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Serum and Cerebrospinal Fluid Immunoglobulins M, A, and G in Japanese Encephalitis

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A comparison was made of virus-specific immunoglobulin M (IgM), IgA, and IgG detected by capture or indirect enzyme immunoassay in serum and cerebrospinal fluid of patients with Japanese encephalitis. The IgM capture enzyme immunoassay was more sensitive than assays for other isotypes of viral antibody; IgM was detected in 75% of specimens collected ≤4 days after the onset of illness. Specific IgA was detected in both serum and cerebrospinal fluid; however, IgA levels were significantly lower than IgM levels.

Worldwide, Japanese encephalitis (JE) virus is the leading cause of epidemic encephalitis (10). In the Far East, Southeast Asia, and the Indian subcontinent, more than 40,000 cases are reported annually. Case fatality rates remain as high as 25% in children, and more than 30% of survivors have significant neurologic sequelae. JE virus, a flavivirus related antigenically to the agents of St. Louis and Murray Valley encephalitis, is transmitted by various *Culex* mosquitoes. Endemic infections occur chiefly in rural rice-producing areas where the principal vector, *Culex tritaeniorhynchus*, is found in conjunction with domestic pigs, the primary vertebrate amplifying host. In temperate locations, JE epidemics occur at intermittent intervals. Despite the availability of an effective vaccine, JE remains a significant public health problem of children in many rural areas of Asia (6). In China, where the disease is endemic, over 10,000 cases occur annually, with a case fatality rate of 10% (10).

Burke previously described an immunoglobulin M (IgM) capture enzyme immunoassay (EIA) for the rapid diagnosis of JE (1–4) and showed that JE antibody levels were correlated with outcome. We extend these observations by describing the detection of JE virus-specific IgA in serum and cerebrospinal fluid (CSF), and we compare the elaboration of virus-specific IgA, IgM, and IgG in these fluids.

MATERIALS AND METHODS

Human specimens. Sera and CSF were obtained from 20 children, 1 to 10 years old, with clinical features of viral encephalitis admitted to hospitals in Shanghai and in Luan, Anhui province. CSF samples were available from 14 of the 20 patients.

Immunoassays. All immunoassays were performed at the Department of Epidemiology, Shanghai Medical University. IgM or IgA was captured onto 96-well Immulon II polystyrene plates (Dynatech Laboratories, Inc., Alexandria, Va.) with anti-human μ- or α-chain-specific goat antibodies, respectively (Jackson Immunoresearch Laboratories, Avondale, Pa.). Serum was added at a 1:100 dilution, and CSF was tested at a 1:10 dilution. Purified JE virions were added at an optimum dilution, followed by a flavivirus group-reactive monoclonal antibody (6B6C-1) conjugated to horseradish peroxidase as a detector (8, 9). Color was developed with *ortho*-phenylenediamine.

IgG was detected by indirect EIA by adding a 1:1,000 dilution of serum or a 1:100 dilution of CSF to plates previously coated with purified JE virions. A α-chain-specific anti-human IgG antibody conjugated to horseradish peroxidase was added to detect specific antibody.

Results were expressed as a ratio of the sample absorbance to background absorbance (the absorbance associated with diluent). Ratios above 2 were considered significant (7, 9).

RESULTS

JE virus-specific IgA, IgM, and IgG were detected in acute- and convalescent-phase serum samples and in CSF (Table 1). The IgM capture assay identified 75% of patients (9 of 12) whose sera or CSF samples were obtained ≤4 days after onset of illness. All samples from day 5 postonset or later had detectable IgM except one late-convalescent-phase serum sample drawn >4 months (145 days) after onset (patient 19). However, in five other patients, IgM remained detectable in serum samples from 145 days after onset. Absorbance ratios were significantly higher in CSF than in serum; among 16 instances in which serum and CSF samples were drawn within 2 days of each other, the mean absorbance ratios were 5.9 and 12.9, respectively [$t_{(15)} = 5.398$, $P < 0.0001$].

IgG was not detectable in either CSF or serum before day 6. IgG absorbance ratios in serum and CSF were not significantly different. In 16 of 20 cases, IgG absorbance ratios converted from insignificant to significant levels in paired sera. In four cases, IgG was present in both specimens, but absorbance ratios rose from 3.9- to 14.4-fold, and 4-fold rises in titer were demonstrated (not shown).

IgA was found in only 6 of 16 acute-phase serum specimens (drawn within 1 week of onset) (37.5%) and in 4 of 10 CSF samples obtained in the acute phase. IgA absorbance ratios were significantly lower than IgM absorbance ratios in both CSF and serum and in both acute- and convalescent-phase samples. There were no instances when specific IgA was detected in the absence of IgM. Conversely, among 37 serum samples and 20 CSF samples with specific IgM, IgA was detected in only 25 (67.6%) and 15 (75%), respectively.

None of the patients died; therefore, correlations between the outcome of infection and the height of absorbance ratios could not be evaluated in this series of patients.

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TABLE 1. Serum and CSF IgM, IgG, and IgA antibodies to JE virus

Patient no.	Sex[a]	Age (yr)	Serum				CSF			
			Day of illness when serum was obtained	Absorbance ratio[b]			Day of illness when CSF was obtained	Absorbance ratio		
				IgM	IgG	IgA		IgM	IgG	IgA
1	F	3	3	—[c]	—	—	3	—	—	—
			16	6.8	14.8	2.8	20	14.6	23.0	5.3
2	M	5	4	7.9	—	2.4	4	13.8	—	2.2
			15	8.7	17.6	6.8	17	17.4	10.6	12.2
3	F	4	6	5.0	—	2.6	5	11.2	—	—
			14	9.2	6.2	6.4	16	15.4	7.4	14.0
4	M	2	8	6.5	—	—	10	18.6	6.2	7.5
			17	8.0	18.2	2.4	16	16.8	12.6	6.5
5	M	3	4	3.0	—	—	3	3.2	—	—
			13	2.5	17.4	6.8	13	12.0	9.4	7.3
6	M	5	4	—	—	—	5	22.1	—	4.0
			40	6.7	23.9	2.6	11	26.1	5.3	8.0
7	F	4	3	3.1	—	2.6	8	23.1	10.0	2.8
			33	5.0	25.3	3.0	26	26.3	19.4	4.8
8	F	1	4	—	—	—	4	6.4	—	—
			14	6.2	9.8	—				
9	M	4	3	8.0	—	—	2	11.2	—	2.2
			10	7.9	13.0	6.4				
10	M	1	10	8.7	3.2	—	8	15.6	3.0	2.2
			17	8.6	16.0	3.0				
11	M	6	11	7.9	—	—	11	17.0	—	2.2
			17	8.2	5.4	3.4				
12	F	5	6	5.4	2.1	6.8	6	12.4	18.2	27.5
			15	5.6	30.2	16.0				
13	F	8	4	3.8	—	—	4	3.6	—	—
			13	5.1	20.4	2.4				
14	M	3	5	8.5	—	—	5	9.4	—	—
			13	9.5	6.2	2.8				
15	F	3	3	5.8	8.2	2.5				
			145	3.8	32.2	16.5				
16	F	8	10	8.5	—	—				
			145	3.8	13.4	—				
17	M	10	2	2.2	—	—				
			145	2.8	6.0	—				
18	M	2	3	9.3	—	—				
			145	4.8	14.8	2.5				
19	F	4	4	4.5	—	—				
			145	—	22.6	—				
20	F	3	6	13.0	2.4	3.5				
			145	6.5	15.2	2.0				

[a] F, Female; M, male.
[b] See text.
[c] —, Absorbance ratio <2.0.

DISCUSSION

Our findings parallel those of Burke and others (1–4) and confirm the sensitivity of the IgM capture assay in identifying JE patients at an early stage of illness. However, the adequacy of testing a single acute-phase specimen in the diagnosis of JE is a function of when the acute-phase serum is drawn; only 75% of patients with a specimen from ≤4 days after onset were reactive. Studies by Burke (1) showed that low levels of specific IgM and IgM in CSF and serum were predictive indicators of a fatal outcome in JE. All of our study patients survived; therefore, there may have been a bias toward finding higher levels of specific antibody in our assays. If so, the sensitivity of IgM capture EIA might have an even lower overall sensitivity in serum samples obtained very early in the course of illness. In studies by Burke, 100% of serum and CSF samples taken at 7 days after onset were reactive, indicating the continued need to obtain serial serum specimens from some patients with suspected JE. While all JE patients will have detectable IgM by day 7, peak IgG levels appear between days 7 and 30. Therefore, measuring specific IgM, particularly in CSF, remains the most rapid means by which the serologic diagnosis of JE can be made (1).

The IgM capture EIA was more sensitive than IgA capture for the diagnosis of JE. Virus-specific IgA in the serum and CSF of patients with St. Louis encephalitis (9), a related flavivirus infection, was recently described. This study also found that the capture EIA for IgA was less sensitive than that for IgM. Studies of a murine model of alphavirus encephalitis indicate that local production of anti-viral IgA in brain exceeds that of IgG or IgM (5). The comparisons of IgM and IgA antibody responses in JE and St. Louis encephalitis patients does not indicate a similar pattern in these flavivirus infections.

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