferon in the CSF due to intrathecal administration of exogenous HIF, could cure the patient. Up to now only one case has been published on intrathecal interferon treatment of a newborn infant suffering from HSV encephalitis (13). Here interferon failed to protect the baby from death. However, from this single case no final conclusion can be drawn.

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