DETECTION OF IgM ANTIBODIES FROM CEREBROSPINAL FLUID AND SERA OF DENGUE FEVER PATIENTS

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Abstract. During the dengue epidemic from late 1987 to 1989, 6 specimens of cerebrospinal fluid (CSF) and sera for IgM detection were collected from 4 cases virologically confirmed dengue patients who had neural symptoms. Another 20 serum specimens, which had been diagnosed as dengue infection either virologically or serologically, were sent to the laboratory from Kaohsiung Medical College Hospital. All these specimens were also taken to detect the existence of IgM.

The results showed that IgM could be detected from 14 out of 20 serum specimens. One of the positive specimens showed IgM can last up to 252 days after onset of illness.

In addition, IgM was detected from both CSF and sera of all four dengue patients with neural symptoms. The IgM titer in CSF ($\leq$ 1:20) was always lower than that in serum ($\geq$ 1:80). Two cases with sequentially collected specimens showed the fading of IgM titer in CSF. As a matter of fact, it became undetectable about a month after onset of illness, which is apparently different from the situation in serum.

INTRODUCTION

There was an epidemic of dengue fever, resulting in more than 10,000 reported cases, occurring in southern Taiwan during late 1987 and throughout 1988. In 1989, sporadic cases were still reported (Department of Health, 1987, 1989). This was the first outbreak in this area since 1945, except that there was a limited outbreak of type 2 dengue fever occurring in Liuchiu Island in 1981.

Serological tests on the virus infection are very important for serodiagnosis to confirm clinical diagnosis and for epidemiological survey to understand the spreading of the virus infection in a community. Recent advances in serology introduced the principle of ELISA (Engvall and Perlman, 1971). It has been known to be a technology possessing several advantages in the field of diagnostic and epidemiological virology (Sever and Madden, 1977). Since IgM antibodies were reported to be quite type-specific and appear earlier than IgG antibodies in flavivirus infection (Westaway, 1986a, b), assay of IgM antibodies was recently used to detect the infection of Japanese encephalitis (JE) in Thailand by using IgM-capture immunoassays on patients' sera or cerebrospinal fluid (Burke and Nisalak, 1982; Burke *et al*, 1982). At present, IgM antibodies apparently become a significant indicator for diagnosis of dengue infection (Botros *et al*, 1989; Chow and Hsu, 1989) and epidemiological survey of the related infection (Chen, unpublished data).

In recent years, some unusual clinical manifestations of dengue virus infections have been reported in Southeast Asia. These unusual manifestations include central nervous system involvement (Gubler *et al*, 1983, Sumarmo *et al*, 1983; George *et al*, 1984; George *et al*, 1988), cardiac abnormalities (George *et al*, 1983) and hepatitis (Nimmannitya, 1987). In Taiwan, neurologic disorders associated with presumed dengue infection were mentioned during the epidemics in 1929 and 1942-1945. Dengue infection complicated with neural symptoms has been seen in some of patients during the epidemic in 1987-1988 in southern Taiwan. Some specimens, including serum and CSF, have been collected to detect specific IgM antibodies by MAC-ELISA method.

MATERIALS AND METHODS

CSF and serum specimens

Cerebrospinal fluid (CSF) and serum were

collected from four cases, totaling 6 CSF specimens, of virologically confirmed dengue fever patients with neural symptoms. Another 20 serum specimens were collected from hospitalized dengue fever patients admitted into Kaohsiung Medical College Hospital. All patients were diagnosed as dengue fever either virologically or serologically. The serological analysis for diagnosis was done using a hemagglutination-inhibition (HI) test by the National Institute of Preventive Medicine, Taiwan.

IgM-capture (MAC) ELISA

The MAC-ELISA for IgM detection was followed. Briefly, plates (Linbro Co) were coated with 100 µl goat anti-human IgM diluted to 1:200 with 0.1 M carbonate buffer. The coated plates were stocked at 4°C until use. Before running the specimen, the plates were washed with PBS, pH 7.4. Then to the wells was added 4% bovine serum albumin to block the uncoated part of the wells. The plates were incubated for 15 minutes at 37°C and washed with PBS five times. Then was added 0.05 ml specimen serum diluted to 1:10 in 0.05% BSA-PBS, incubated at room temperature for two hours and washed again. 0.05 ml (15 HA units/0.05 ml in 20% normal human serum, acetone extracted) of dengue antigen extracted from mouse brain was added, incubated overnight at 4°C and washed again. There was add 0.025 ml of HRP-conjugated 6B6C-1 monoclonal antibody diluted to 1:6000 in 20% normal human serum in PBS, pH 7.4, to each well, incubated for 1 hour at 37°C, the plates washed with PBS, and 0.1 ml ABTS substrate solution was added to each well, incubated 30 minutes at 37°C, then allowed to stand at room temperature for 2 hours. The optical density was read with a Titertek Multiskan 310 at 405 nm.

RESULTS

During the period from late 1987 through 1989, there was a dengue epidemic, primarily type 1, occurring in southern Taiwan. Some cases had shown neural symptoms (Table 1), which include weakness, muscular atrophy, loss of tendon reflexes, sensory impairment, motor fiber disturbance, gait disturbance, pain hyperpathia, and tingling. In addition, both protein amount and cell count in CSF were increased. Four cases, totaling 6 CSF specimens, and another 20 serum specimens which were collected from patients diagnosed as dengue fever either virologically or serologically were received from Kaohsiung Medical College Hospital. MAC-ELISA was carried out for all these specimens to detect specific IgM antibodies to dengue viruses.

Table 1

The CNS symptoms in observed dengue patients.

1. weakness
2. muscular atrophy
3. loss of tendon reflexes
4. sensory impairment
5. motor fiber disturbance
6. gait disturbance
7. pain
8. hyperpathia
9. tingling
10. CSF: protein amount ↑
cell count ↑

Twenty serum specimens were collected between 5 and 275 days after onset of illness (Table 2). Among these, 14 specimens turned out to be positive to IgM antibodies. All specimens collected earlier than 106 days after onset of illness appeared IgM-positive. Unusually, one of the positive specimens showed that IgM antibody was still detectable in the blood circulation up to 252 days after onset of illness.

For 6 specimens from four cases with neural symptoms, both CSF and serum were taken and were processed for MAC-ELISA to detect specific IgM antibodies. All these specimens were collected from patients at 9, 13, 20, 21, 23, and 33 days after onset of illness, respectively. The results revealed that the titer of IgM antibodies in CSF was always lower than that in serum even though both specimens were taken from the same patient (Table 3). The result showed that all titers of IgM antibodies in 6 serum specimens were equal or higher than 1:80 while those in CSF specimens were equal or lower than 1:20. The specific IgM antibodies from two cases with paired specimens decreased within a month after onset of illness. One of the specimens showed that the titer of IgM

Table 2

Detection of IgM antibodies from sera of patients with dengue fever.

Cases	Days after onset of illness	Infection + / −	Diagnosis
1	5	+	virologically confirmed
2	7	+	"
3	9	+	"
4	13	+	"
5	14	+	"
6	20	+	"
7	21	+	"
8	23	+	"
9	30	+	serologically diagnosed
10	33	+	virologically confirmed
11	33	+	"
12	60	+	serologically diagnosed
13	106	+	virologically confirmed
14	218	−	"
15	245	−	serologically diagnosed
16	252	+	virologically confirmed
17	265	−	"
18	271	−	"
19	273	−	"
20	275	−	serologically diagnosed

Table 3

IgM antibodies in CSF and sera of patients with dengue fever.

Cases	Days after onset of illness	Titer in CSF	Titer in serum
1	9	1:10	≥ 1:80
2	13	1:20	≥ 1:80
	23	1:10	≥ 1:80
3	21	1:10	≥ 1:80
	33	Neg*	≥ 1:80
4	20	1:10	≥ 1:80

* Neg indicates negative reaction

antibodies decreased from 1:20 at day 13 after onset of illness to 1 : 10 at day 23; the other became negative at the 33rd day after onset of illness.

DISCUSSION

Serum IgM antibodies can be detected from dengue patients at 1 day after onset of the disease

(Chow and Hsu, 1989). Thus, it was not surprising to detect IgM antibodies in the patient's serum at 5 days after illness in this study. Usually, the titer of IgM antibodies in the serum may persist for about 30-90 days (Nogueira *et al*, 1988; Innis *et al*, 1989) or may extend to 150 days (Chow and Hsu, 1989). It, however, was still detectable in one specimen which was collected at 252 days after onset of the disease. But two serum specimens which were collected at 218 and 245 days after illness showed negative reactions, the time of IgM persistence in human serum actually depends on individual variation. It should not be unrealistic to assume that the "recent infection" of dengue fever diagnosed by IgM antibody detection, at least for some cases, might reflect an infection up to 8 months previously.

However, the situation of IgM in CSF seems to be different. Two cases each with two consequent specimens showed that IgM antibody will fade within a certain period of time. In fact, it may disappear from CSF one month after onset of illness. The duration of IgM in CSF is evidently shorter than that in the serum. Dengue antibodies have never been detected in the CSF by hemagglutination-inhibition test (Gubler *et al*, 1983). It is apparent that the increased sensitivity of MAC-ELISA has changed this finding.

To date, CNS symptoms have been diagnosed a variety of patients who contracted dengue fever (Sumarmo *et al*, 1983; George *et al*, 1984; George *et al*, 1988). It is known that the local humoral immune response to inflammatory diseases of the CNS can be reflected by an increase of gamma-globulin (Siemes and Siegert, 1983). The detection of immunoglobulin in CSF is possibly due to increased permeability of the blood-brain barrier to proteins and to local antigenic stimulation resulting in the proliferation of clones of lymphocytes, which release antibodies into the CSF (Siemes and Siegert, 1983). For 4 dengue fever patients who showed CNS symptoms in this study, all of them showed IgM antibody in both CSF and sera. Because detection of virus-specific IgM may also possibly reflect the persistence of virus (Ravi *et al*, 1989), the detected IgM antibodies may arise by two ways: 1) passage through the blood-brain barrier due to the increased permeability, 2) be stimulated by the virus infection. These results have shown that in the patients who appeared to have neural symptoms the titers of IgM antibody in CSF were usually lower than those in sera. Evasion of dengue virus into the cerebrospinal system, therefore, might be a possible way although it is probably only a minor event due to the inappropriate target organ. However, there is no good evidence, thus far, that dengue viruses actually cross the blood-brain barrier to invade the nervous system and replicate there (Gubler *et al*, 1983). As a result, IgM antibodies detected in CSF are, perhaps, not caused by the direct stimulation of dengue virus.

Low level or absence of virus-specific IgM in CSF has been reported to be associated with fatal outcome of Japanese encephalitis (Burke *et al*, 1985; Ravi *et al*, 1989). Immunoglobulins are known to act directly against viruses by attaching to and neutralizing them, so that IgM might be one of important protection factors in encephalitis. Surely, on the other hand, detection of low level or absence of virus-specific IgM may result from its fast disappearance in CSF.

The manifestation of encephalopathy in dengue patients has been reported in various areas. The detection of IgM antibody was known to be associated with the patient's clinical symptoms. In the case of Japanese encephalitis, the presence of virus in CSF statistically correlated with a fatal outcome (Burke *et al*, 1985). Unfortunately, virus isolation from CSF, in this study, has not been tried. Therefore, whether or not neural symptoms of those patients were caused by the presence of dengue virus in their CSF can not be defined at this moment.

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