Epidemiology and Seroimmunological Studies on Aseptic Meningitis

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(Received October 30, 1964)

In recent years a number of investigators[1)~3)] have reported the etiological analysis of viral diseases of the central nervous system, and an increasing array of immunotypes of ECHO virus has been implicated in the causation of broad spectrum of acute central nervous system diseases.[4)~8)]

In Japan, epidemic outbreaks of aseptic meningitis have been experienced as often during the past several years as in foreign countries. We made studies on about 155 cases of aseptic meningitis, epidemiologically, serologically and virologically for the past 5 years, from 1959 to 1963 in Kansai districts, mainly in Kyoto and Matsuzaka city. In our country, furthermore, live attenuated Polio vaccine (Sabin) has been administered since 1961 and patients suffering from paralytic Polio have decreased in number. The advent of Polio vaccine has heightened the need to elucidate further and to compare the frequency with which various enteroviruses, as well as certain non-enteric viruses, are associated with aseptic meningitis.

Methods

Specimens for laboratory study were as follows: as a rule two stools were collected at one to three day intervals during the acute phase of illness; one cerebrospinal fluid specimen; two to three blood specimens, taken, when the patient was first seen, about 14 days after onset of illness, and when possible, again about four to five weeks after onset.

Isolation and Identification of Viruses. Stool specimens and cerebrospinal fluid were inoculated into FL cell and monkey kidney cell cultures for the detection of cytopathogenic agents. Stools were triturated to make suspension of about 10 per cent in Hanks' balanced salt solution with Penicillin and Streptomycin. The crude suspension was spun in a centrifuge at 7,000 r. p. m. for 30 minutes. Two-tenths ml of the supernatant fluid was inoculated into each of 2 culture tubes of FL and monkey kidney cells, respectively, and incubated at 37°C for 2 hours without rolling; the inoculum was then removed and replaced with 1 ml of YLH-maintenance medium. The tubes were observed daily for

cytopathogenic changes. Subsequent passages were made by inoculating 0.1 ml of infected tissue culture fluid into fresh tubes and these were then observed for 7 days. All tissue cultures were incubated at 37°C with rolling. All viruses obtained were identified by neutralization with rabbit or monkey immune antisera. Antisera against the following enterovirus types were available: Poliovirus, types I, II and III: Coxsackie virus group B, types 1–6: ECHO virus, types 1–28. For virus identification neutralization tests were made by combining equal amounts of antisera and a dose of unknown isolated virus calculated to contain approximately 100 TCD_{50} units in 0.1 ml.

Serological Tests. All sera were regularly tested for complement fixing (CF) antibody to the viruses of Polio I (Mahoney), II (MEF-1) and III (Saukette), ECHO (antigen of types 4, 6, 7 and 9 were pooled), Coxsackie group B (antigens of types 1 through 6 were pooled), Mumps (Enders), Adeno type 3 (G. B.), and Japanese encephalitis (Nakayama). As CF antigen the undiluted fluids from FL or monkey kidney tissue cultures infected with enteroviruses were used. The soluble antigen of Mumps was made from infected allantoic membrane. The antigen of Japanese encephalitis was made from the brain tissue of mice d. d. strain infected intracerebrally with Japanese encephalitis virus (Nakayama strain). For those cases from which enteroviruses were isolated, CF antibody for one of the prototype viruses of the same type as the virus isolated, and neutralizing antibody for the same virus were also tested. Tissue culture neutralization tests were made by combining equal amounts of serum and a dose of isolated virus calculated to contain approximately 100 TCD_{50} units in 0.1 ml.

Hemagglutination Inhibition (HI) Test. Cases of Mumps and Japanese encephalitis diagnosed clinically or serologically were tested for HI antibodies, in addition to CF tests. The antigen of Mumps was allantoic fluid from chick embryos infected with the Enders strain. The antigen of Japanese encephalitis was made from brain tissue of mice infected intracerebrally with Japanese encephalitis virus (Nakayama strain).

A four fold or greater rise in antibody titer between acute-phase and convalescent-phase sera was considered necessary to permit the positive diagnosis of recent infection. A two fold rise in antibody titer between them was considered necessary to permit the probable diagnosis. When both acute and convalescent CF antibody titers were 1 : 8, a CF antibody titer was 1 : 32 or greater in a single specimen, or when an HI titer was 1 : 320 or greater in Japanese encephalitis, these were considered sufficient to permit the probable diagnosis, even in the absence of a demonstrative rising titer.

Results

Figure 1 presents annual and sex distribution of aseptic meningitis in Kyoto and Matsuzaka during the past 5 years. One hundred and fifty-five cases of

aseptic meningitis were experienced and consisted of 98 males and 57 females showing that the male suffered more often than female, similar to the general tendency in Japan. Figure 2 presents age distribution of aseptic meningitis. It shows the peak in childhood and early school age, the same as generally reported, and the other peak of infants which is seen in the cases in Kyoto is due to Poliovirus as mentioned later. Figure 3 shows monthly incidence. Cases

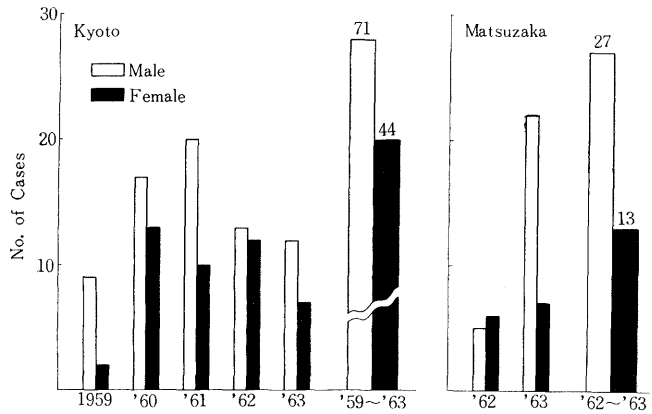

Fig. 1. Annual and Sex Distribution of Aseptic Meningitis

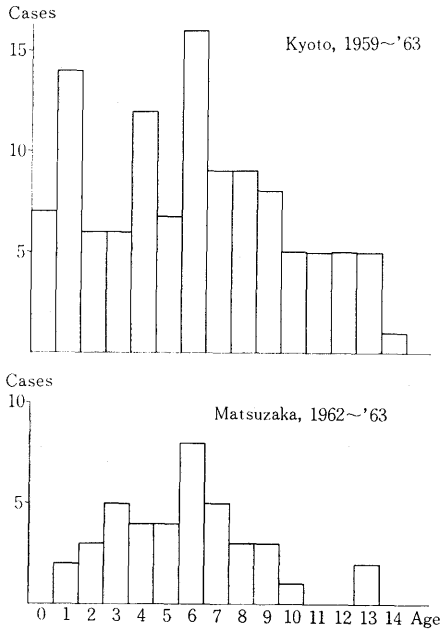

Fig. 2. Age Distribution of Aseptic Meningitis

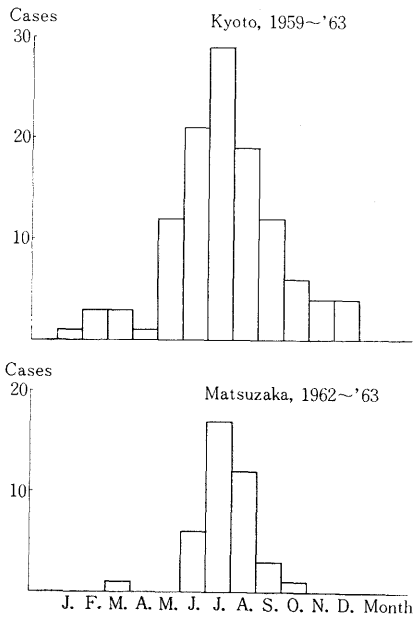

Fig. 3. Seasonal Distribution of Aseptic Meningitis

of aseptic meningitis were concentrated mostly in summer in Kyoto and Matsuzaka, as expected.

Serological studies were carried out on 108 out of 115 cases during 5 years from 1959 to 1963 in Kyoto, and 31 out of 40 cases during 2 years from 1962 to 1963 in Matsuzaka, respectively. Figures 4 and 5 present the etiological diagnosis of aseptic meningitis divided into three groups in our study. Serological diagnosis of aseptic meningitis was established in 44 cases out of 67 cases (65.7 %) on the lst group (Kyoto, 1959–1961), including cases of probable diagnosis as follows: Polio, 20 cases (29.9%), ECHO, 5 cases (7.5%), Coxsackie B, 10 cases (14.9%), Mumps, 7 cases (10.4%), Adeno, 1 case (1.5%), Japanese encephalitis, 1 case (1.5%), and in 29 out of 41 cases (70.7%) on the 2nd group (Kyoto, 1962–1963) as follows: ECHO, 10 cases (24.4%), Coxsackie B, 4 cases (9.8%), Mumps, 11 cases (26.8%), Adeno, 2 cases (4.9%), Japanese encephalitis, 1 case (2.4%), mixed infection, 1 case (2.4%), and in 26 out of 31 cases (83.9%) on the 3rd group (Matsuzaka, 1962–1963) as follows: ECHO, 8 cases (25.8%), Coxsackie B, 1 case (3.2%), Mumps, 14 cases (45.2%), Adeno, 1 case (3.2%), Japanese encephalitis, 1 case (3.2%), mixed infection, 1 case (3.2%). Figures 6, 7 and 8 illustrate the monthly incidence of 6 agents and others in detail. As mentioned before, attenuated Polio vaccine (Sabin) has been administered in our country since 1961. It has been done on a very large scale, and about fourteen

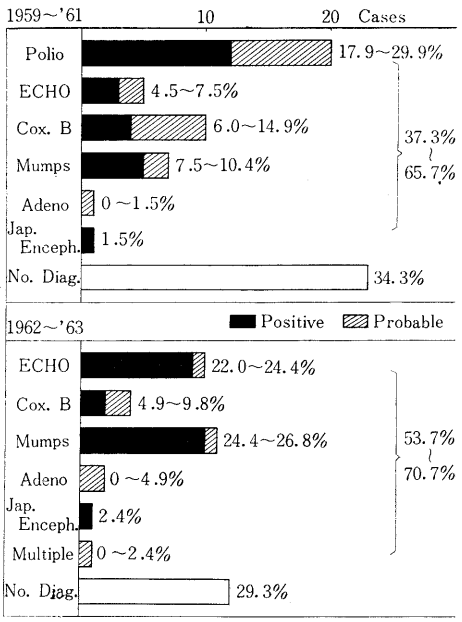

Fig. 4. Immunological Diagnosis of Aseptic Meningitis (Kyoto)

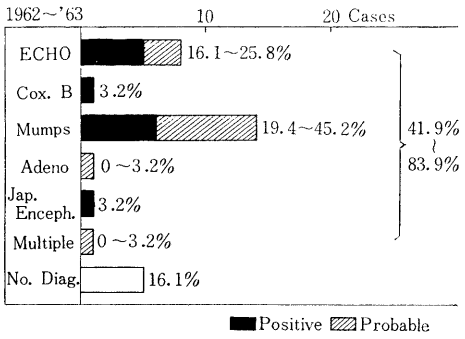

Fig. 5. Immunological Diagnosis of Aseptic Meningitis (Matsuzaka)

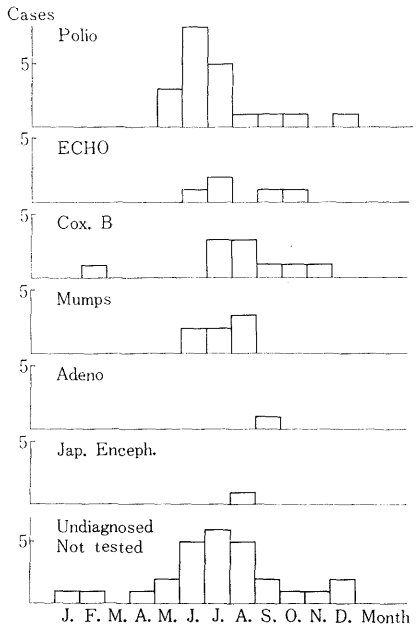

Fig. 6. Seasonal Distribution of Aseptic Meningitis Differentiated by Viral Agent (1) Kyoto, 1959～1961

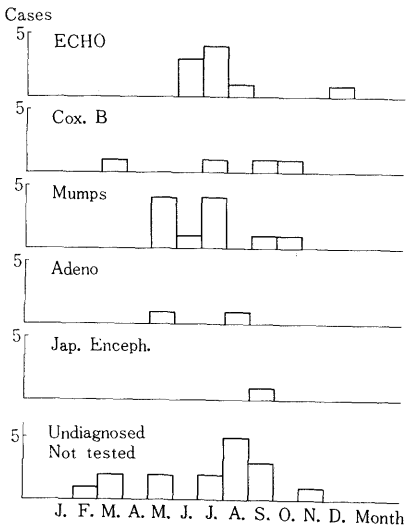

Fig. 7. Seasonal Distribution of Aseptic Meningitis Differentiated by Viral Agent (2) Kyoto, 1962～1963

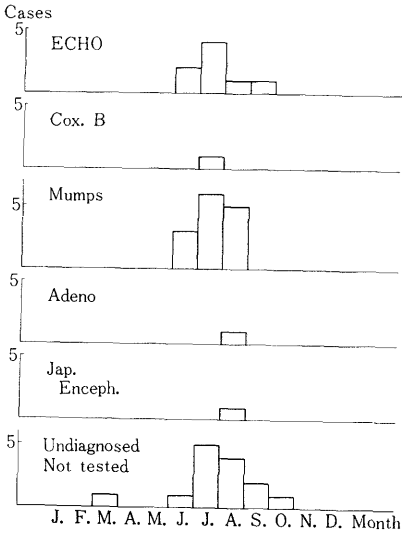

Fig. 8. Seasonal Distribution of Aseptic Meningitis Differentiated by Viral Agent (3) Matsuzaka, 1962～1963

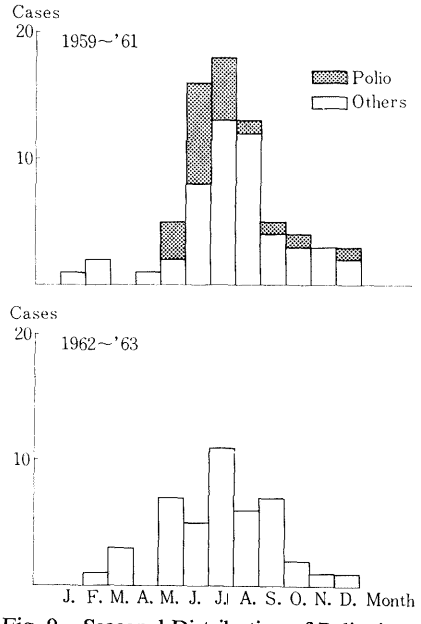

Fig. 9. Seasonal Distribution of Poliovirus and Other Agents Associated with Aseptic Meningitis (Kyoto)

Table 1. Viral Isolation from Stools and Cerebrospinal Fluid

Districts	Kyoto										Matsuzaka				Total	
Years	1959		1960		1961		1962		1963		1962		1963			
Materials																
Agents	Stool	CSF	Stool	CSF	Stool	CSF	Stool	CSF	Stool	CSF	Stool	CSF	Stool	CSF	Stool	CSF
Polio I			2		4										6	
Polio II																
Polio III																
ECHO 4										2					2	
ECHO 6										3			1		4	
ECHO 7			2	1											2	1
ECHO 14										1			1		2	
ECHO Unknown			4												4	
Cox. B 2										1					1	
Cox. B 3													1		1	
Cox. B 5			1												1	
Cox. B Unknown			1												1	
Unknown					1										1	
Total Isolated			10	1	5				7				3	25 30.9%	1	1
Negative	2	1	9	2	14	7	14	10	16	21			1		56	41

Table 2. Relationship between Enteroviruses Isolated and Their Antibody Responses in Cases of Aseptic Meningitis, 1962-'63

Case No.	Age	Sex	Virus Isolated from Stool	CF and NT Antibody Responses													Serological Diagnosis
				Antigen	Polio CF			ECHO CF			Coxsackie B CF			Mumps	Adeno	Jap. B Enceph.	
				Day of Disease	I	II	III	Pooled[1] Antigen	Single[2] Antigen	NT[3]	Pooled[4] Antigen	Single Antigen	NT	CF	CF	CF	
1	5	♂	ECHO 4	3	<4	8	<4	4	4	16	<4			<4	32	<4	ECHO 4
				21	4	16	4	16	8	256	4			<4	32	<4	
2	7	♂	ECHO 4	4	4	4	8	8	4	<4	<4			<4	<4	<4	ECHO 4
				19	4	8	8	16	8	64	4			<4	4	<4	
3	4	♂	ECHO 6	4	<4	16	4	4	8	<4	4			<4	<4	<4	ECHO 6
				22	<4	16	4	16	32	256	4			<4	<4	<4	
4	9	♀	ECHO 6	2	<4	<4	<4	<4	4	<4	4			<4	8	<4	ECHO 6
				16	<4	<4	<4	8	8	16	4			<4	8	<4	
5	1	♀	ECHO 6	1	<4	4	<4	8	32	4	8			<4	<4	<4	ECHO 6
				19	4	<4	4	16	64	256	4			<4	<4	<4	
6	9	♂	ECHO 6	2		<4		<4	4	8	<4			<4	<4	<4	ECHO 6
				14		<4		8	8	128	<4			<4	<4	<4	
7	8	♀	ECHO14	3	<4	<4	<4	8	4	16	8			<4	<4	<4	ECHO14
				16	<4	4	<4	8	16	32	16			4	4	4	
8	6	♂	ECHO14	2		4		4	<4	<4	<4			<4	<4	<4	Undiag.
9	6	♀	Cox. B 2	2	<4	4	<4	8			<4	4	<4	4	4	<4	Cox. B 2
				16	<4	<4	<4	8			8	8	4	4	4	<4	
10	4	♂	Cox. B 3	2		<4		8			8	32	8	<4	8	<4	Cox. B 3
				17		<4		8			8	32	256	<4	8	<4	

1) ECHO Virus Types 4, 6, 7 and 9 Are Pooled.
2) One of the Prototype ECHO Viruses Which Is the Same Type as the Virus Isolated.
3) Neutralizing Antibodies to Virus Isolated.
4) Cox. B Virus Types 1~6 Are Pooled.

million children were inoculated in 1961. The cases of paralytic Poliomyelitis were about 200 in 1962 and about 150 in 1963, respectively, and they were about one tenth of the cases in the previous years. The administration of attenuated Polio vaccine gave a great influence to the etiology of aseptic meningitis, as shown distinctly in Figures 4, 5, 6, 7 and 8. Polioviruses played a leading role as an agent of aseptic meningitis in 1959–1961 in Kyoto as mentioned above (Figure 4 above and Figure 6), but they vanished in 1962–1963 in Kyoto and Matsuzaka also (Figure 4 bottom and Figures 7 and 8). On the other hand, ECHO and Mumps viruses show a somewhat higher percentage than Polio (Figure 4 bottom and Figure 5). Figure 9 shows the comparison of seasonal incidence of Polio and other agents by month during the periods 1959–1961 and 1962–1963, presenting no Polio cases since 1962. Figure 10 shows age distribution of Polio and other agents during the periods 1959–1961 and 1962–1963, also presenting no Polio cases since 1962.

Table 1 presents virological studies. The types of enteroviruses isolated from stools each year are shown. During 5 years, stools from 81 cases were tested and enteroviruses were isolated from 25 cases (30.9%). There were Polio-I, 6 strains, ECHO-4, 2 strains, ECHO-6, 4 strains, ECHO-7, 2 strains, ECHO-14, 2 strains, ECHO type unknown, 4 strains, Coxsackie B types 2, 3 and 5, 1 strain, respectively, and Coxsackie B type unknown, 1 strain and unknown

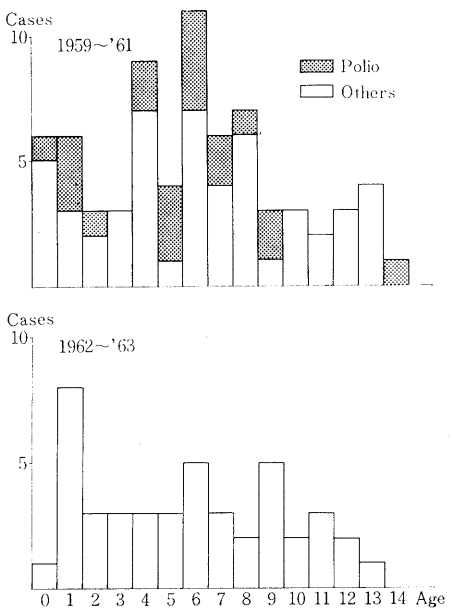

Fig. 10. Age Distribution of Poliovirus and Other Agents Associated with Aseptic Meningitis (Kyoto)

virus, 1 strain, respectively. From cerebrospinal fluid only ECHO-7, 1 strain was isolated.

Table 2 shows enteroviruses isolated and results of serological tests on the isolated enteroviruses cases. Sera from the same patient were tested simultaneously against CF antigens for ECHO or Coxsackie group B and neutralization antigens for viruses isolated. As for CF antigens two kinds were used. One of them was derived from one of the prototype ECHO viruses of the same type as the virus isolated, the other was a pooled antigen, which involved the pooling of ECHO types 4, 6, 7 and 9 or Coxsackie group B types 1-6. These three methods of serological tests were compared for their sensitivity and potency as shown in Table 2. It is clear that case numbers 5, 7 and 10 show somewhat different antibody titers between the two ways of CF tests, but others show similar antibody titers between them. On the other hand, neutralization test for virus isolated shows a more specific and sensitive rise in antibody titer. ECHO and Coxsackie viruses are divided into many seroimmunotypes, and many of them have been implicated in the causation of aseptic meningitis. It is difficult to conduct CF tests covering all these antigens. To solve this problem, the above-mentioned pooled antigens of ECHO or Coxsackie group B viruses were used with the intention of comparing the sensitivity, specificity and potency with the homotypic CF antibody response of patients who excreted enteroviruses. These pooled antigens contain 2 units of antigen, considered desirable for CF tests, and it is considered possible that these methods might be adopted, to some extent, especially in cases from which viruses have not been isolated.

Discussion

The results of virus isolation attempts and serological examinations are summarized according to the clinical diagnosis of the patients in Figures 4, 5 and Table 1.

Poliovirus had played a leading role universally as an agent of aseptic meningitis before the administration of Polio vaccine: Schnyder[9] reported 21% of Poliovirus as an agent of aseptic meningitis, Wiesmann[28] 16%, Pulver[10] 16%, Lippelt[11] 10.3%, Hennessen[12] 23.5% and Kato[13], one of our colleagues, reported 28%, respectively. In our study, Poliovirus occupied 29.9% as shown in Figure 4 above as an agent of aseptic meningitis during the period of 1959-1961 in which the administration of Polio vaccine was not yet prevailing. But after the administration of Polio vaccine, Poliovirus disappeared as an agent of aseptic meningitis as shown in Figure 4 bottom and Figure 5. As for the diagnosis of Polio, the rules for the diagnosis mentioned before was not necessarily adopted, considering the influence of Polio vaccine. After receiving the Polio vaccination there were 6 cases of aseptic meningitis, who demonstrated 1 : 8 or 1 : 16 rise as

a reciprocal of serum dilution in Polio antibody titer. No virus was isolated from them either. Although in some cases it was not known whether they had taken Polio vaccine or not, they could not be admitted as Poliovirus infection cases. Since Polio vaccination in 1961, a great amount of Polio vaccine viruses has already become widespread in Japan, and it is said that most people may be exposed to Polio vaccine virus, whether they take Polio vaccine or not. They might easily have antibody response by vaccine intake. But neither Poliovirus nor any significant (four fold or greater) antibody titer rise in the present cases was found. Thus, no confirmed Poliovirus infection is shown both in Kyoto and Matsuzaka. On the other hand, ECHO, Coxsackie and Mumps viruses are associated with some cases of aseptic meningitis instead of Poliovirus. How can Poliovirus infection be diagnosed after the vaccination? Does it come from the vaccine or from a wild strain? Several methods have been proposed for the purpose of serological identification of Polioviruses but still no absolutely sure method of solving this problem is known. It should be noted, however, for the purpose of diagnosing Polio, that a careful examination for detecting Poliovirus and its immunologic response is needed; and also a complete absence of any other virus infection. On the other hand, in recent years newly recognized enteroviruses have been reported in the causation of a broad spectrum of acute central nervous system diseases. Since the advent of Polio vacciation, Poliovirus infections have ceased to be the leading cause of paralytic and non-paralytic diseases of the central nervous system. But it is felt that continuous research for these diseases is necessary to determine whether the suppression of illness due to Polio vaccine is a transient phenomenon or not.

As to ECHO virus as an agent of aseptic meningitis many reports have been published: Méndez-Cashon[14] isolated ECHO-1 virus, Jhala[15] isolated ECHO-23 virus, Sabin[16] isolated ECHO-22 virus from aseptic meningitis, respectively, and Bastianni strain,[17] Price strain,[18] Giles strain[19) 20] and Frater strain belonging to ECHO-30, and Caldwell strain[21] and 4331-S strain[22] belonging to ECHO-31 which were serologically classified by Wenner,[23] were isolated from aseptic meningitis also. As to epidemics of ECHO-4, there are fairly many reports of Lehan,[24] Chin,[25] Melnick,[26] Meyer,[27] Lepow[28] and Gillfand[29] et al. Two strains of ECHO-4 were isolated from aseptic meningitis in 1963 in Kyoto in our study. It was found that the marked affinity of these 2 strains of ECHO-4 virus to FL cell is a distinguishable characteristic. ECHO-6 is a typical ECHO virus with clinical symptoms such as aseptic meningitis or exanthema, and its epidemic has been often universally experienced.[26) 27) 29) 30] Regarding ECHO-7, 2 strains were isolated in 1960 in Kyoto in our study. As most cases in which ECHO-7 virus was isolated, reported up to the present time, have been from healthy infants[31)32)33] or infants suffering from summer diarrhoea[34] and furthermore, that there are very few reports or ECHO-7 virus as an agent of diseases

of the central nervous system, it should be noteworthy as a rare case in the world that ECHO-7 virus was isolated from cases of aseptic meningitis.

Concerning Coxsackie virus, according to many reprots, it is well known that B group type 5 strain has a special affinity to the central nervous system. In our country, we have experienced a moderately big epidemic of aseptic meningitis due to Coxsackie virus B-5 in 1960 and 1961.[35) 36) 37)]

Summary

One hundred and fifty-five patients with aseptic meningitis syndrome were investigated serologically, virologically and epidemiologically in Kyoto and Matsuzaka during the peiod 1959-1963. Laboratory procedure included attempts to isolate enteroviruses from stools and cerebrospinal fluid, and antibody determinations for Polio, ECHO, Coxsackie group B, Mumps, Adeno, and Japanese encephalitis viruses.

Cases of aseptic meningitis were encountered mostly in summer, as expected. Males moderately outnumbered females, as generally reported. Age distribution showed a peak in childhood and early school age.

Aseptic meningitis showed diverse etiologies, associated most frequently with Polio, ECHO, Coxsackie group B and Mumps viruses. A viral etiology could be established for 65.7% and 70.7% in Kyoto, and 83.9% in Matsuzaka with serological techniques employed. It was shown that 25 strains of enteroviruses, including Poliovirus type I, ECHO virus types 4, 6, 7 and 14, Coxsackie group B virus types 2, 3 and 5, were recovered from stools of patients. Only one strain of ECHO virus type 7 was isolated from cerebrospinal fluid.

Comparison of cases of aseptic meningitis during the period 1959-1961, before the administration of Polio vaccine, with the cases during the period 1962-1963, after administration, was made. In the former, Poliovirus played a leading role as an agent of aseptic meningitis, but in the latter, the association of Poliovirus was not demonstrated. And no evidence was found that Polio mass vaccination might have any influence upon the other virus patterns.

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