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ORIGINAL COMMUNICATION

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Incidence and pathogenesis of clinical relapse after herpes simplex encephalitis in adults

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Abstract *Objectives* To study the occurrence of relapse of herpes simplex encephalitis (HSE) and to find out whether soluble activity markers in cerebrospinal fluid (CSF) indicate direct viral or immune-mediated events. *Methods* A consecutive series of 32 adult survivors of HSE were followed to determine the incidence of clinical relapse of HSE. Four patients had neurological deterioration interpreted as relapsing HSE. Four non-relapsing HSE cases were selected as matched controls. Fiftynine batched, paired CSF and serum samples from the eight HSE patients were analysed for soluble activity markers, predominantly cytokines and mediators (interferon-γ, soluble CD8, tumour necrosis factor-α, and interleukin-10), amount of HSV-DNA and markers of glial and neuronal destruction (neurofilament protein, glial fibrillary acidic protein, S-100-β, and neuron specific enolase). *Results* Relapse of HSE was diagnosed in 3 of 26 (12 %) acyclovir-treated patients (5 episodes during 6.1 years of follow-up) and in 1 of 6 vidarabine-recipients. All relapses occurred from 1 to 4 months after acute HSE, except for a second relapse after 3.3 years in one patient. Computer tomography at relapses revealed few abnormalities apart from those found during the primary disease. Intravenous acyclovir and corticosteroids were given for 7–21 days in all the relapse patients. All relapse patients seemed to recover to the pre-relapse condition. HSV-DNA was demonstrated in CSF in all patients during the acute stage but not in any of 13 CSF samples taken during relapse phases. The HSV viral load during the acute stage of HSE was not higher or of longer duration in the relapsing patients than in the non-relapsing HSE controls. The levels of sCD8 were increased in nearly all CSF samples tested with peaks of sCD8 at one month of acute HSE. In all episodes of relapse, sCD8 peaks were detected during the first week at high levels. CSF levels of neuron-specific enolase, S-100 and glial fibrillary acidic protein were markedly lower at relapse than at the acute stage of HSV-1 encephalitis. *Conclusion* The lack of demonstrable HSV DNA in CSF, the lack of acute CSF signs and the lack of signs of neural and glia cells destruction indicate that a direct viral cytotoxicity is not the major pathogenic mechanism in relapse. Instead, the pronounced CSF proinflammatory immunological response and the relative lack of CSF anti-inflammatory cytokine IL-10 response suggest immunologically-mediated pathogenicity.

Key words relapse of herpes simplex encephalitis · cerebrospinal fluid · HSV-DNA · soluble CD8 and interleukin-10 · neurofilament protein

Introduction

Herpes simplex encephalitis (HSE) is a disease with high mortality as well as significant morbidity in the survivors. In two studies, one Swedish and one American, on acyclovir versus vidarabine in HSE, respectively, 44 % of the 27 and 62 % of the 32 acyclovir-treated patients died or had severe sequelae 12 months after HSE and 50–54 % of the vidarabine-treated patients died [1, 2].

Relapse after HSE has been reported in anecdotal cases in adults [1–6] and in children [7–10]. Persistence or recurrence (reactivation) of infection with herpes simplex virus (HSV) [4–6] and/or immunologically mediated demyelinisation of the brain tissue [3] have been suggested as causes of clinical relapses after HSE. Different mechanisms might be the case in different individuals, in children versus adults, and include viral replication or an immuno-inflammatory process. Analysing surrogate markers of inflammation and cell destruction in the CSF is a way of exploring this issue. Soluble inflammatory mediators can be demonstrated in the CSF in viral encephalitis [11–14]. In the first month of acute HSE, we have demonstrated different kinetics in CSF of interferon-γ (IFN-γ), and of neopterin and soluble CD8 (sCD8). The last of these was persistent for years after the HSE [11, 12].

The cells of the brain contain several proteins that are more or less unique for the central nervous system (CNS). These proteins leak into the CSF reflecting the status of different cell types in the CNS and have been measured in both chronic neurodegenerative and more acute CNS diseases such as brain infarction and herpes simplex encephalitis [15]. Markers for neural and glia cells destruction have been used to determine the extent of CNS damage [15–20]. In HSE astroglial destruction markers such as S-100 protein and glial fibrillary acidic protein (GFAP) as well as markers for neural destruction, neuron specific enolase (NSE) and neurofilament protein (NFL) increased in the CSF to extremely high levels in the acute stage [20]. The concentrations of these cell damage markers decreased within 45 days except for NFL which had a peak concentration after 1 month.

The aim of the present study was to study the incidence of relapse of HSE in a prospective cohort of HSE patients, to describe the presentation of relapse, and to explore, also in matched, non-relapsing HSE controls, whether the CSF dynamics of various soluble markers could yield information about possible events leading to relapse of HSE. The activity markers were quantitative HSV DNA, cytokines and mediators (IFN-γ, sCD8, and Interleukin-10, IL-10), and glial and neuronal destruction markers (NFL, GFAP, S-100-β, and NSE).

Patients and methods

Patients

A total of 32 consecutive patients were treated for acute HSE and survived the acute phase at the Department of Infectious Diseases, Danderyd Hospital, Stockholm, during a 22-year period, 1977–95. Of these, 26 had been treated with acyclovir for 10–21 days and 6 with vidarabine. The 32 consecutive patients were followed for 6.1 years (range 24–228 months) after HSE. Their median age was 51.7 years (range 17–89) and 21 (57 %) were females. The long-term follow-ups of the patients were mainly done by two of the authors. Four patients did have clinical neurological deterioration interpreted as relapsing HSE. Four non-relapsing HSE cases were selected by age, year of onset and stage of disease as matched controls. Soluble activity markers, predominantly cytokines and mediators (IFN-γ, sCD8, tumour necrosis factor-α, TNF-α, and IL-10), quantitative HSV-DNA and glial and neuronal destruction markers (NFL, GFAP, S-100beta, and NSE) were analysed in 59 batched, frozen (–70 °C), paired CSF and serum samples from the 8 HSE patients (Table 1).

The virological diagnosis of HSE was made by intrathecal HSV serology and, since 1990, also by PCR for the detection of HSV DNA in CSF [1, 21, 22]. HSV-DNA was demonstrated retrospectively in CSF of all the HSE patients.

Clinical definition of relapse

A relapse was considered to have occurred if there were acute, suddenly appearing, new or aggravated symptoms and signs of focal encephalopathy with or without fever and without neurological symptoms secondary to severe pneumonia, septicaemia, metabolic disorder, or other disease. Transient seizures, not giving rise to sequels and occurring without concomitant symptoms, were not considered to constitute a relapse.

HSV assays

Quantitative HSV-DNA assays based on the nested polymerase chain reaction (PCR) were performed in duplicate as previously described [23]. Intrathecal IgG antibodies to HSV were determined by IgG capture ELISA using type-common antigen as described earlier [22].

Methods for cytokines and other immune activation markers

IFN-γ and IL-10 were determined by sandwich enzyme immunoassays (EASIA) developed by Medgenix Diagnostics SA (Fleur, Bel-

Table 1 Fifty nine paired CSF and serum samples drawn from 4 HSE patients with relapse (total, at relapse from acute onset, and at relapse phase) and from 4 HSE patients without relapse

	Day 1–7	Day 9–19	Day 20–39	Day 46–57	Mo. 3–6	Mo. 9–15	Mo. 26–79
HSE with relapse	6	5	4	2	9	4	6
Relapse after onset			2	2	8		3
Relapse Phase	6	3	4				
HSE without relapse	6	4	3	3	3	3	1

gium) and were performed according to the manufacturer's instruction. Soluble CD8 was determined using an enzyme immunoassay from T Cell Diagnostics (Cambridge, MA). CSF samples from 22 neurologically healthy individuals aged 51–69 years (median 63) undergoing spinal anaesthesia for transurethral examinations were used as controls.

Methods for glial and neural destruction markers

GFAP levels were measured with an ELISA as earlier described [20]. The sensitivity of the assay was 16 ng/l. Reference values were: 2–20 years: < 175 ng/L, 21–60 years: < 750 ng/L, 61–75 years: < 1250 ng/L. The concentration of the light subunit NFL was analysed by an ELISA [15]. The sensitivity of the assay was 125 ng/l. Reference values were: < 60 years: < 250 ng/L, 60–70 years: < 380 ng/L. The S-100 protein and NSE concentrations were measured by means of commercially available luminescense immunoassay (LIA) methods (AB Sangtec Medical, Bromma Sweden). The sensitivity of the S-100 protein assay was 0.02 µg/l and the sensitivity of the NSE assay was 1.0 µg/l. Reference values were: S-100: ≤ 5.0 µg/L, NSE: < 10.5 µg.

Results

Relapse of HSE was diagnosed in 3 of 26 (12 %) acyclovir-treated patients and in 1 of 6 vidarabine recipients. Three acyclovir recipients had five episodes of relapse. The risk of having a relapse was greatest during the first four months of HSE when 3 (12 %) patients had four relapses and only one further relapse occurred later, at 3.3 years after the onset of HSE. Six patients suffered from occasional epileptic seizures, which were not related to a diagnosed relapse. The relapsing patients had been treated for 10–21 days with intravenous acyclovir, 10–15 mg/kg t. i. d. initiated at 1–3 days after signs of acute HSE, except for one vidarabine recipient. Corticosteroids (dexamethazone for 6–10 days) were given in 3 of the 4 patients. During the six relapse episodes, the four patients received intravenous acyclovir, 10 mg/kg three times daily during 10–14 days, in 5 of 6 cases in combination with corticosteroids during 7–23 days. After the relapse episodes the patients recovered to the pre-relapse level within 2–8 weeks.

The clinical presentations in the four HSE patients with relapse indicate different pathobiology in cases no. 1 and no. 4. On CT, progressive attenuation with mass effect was present in case no. 1 and no attenuation but, on MRI, multifocal necrotic lesions were observed in case 4.

Data on the four relapsing HSE patients are summarized in Tables 2 and 3. Data from another case report from our Department of Infectious Diseases, Karolinska University Hospital, are included in the Tables (Hjalmarsson et al. in manuscript [24]). It concerns a 26-year-old man who fell ill in 2001 with a predominantly right-sided temporal HSE but relapsed subacutely within seven months with left-sided temporal HSE with severe sequels.

Table 2 Symptoms, findings and treatment at acute HSE

Case no	Sex, Age (years)	Symptoms	Initial EEG	Initial CT scan	CSF leucocytes at start txm	CSF protein at 8–13 days	Treatment after Neurological Symptoms		Hospitalisation
							IV Acyclovir	Corticosteroids	
1	F 22	headache, fever, left hemiparesis and facial paresis, seizures	right, focal abnormality	no abnormality	35/0.7	54/1.5	Vira-A, day 5, during 10 days	Dex 16 mg 5 days tapered 5 days	15 days
2	M 67	fever, decreased consciousness, personality changes, seizures	left, sharp wave temp. abnormality	no abnormality	350/0.8	41/1.1	day 1, during 11 days	0	4 weeks
3	M 67	fever, headache, confusion, somnolence, seizures	right, focal abnormality	right temporal attenuation	132/2.6	56/1.6	day 1, during 12 days	Dex 16 mg 6 days	3 weeks
4	M 71	fever, headache, seizures, decreased consciousness	right, sharp wave abnormality	no abnormality	44/0.26	28/0.39	day 3, during 20 days	Dex 16 mg 7 days	4 weeks
Case Report	M 26	headache, vomiting, fever somnolence	right, focal abnormality	right frontal, major temporal lobe	104 1165 (alb)	124 486 (alb)	day 7, during 21 days	Dex 14 days	3 weeks

Table 3 Symptoms, findings and treatment at relapse of HSE

Case no	Relapse after acute txm	Symptoms	EEG	CT-scan	MRI	CSF Leucocytes	CSF protein	Treatment at relapse		Hospitalisation	Recovery of Relapse	Sequelae
								IV Acyclovir	Corticosteroids			
						at start txm	at 3–4 weeks					
1	13 days	acute headache, vomitings, left-sided hemisymptoms	increased focal abnormality	mass effect with dislocation of midline			20/0.8	day 7, during 10 days	Dex 16 mg 6 days, tapered 17 days	21 days	yes	Mild/No, epileptic seizures after 4–9 yrs
2	4 months	sudden dysphasia, confusion, seizures	triphasic, sharpwave focality	unchanged left temp. attenuation	T2 gliosis, oedema?	8/0.36	4/0.4	day 5 during 14 days	Dex 2 days and Pred 50 mg tapered during 16 days	31 days	yes	
	41 months	sudden dysphasia, fever, right dysaesthesia	triphasic, sharpwave focality	unchanged left temp. attenuation		0.26	0.33	day 1 during 10 days	Dex 16 mg 3 days, tapered 5 days	13 days	yes	Moderate epileptic seizures
3	3 months	acute nausea, vomiting, somnolence, disorientation, decreased memory, slight hemi-symptoms	unchanged	unchanged		8/1	10/1	day 1 during 9 days	Pred 60 mg tapered during 8 days	10 days	yes	Moderate
4	13 days	faintings, changed personality, hallucinations	ND	no abnormalities		28/	12/0.3	day 2 during 14 days	no	16 days	yes	
	8 weeks	acute confusion, disorientation anxiety	bilat dysrhythmia	discrete attenuation of right parietal lobe	multifocal, cortical haemorrh. lesions right temp, front, pariet. thalamus, occip.	5/0.28	1/0.16	14 days	Dex 16 mg 3 days, tapered 5 days	40 days	yes	Severe
Case Report	7 months	during 1 month fatigue, memory impairment	unchanged		MRI progress right temporal and frontal lobes, new lesions in left temporal and frontal lobes.	8 262 (alb)	ND	day 45, 21 days	no	22 days	no	Severe memory impairment

CSF Findings in acute and relapse phases of HSE

CSF leucocytes and protein

In contrast to the more or less pronounced pleocytosis (mostly mononuclear) and elevated CSF protein concentrations during the acute stage of HSE, only subtle changes in these parameters were detected during the relapses (Tables 2 and 3). In case no. 1, however, CSF protein had, six days after onset of relapse symptoms, increased to 1.9 g/l, from values of 0.7–0.8 g/l during treatment.

HSV-DNA in CSF and intrathecal HSV serology

HSV type 1 was identified in all except relapse case no. 1, who had a HSV type 2 infection. HSV DNA was demonstrable during 1–13 days after onset of neurological symptoms of HSE. Thirteen CSF samples from the four relapsing patients were taken during the first 32 days after relapse; all of them HSV DNA negative. The HSV viral load during the acute stage of HSE was not higher or of longer duration in the relapsing patients than in the non-relapsing HSE controls (Fig. 1). Intrathecal HSV IgG antibodies were not found during 1–7 days after onset of neurological symptoms of HSE but in all the other samples taken later except the sample collected 6.5 years after the acute infection in patient no. 1 (data not shown).

Interferon-γ, sCD8 and interleukin-10 in CSF

IFN-γ levels in CSF were elevated to 1.4–6.8 μ/L on days 1–10 (reference values < 1.0 μ/L) of the acute phase of four HSE patients, on days 6 and 12 of the relapse phase in two patients (1.3 μ/L), and in two of four HSE patients without relapse (6.4–38 μ/L). Soluble CD8 levels were increased in nearly all CSF samples tested with peaks of sCD8 at one month of acute HSE. In all episodes of relapse, sCD8 peaks were detected during the first week at high levels (89–1030 μ/ml).

IL-10 reached high levels in CSF (4–63 μ/ml) during the first week of acute HSE in all patients. By contrast, IL-10 was undetectable during 4 relapses in 3 patients. Thus, the sCD8/IL-10 ratios were higher during the relapses than in the acute stage of disease (Fig. 5).

Glial and neuron destruction markers in CSF

The GFAP levels were increased above reference values mainly during the first month after the acute HSE (Fig. 2). During the six relapse phases, GFAP was not elevated, except slightly in one patient, NSE presented with a similar pattern as GFAP, as can be seen by Fig. 3. It was interesting to note that in the additional case report, summarized in Tables 2 and 3, NSE and GFAP levels were increased at four months after acute onset of HSE, being about two months before slowly progressive deterioation in a contralateral temporal encephalitis.

By contrast to GFAP and NSE, NFL was significantly elevated in all except one patient up to 6 months and in 2 of the 4 patients at 9–15 months (Fig. 4). The relapse cases had high NFL levels during their relapse phase. One patient with a second relapse at 41 months peaked to a high level (3940 ng/L) from 640 ng/L at 14 months. The case report had high NFp levels as can be seen in Fig. 4.

Discussion

The frequency of relapses after HSE has been reported to range between 5 % and 26 %, where the higher num-

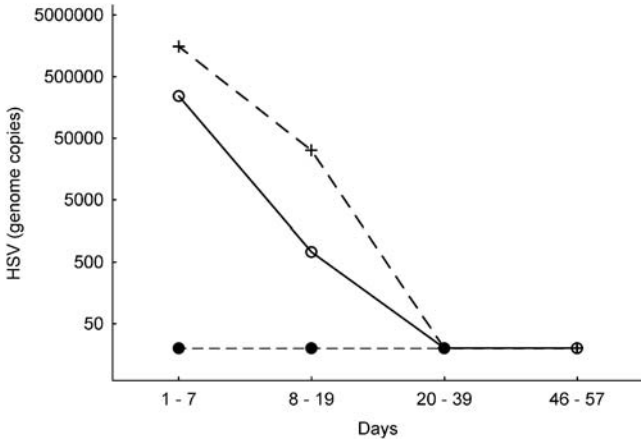

Fig. 1 HSV DNA viral load (mean levels) in CSF of four HSE patients with relapse (—○— from acute onset; – – ● – – during relapse phase), in four HSE patients without relapse (– – + – –)

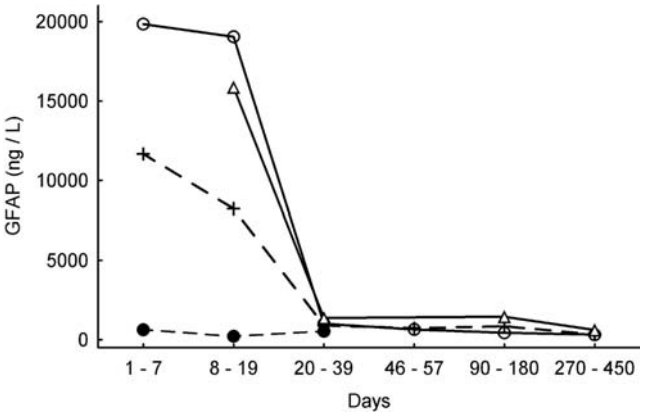

Fig. 2 Mean levels of GFAP in CSF of four HSE patients with relapse (—○— from acute onset; – – ● – – during relapse phase), in four HSE patients without relapse (– – + – –), and in one additional HSE case with subacute relapse △—▽. Reference values were: 21–60 years < 750 ng/L, 61–75 years < 1250 ng/L

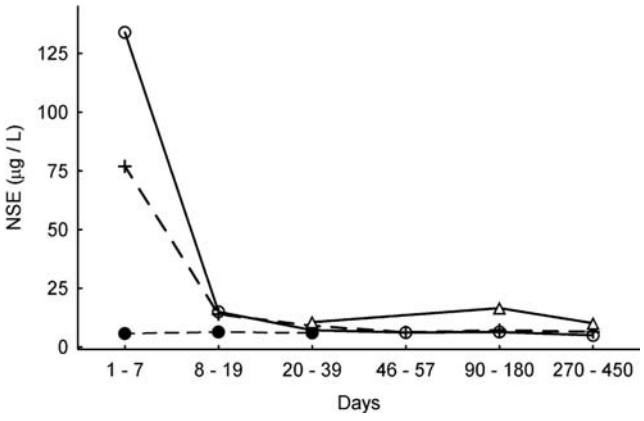

Fig. 3 Mean NSE levels in CSF of four HSE patients with relapse (—○— from acute onset; – –●– – during relapse phase), in four HSE patients without relapse (– – + – –), and in one additional HSE case with subacute relapse △—▽. Reference values were ≤ 10.5 µg/L

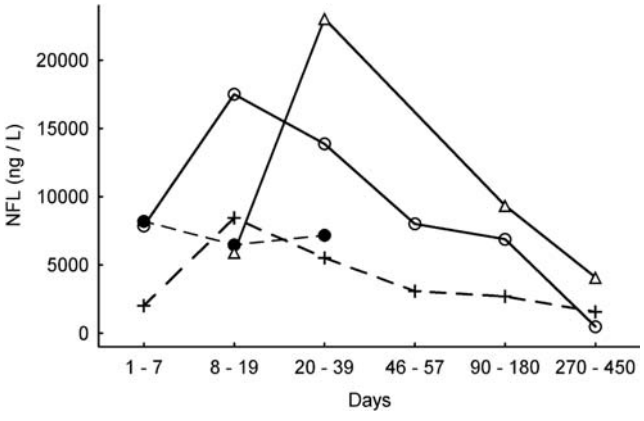

Fig. 4 Mean levels of NFL in CSF of four HSE patients with relapse (—○— from acute onset; – –●– – during relapse phase), in four HSE patients without relapse (– – + – –), and in one additional HSE case with subacute relapse △—▽. Reference values were: < 60 years < 250 ng/L, 60–70 years: < 380 ng/L

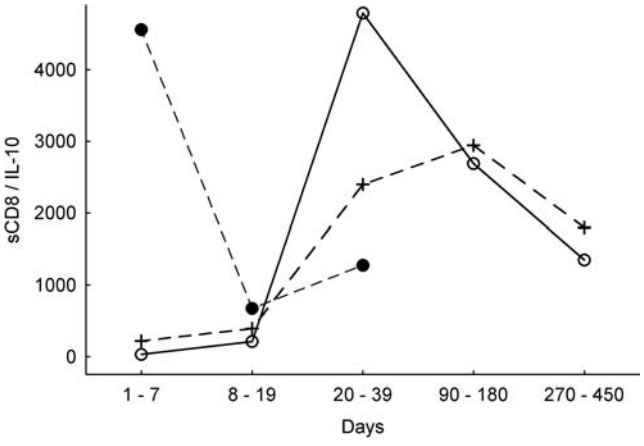

Fig. 5 Mean levels of sCD8/IL-10 ratios, representing pro-inflammatory/anti-inflammatory activity in CSF of HSE patients with relapse (—○— from acute onset; – –●– – during relapse phase), in HSE patients without relapse (– – + – –)

bers have been reported in children [9, 10, 25]. Thus, our finding that 4 out of 32 adult patients (13 %) had one or more relapses constitutes a percentage within the expected range. One relapse occurred in a patient treated with vidarabine, an antiviral substance not used today because of less efficacy compared with acyclovir.

As most relapses occur within a relatively short time after the withdrawal of antiviral therapy, it might be expected that the relative efficacy of the antiviral therapy is of importance for the frequency of relapses. In a series of 27 children with acute HSE, 7 (26 %) had a relapse of HSE within 18 days after withdrawal of intravenous acyclovir [25]. In 2 of those 7, HSV DNA reappeared temporarily in the CSF. The total dose during initial acyclovir therapy was significantly lower in the relapse group than in the control group. However, in our series of adults with relapse of HSE, HSV-DNA was not demonstrable in any of 13 CSF samples collected during the first month of relapse.

The clinical courses of the relapses in the present study were generally milder than those observed during the acute HSE. Several cases of severe, lethal relapses of HSE after vidarabine or acyclovir therapy have been described, however, and often – as in our study – within two weeks to four months after the initial antiviral treatment [3–10]. Severe syndromes with choreoathetosis have been reported, particularly in children with relapsing HSE [25–27]. Acute perivenous demyelinating disease, post-infectious encephalopathy without demyelinisation or perivascular inflammation, oedema, and gliosis have been observed in brain biopsy specimens from relapse episodes [3, 4, 6]. By contrast, virus was not demonstrable in the brain biopsy specimens, but in situ hybridisation techniques were not always used. In brain from a fatal HSE case with relapse, HSV-1 antigen was recently detected by immunohistochemistry, and HSV-1 DNA by in situ hybridization [28].

The present study was conducted during a long period of time and still we could only include three HSE patients with non-severe, clinical relapse after intravenous acyclovir for 10–21 days. During the study period radiological diagnostic methods have improved and magnetic resonance imaging (MRI) has been introduced. MRI is a better tool for early diagnosis of HSE and is more sensitive than CT for studying changes during suspected relapse. In the present study CT at relapse showed no major changes compared with the acute phase. In future studies of relapse MRI would preferably replace CT.

The pathogenic mechanisms are important to understand in order to give the optimal treatment. As yet there have been no studies on relapse treatment. Our patients were treated mostly with a combination of intravenous acyclovir for 10–14 days and corticosteroids. Corticosteroids may be important for their anti-inflammatory effect. To obtain further information on the pathogene-

sis of HSE we studied the pattern of various cytokines and markers of neuron and glia cell destruction in the CSF sampled from patients with acute HSE and followed or not by relapsing HSE.

The cytokines were chosen to be representative of pro-inflammatory (IFN-γ and TNF-α) as well as anti-inflammatory (IL-10) substances [11, 29–34]. An additional marker used was soluble CD8 which is considered to reflect the activity of CD8 cytotoxic cells [35]. IFN-γ, which is often described as the prototype Th1 cell cytokine, is produced by Th1 cells but also by NK cells and CD8 positive cells [35]. IFN-γ activates macrophages, induces MHC class II antigen and, similar to TNF-α, augments the expression of ICAM-1 on cultured endothelial cells of cerebral origin [29]. TNF-α has been found to be toxic to myelin. Thus, IFN-γ as well as TNF-α are proinflammatory cytokines that may have pathogenic effects in, for example, CNS infection. In our earlier studies on acute HSE, we found early and rapid increases of IFN-γ in CSF but no, or moderate, rises of TNF-α [11].

IFN-γ is inhibited by the Th2-type cytokine IL-10, which was originally described as "cytokine synthesis inhibitory factor" [29]. Although IL-10, like all cytokines, has multiple biological activities, its main effect remains its capacity to inhibit the synthesis of IFN-γ [38]. The powerful inhibitory effect of IL-10 on IFN-γ production is illustrated by the observation that IL-10 administration to mice can inhibit shock induced by Staphylococcal enterotoxin B sepsis in these animals [37]. The antagonistic effect of IL-10 on IFN-γ is reciprocal [38]. It seems therefore likely that the balance between IL-10 and IFN-γ is of importance for the course of the immunological response in HSE.

Relapses were associated with markedly increased levels of soluble CD8 in CSF and, in half the relapses, with increases in levels of the pro-inflammatory cytokine IFN-γ. By contrast, levels of the anti-inflammatory cytokine IL-10 were usually low and did not show any significant increase during the relapses. Compared with acute HSE, relapses were associated with significantly lower CSF levels of IL-10, whereas those of sCD8 were significantly higher in relapses than in primary HSE. Although the levels of IFN-γ were lower in relapses than in acute HSE, the IFN-γ/IL-10 ratios still showed a tendency to be higher in relapses than in the acute stage of HSE. These results suggest that the pro-inflammatory cytokines, although present in lower concentrations during relapse than during acute HSE, are not counteracted to the same extent during the former as during the latter phase of the HSV infection. In the absence of widespread focal damage caused by replication of HSV, it seems possible that immunological cytotoxicity plays a major role in the pathogenic events occurring during relapse. This interpretation is supported by the results of measurement of sCD8, which may be used as a marker for T-cell mediated cytotoxicity [35].

The markers of neuron and glia cell destruction were markedly elevated in the acute stage of encephalitis in both patients with later relapse as in those without. The levels reached the concentrations previously observed and reflect acute cell damage [20]. Thereafter, the CNS cell markers decreased over time in a similar pattern in both groups. If a viral rebound had occurred in the relapse group one would have expected increased CSF concentrations in a second peak or at least a slower decrease of the neural and glia cells markers. These data indicate that different pathogenic mechanisms are responsible for the relapse compared with the acute haemorrhagic cytopathogenicity at the acute stage. Even though there was no demonstrable HSV-DNA in the CSF of relapsing patients, there may well be local viral replication in the brain tissue in the absence of any leakage of virus into the CSF. In fact, it may be presumed that an outburst of viral replication, triggered by unidentified stimuli, may be responsible for the immunological events associated with the development of relapses.

In addition, we measured soluble Fas (sFas), a proinflammatory molecule that is involved in regulation of apoptosis induced by Fas ligand, in serum and CSF in three HSE patients with relapses. Elevated levels of sFas in plasma or CSF have been found in patients with HIV-1, lymphoproliferative disorders, multiple sclerosis and autoimmune disease like systemic lupus eryhematosus [39–41]. Our observation on increase of sFas levels in CSF of the HSE patients but only in 3/34 non-HSE encephalitis cases and not at all in CSF from 16 healthy controls gave an additional indication of the importance of immune activation in pathogenesis of HSE during the course of relapses.

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