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Diagnosis of Herpes Simplex Virus Encephalitis by Detection of Virus-Specific Immunoglobulins A and G in Serum and Cerebrospinal Fluid by Using an Antibody-Capture Enzyme-Linked Immunosorbent Assay

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An enzyme-linked immunosorbent assay was evaluated for detection of intrathecal synthesis of immunoglobulin G (IgG) and IgA antibodies to herpes simplex virus (HSV) in patients with HSV encephalitis (HSVE). Since the antibody-capture principle was used and the assay was carried out at the saturation level of the anti-IgG- or anti-IgA-coated solid phase, correction for blood-brain barrier leakage was not needed. A total of 34 pairs of serum and cerebrospinal fluid specimens obtained from 20 patients with HSVE were examined. Intrathecal synthesis of HSV IgG and IgA was detected from day 7 after the onset of illness in patients with HSVE. Specimens from all 19 patients from whom paired serum and cerebrospinal fluid specimens were obtained at more than 10 days after the onset of illness were positive. Intrathecal synthesis of HSV IgG and IgA was not detected in patients with HSVE before day 7 of illness or in any of the 16 control patients with other causes of (meningo)encephalitis. Use of the antibody-capture enzyme-linked immunosorbent assay for HSV IgG and IgA allows the rapid diagnosis of HSVE during the second week of illness.

In the developed world, herpes simplex virus (HSV) is probably the most frequent cause of sporadic acute viral encephalitis. Before treatment with antiviral agents became possible, prognosis was poor and mortality was about 70% (20), and even after the advent of effective antiviral drugs, morbidity and mortality are still considerable (13, 14, 19, 20). The success of treatment highly depends on early diagnosis and treatment (14, 19). Since clinical diagnosis is not sufficiently sensitive and specific, laboratory examination of specimens from patients must provide confirmation of suspected HSV encephalitis (HSVE). Detection of the virus in brain biopsy material is still considered to be the most definitive proof of HSV infection, but most physicians are reluctant or even refuse to allow brain biopsies to be performed for diagnosis of the infection. Therefore, a need exists for sensitive, noninvasive diagnostic methods that allow early diagnosis of the disease.

Several studies (7, 9, 13–15, 18) have shown that an increased level of HSV-specific antibodies in cerebrospinal fluid (CSF) relative to that in serum is indicative of intrathecal HSV antibody synthesis, and thus of HSV infection of the central nervous system (CNS). When this method is used, however, correction must be made for possible leakage of the blood-brain barrier (9, 13–15). In a previous study on HSV antibody production (8, 18), we observed that the proportion of HSV-specific immunglobulin A (IgA) was relatively increased in the CSF of a small number of patients with HSVE compared with that in serum and that this could be used as a diagnostic criterion. Since an antibody-capture enzyme-linked immunosorbent assay (ELISA) was used, correction for possible leakage of the blood-brain barrier was no longer needed.

In this study we extended these observations by examining a larger number of patients suspected of having HSVE for intrathecal production of both IgA and IgG antibodies to HSV to obtain a more rapid and specific diagnosis of HSVE.

MATERIALS AND METHODS

Serum and CSF. Thirty-four pairs of serum and CSF specimens were obtained from 20 patients with HSVE. Diagnosis was obtained by brain biopsy (n = 3), by a significant, fourfold HSV antibody titer rise in CSF (n = 19) in the absence of an adenovirus antibody titer rise in the same CSF samples, or by both methods. As a control group, we examined paired serum and CSF specimens from 16 patients with other causes of (meningo)encephalitis, including four patients with varicella-zoster virus meningoencephalitis, two with mumps meningitis, one with Creutzfeldt-Jakob disease, and one with the Guillain-Barré syndrome. In the other patients, HSVE was excluded because of the absence of elevated or rising HSV antibody titers in successive CSF and serum samples. Specimens used in this study were not taken before day 12 after the onset of illness.

Antibody-capture ELISA. The previously described antibody-capture ELISA (16–18) was used for the detection of HSV-specific IgA and IgG antibodies in serum and CSF specimens. The concentrations of anti-human IgA and IgG used in this study were determined by checkerboard titration. Briefly, wells of polyethylene terephthalateglycol microdilution plates (no. 6595; Costar Europe, Badhoevedorp, The Netherlands) were coated overnight at 4°C with 0.120 ml of heavy-chain-specific anti-human IgA or IgG (Cappel Laboratories, Cochranville, Pa.), each of which was used at a dilution of 1:1,000 in 0.01 M Tris buffer (pH 9.0). After the wells were washed four times with 0.01 M phosphate-

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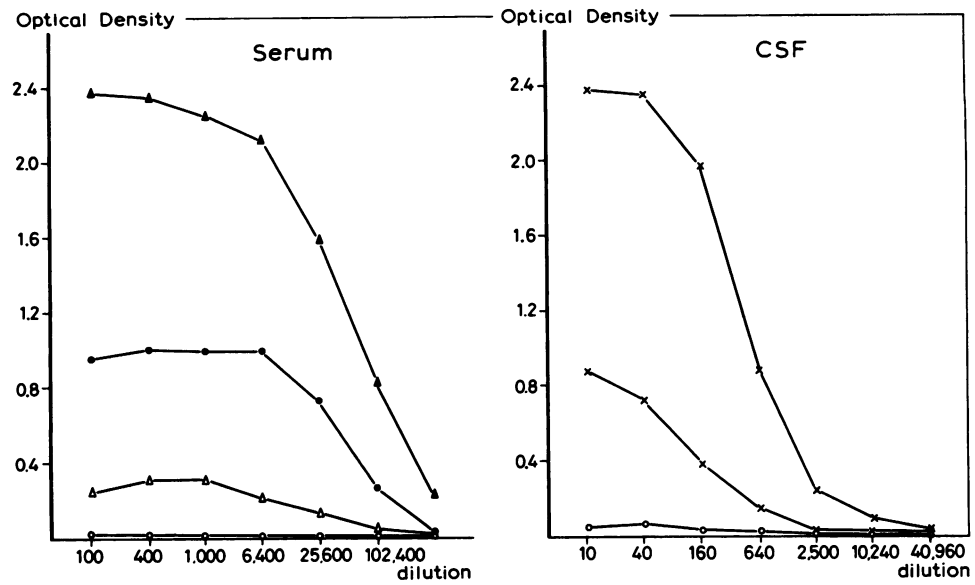

FIG. 1. Dose-response relationship of one negative (○) and three positive (△, ▲, ●) serum specimens and of one negative (○) and two positive (×) CSF specimens in the antibody-capture ELISA for IgG antibody to HSV.

buffered saline (pH 7.2) containing 0.05% Tween 20, 0.100 ml of the serum or CSF specimens diluted 1:50 and 1:5, respectively, in phosphate-buffered saline–Tween 20 containing 2% fetal bovine serum and 0.005% Merthiolate (PFT-M) was added to each of two wells and incubated for 2 h at 37°C. Thereafter, the plates were washed again four times and 0.100 ml of horseradish peroxidase-labeled HSV antigen diluted in PFT-M containing 0.100 mg of control antigen per ml was added to each well and incubated overnight at 4°C. After the wells were washed again four times, 0.100 ml of the substrate solution was added to each well. Substrate solution was prepared immediately before use by dissolving 4 mg of *o*-phenylenediamine per ml in 0.05 M citrate buffer at pH 5.2, followed by the addition of 0.15% of a 30% solution of H_2O_2. After 10 min of incubation at room temperature, the reaction was stopped by adding 0.150 ml of 3 N H_2SO_4. The absorbance was determined by photometry (Titertek Multiskan; Flow Laboratories, Irvine, United Kingdom), using the buffer control as a blank. A weakly positive control serum sample was used as a reference to determine the positive or negative cutoff value in each plate. The HSV antigen was prepared by sucrose gradient centrifugation from Vero cells infected with HSV type 1, strain McIntyre, as described before (17), and consisted mainly of HSV virions. The method of Wilson and Nakane (21) was slightly modified as described previously (16) and was used to conjugate the HSV antigen and horseradish peroxidase (Sigma Chemical Co., St. Louis, Mo.).

Indirect ELISA. IgG antibodies to HSV in serum and CSF specimens were also quantitated by use of an indirect ELISA as described previously (17). Briefly, a 0.120-ml amount of HSV antigen diluted in 0.01 M carbonate buffer (pH 9.5) was added to each well of a microdilution plate. Sera were examined in four fourfold dilutions in PFT-M at a starting dilution of 1:200; CSF was examined at a starting dilution of 1:20. Peroxidase-labeled anti-human IgG (γ-chain specific; Dakopatts, Glostrup, Denmark) was used at a dilution of 1:8,000 in PFT-M. To check for possible leakage of the blood-brain barrier, a similar ELISA for IgG antibodies to adenovirus was carried out.

Other virological methods. For virus isolation, 10% (wt/vol) homogenates of brain biopsy or autopsy specimens were inoculated onto duplicate cultures of primary monkey kidney cells and human diploid fibroblasts (Flow 2002; Flow Laboratories). Levels of viral antibodies were further determined by complement fixation by the microtechnique of Casey (4).

RESULTS

Dose-response relationship of the HSV IgG antibody-capture ELISA. A dose-response relationship was determined by examining fourfold dilutions of one negative and three positive serum specimens and of one negative and two positive CSF specimens (Fig. 1). The dose-response curve showed a plateau phase at low dilutions of serum and CSF. This was due to the saturation of the IgG-binding sites on the solid phase. A similar pattern was previously found in the HSV IgA antibody-capture ELISA (18).

HSV IgG and IgA in serum and CSF of patients with HSVE. Pairs of serum and CSF specimens from patients with HSVE and from control patients were examined for the presence of HSV IgG and IgA. To detect intrathecal production of HSV antibody, we expressed the results as net optical density (OD) of CSF, i.e., the difference between the optical densities in the HSV IgG and IgA assays of CSF and serum specimens. Since both assays were carried out at the saturation level of the solid phase, a positive net OD value should be considered indicative of intrathecal antibody synthesis. Moreover, the results were correlated with the number of days after the onset of illness (Fig. 2 and 3).

Positive net OD values started to emerge 7 days after the onset of illness and reached high values at 10 days or more after the onset of illness. Some of the relatively low net OD values obtained at 14 days or more after the onset of illness were due to a relatively high OD value in the serum sample, thereby diminishing the difference from the OD value in CSF. All net OD values of samples from control patients were not significant (less than 0.050) or were negative. A net OD exceeding 0.100 was considered to be a positive result.

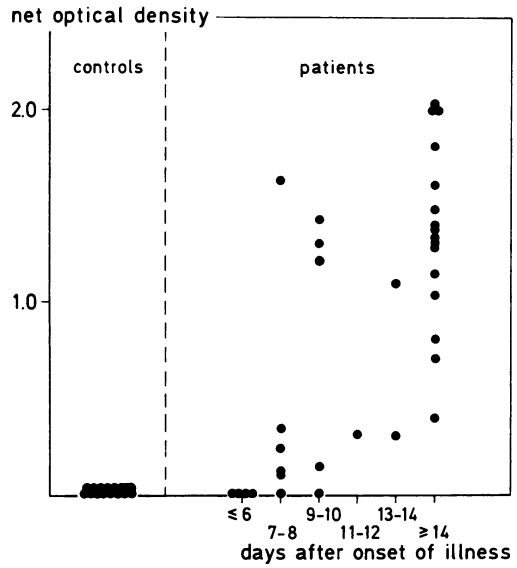

FIG. 2. Difference between OD values of CSF and serum specimens in the HSV IgG antibody-capture ELISA (net OD) in relation to the number of days after the onset of illness.

All patients with HSVE from whom specimens were available at more than 11 days after the onset of illness showed positive net OD values for both HSV IgG and IgA. One patient, who died 4 days after the onset of illness, remained negative for HSV IgG and IgA. HSVE in this patient was diagnosed by virus isolation from autopsy material from the brain.

To illustrate the rapid increase of HSV-specific antibody reactivity during the second week of illness, OD values in the HSV IgG and IgA assays of serum and CSF specimens from four patients are given in Table 1.

DISCUSSION

Determination of HSV-specific antibody levels in CSF has been used previously for the diagnosis of HSVE and has included the detection of a significant antibody titer rise in successive CSF specimens as well as detection of a decreased serum to CSF antibody ratio (7, 9, 12–14, 17). A ratio of 20:1 or lower is considered to be indicative of intrathecal HSV antibody synthesis, and therefore of HSVE (12, 13). However, by using one of these criteria, a correction is needed for possible leakage of the blood-brain barrier. Determinations of albumin or adenovirus-, respiratory syncytial virus-, or measles virus-specific antibody levels have been used for this purpose (7, 9, 13, 14). Furthermore, determination of antibody titers by conventional serology usually has a considerable degree of inaccuracy. By consequence, calculation of antibody titer ratios has an even larger inaccuracy, interfering with the use of antibody titer ratios as a diagnostic criterion. In our study an antibody-capture ELISA was used to determine levels of HSV IgG- and IgA-specific antibodies in serum and CSF specimens. The assays were carried out at saturation levels of the IgG- and IgA-binding sites at the solid phase, and therefore, the proportion of IgG or IgA antibody specific for HSV determined the magnitude of the OD value in the assay. In the present study we showed that in the CSF of patients with HSVE, the proportion of HSV IgG and IgA was higher than that in the corresponding serum. This strongly indicates that HSV IgG and IgA are locally synthesized within the CNS, and therefore implies HSV infection of the CNS. Correction for possible leakage of the blood-brain barrier was no longer needed since a higher proportion of HSV-specific antibodies in CSF compared with that in serum could not be achieved by leakage from serum. The same principle was applied previously in the diagnosis of CNS infections by Japanese encephalitis virus (3) and tick-borne encephalitis virus (6).

Of the 11 CSF specimens taken between 7 and 10 days after the onset of illness, 9 had positive net OD values in the HSV IgG assay, indicating the intrathecal synthesis of HSV-specific IgG antibody. In six of these nine CSF specimens, evidence was also obtained for intrathecal HSV IgA synthesis. Evidence for the synthesis of HSV IgG and IgA in the CNS was found in all CSF specimens taken at more than 10 days after the onset of illness. In contrast, intrathecal synthesis of HSV antibody was not found in CSF specimens from patients with HSVE obtained within 1 week after the onset of illness and in CSF specimens from the 16 patients with other causes of (meningo)encephalitis, of whom 14 had HSV IgG antibody in their serum. These results compare well with those of other investigators (7, 9, 14, 15), who also observed that intrathecal HSV antibody synthesis starts between 5 and 12 days after the onset of illness.

Our results show that by using the antibody-capture ELISA for HSV IgG and IgA, a reliable diagnosis of HSVE can be made at the beginning of the second week after the onset of illness, which is usually only 3 to 4 days after admission to the hospital. The assays are not suitable for use earlier in the disease, when antiviral treatment is most effective (15, 19). Rapid laboratory diagnosis of HSVE during the first week of illness still remains a problem, since recent studies in which HSV antigen detection in CSF was evaluated for the rapid diagnosis of HSVE did not give evidence of a significantly more rapid diagnosis compared with the time for diagnosis by antibody detection systems (2, 5, 11).

Because of the availability of acyclovir (ACV) as a safe and efficacious antiviral agent in patients with HSVE, the use of brain biopsy for the diagnosis of HSVE is no longer an

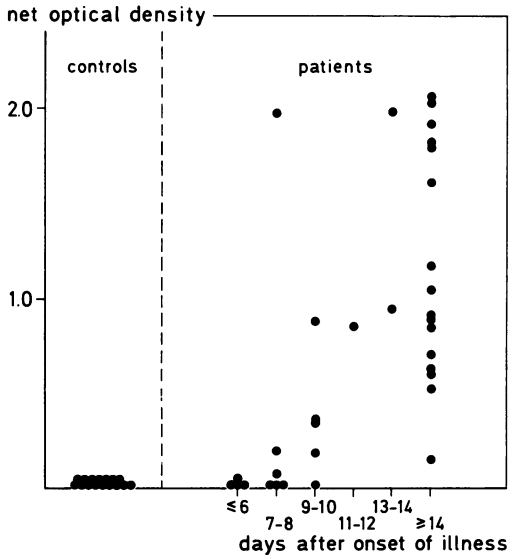

FIG. 3. Difference between OD values of CSF and serum specimens in the HSV IgA antibody-capture ELISA (net OD) in relation to the number of days after the onset of illness.

TABLE 1. Adenovirus- and HSV-specific IgG or IgA antibody determined by antibody-capture ELISA, indirect ELISA, or both[a]

Patient	Specimen	Days after onset of illness	Antibody-capture ELISA (OD)		Complement fixation	Indirect ELISA (EU)[b]	
			HSV IgG	HSV IgA		HSV IgG	Adenovirus IgG
A	Serum	4	0.020	0.114	1:2	28	47
		14	0.242	0.319	1:16	116	19
	CSF	4	0.032	0.000	<1:2	<1	<1
		14	2.055	1.903	1:8	66	<1
B	Serum	10	0.000	0.127	<1:2	18	98
		16	0.046	0.199	ND[c]	25	49
		22	0.630	0.403	1:16	155	44
	CSF	10	0.151	0.318	<1:2	1	<1
		16	2.606	2.522	1:4	48	<1
		22	2.368	2.236	1:16	150	<1
C	Serum	7	0.046	0.090	1:2	95	120
		16	0.105	0.225	1:8	140	100
	CSF	7	0.283	0.080	<1:2	3	<1
		16	1.589	1.101	1:4	67	<1
D	Serum	12	0.068	0.058	ND	162	185
	CSF	12	0.047	0.026	ND	25	20
Positive or negative cutoff value			0.090	0.145			

[a] Serum and CSF specimens were from three patients with HSVE (patients A through C) and one patient (patient D) with the Guillain-Barré syndrome.
[b] EU, ELISA units.
[c] ND, Not done.

attractive alternative. Understandably, on suspicion of HSVE, most physicians are reluctant to perform a brain biopsy and to wait for the results of subsequent laboratory examinations, which may take at least a few days in the case of virus isolation. Instead, most physicians prefer to start treatment with ACV immediately along with retrospective laboratory confirmation. As a result, a number of patients receive unnecessary treatment. This occurs in only a small number of patients, however. Between 33 and 42% (13, 15, 19) of patients suspected of having HSVE were confirmed as such. Together with an annual incidence of approximately 2 cases per 10^6 population (10, 15), fewer than 8 cases per 10^6 population per year are treated unnecessarily with ACV on suspicion of HSVE. In view of the total use of ACV, this number would hardly contribute to the development of ACV-resistant HSV strains. This is particularly true since patients who are treated unnecessarily are not actively replicating HSV at the time of treatment, and therefore, development of ACV resistance is highly improbable.

It is well known that ACV treatment attenuates the HSV antibody response. This, however, has so far not been found to affect the sensitivity of antibody assays for the diagnosis of HSVE (1). No evidence for such an effect was found in this study.

The purpose of this study was to evaluate the suitability of an antibody-capture ELISA to detect HSV IgG and IgA synthesis in the CNS for the rapid, noninvasive diagnosis of HSVE. By using these assays, a specific and sensitive diagnosis of HSVE could be made in a single pair of serum and CSF specimens during the second week after the onset of illness.

The assays were simple and easy to perform and may be useful for gaining insight into the pathogenesis of HSV infections of the CNS, particularly in cases without characteristic focal signs of brain damage. Furthermore, antibody-capture assays for the detection of specific IgG or IgA antibodies may also be useful for the diagnosis of other infections of the CNS, for example, in patients with CNS involvement of infections with the human immunodeficiency virus or *Toxoplasma gondii*.

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