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Two outbreaks of echovirus 14 infection: a possible interference with oral poliovirus vaccine and a probable association with aseptic meningitis

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During 1962 and 1963 we encountered two outbreaks of echovirus 14 infection in northern Japan. In the first, which occurred in Sendai in 1962, it seemed possible that echovirus 14 was interfering with the establishment of infection with Sabin type 1 oral poliovaccine. In the second, which occurred in Aomori in 1963, a small outbreak of aseptic meningitis was observed.

The present communication describes these events and discusses the significance of echovirus 14 infection in man.

MATERIALS AND METHODS

Subjects

In October 1962, babies in an infant institution in Sendai were fed type 1 strain (LSc2ab) of Sabin's oral poliovaccine obtained from the Connaught Medical Laboratories, Canada. Nine babies to whom this vaccine was given were examined. In 1963, fifty nine patients with aseptic meningitis in Aomori City were studied.

Virus isolation

Primary cultures of cynomolgus monkey kidney cells were used. Growth medium for the cell monolayers consisted of Hanks's balanced salt solution containing 0·5% lactalbumin hydrolysate and 5% unheated bovine serum. Maintenance medium was Earle's balanced salt solution containing 0·5% lactalbumin hydrolysate *without serum*.

Faeces and cerebrospinal fluids (CSF) were kept frozen at $-25°$ C. until tested. Stools for virus isolation were prepared in 10% suspension in the maintenance medium, and centrifuged for 10 min. at 3000 r.p.m. Before infection, fluids of the cell monolayers were replaced by 1·0 ml. of the maintenance medium, and 0·1 ml. of the stool extract or 0·2 ml. of CSF was then inoculated. The cultures were incubated at 35–37° C. in a stationary state and observed for at least 7 days. The fluids of any cultures exhibiting marked cytopathic effect were passaged to fresh cell cultures for confirmation of the viral agents.

Identification of virus

Virus strains were identified by the neutralization test, using antisera to polio-viruses 1–3, coxsackie A 9 and B 1-6 viruses, and to echoviruses 1-28. Neutralization of approximately 100 TCD 50 of virus by 20 neutralizing units of antiserum was considered a positive identification.

Neutralizing antibody test

Paired serum specimens from the same person were always tested simultaneously. Sera were heated at 56° C. for 30 min. before testing. Fourfold serial dilutions of the sera were tested against approximately 100 TCD 50 of poliovirus 1, Mahoney strain and of echovirus 14, the current strain, AM-53-63. Serum-virus mixtures were allowed to stand at room temperature for 1 hr. before inoculation into stationary monkey kidney cell culture tubes. Neutralizing-antibody titres were expressed as the reciprocal of the highest serum dilution that completely protected the cells in the presence of approximately 100 TCD 50 of virus.

RESULTS

(1) *Failure of vaccination with Sabin type* 1 *attenuated poliovirus in infants infected with echovirus* 14

Nine babies in the infants' institution in Sendai were chosen to receive Sabin oral poliovaccine as a group of our field trial of the vaccine. On 5 October 1962, faeces and blood specimens were taken before vaccination and examined for enteroviruses and antibody titres. Three days later, 1 ml. of type 1 vaccine virus containing 10^5 or 10^6 TCD 50 was given by mouth. All the babies were under 8 months old at the time of feeding vaccine. Faeces were obtained 10 days and blood specimens 7 weeks after the feeding of vaccine.

Results are shown in Table 1. From the faecal specimens obtained 3 days before feeding vaccine, no virus was recovered in any of the babies tested. From those obtained 10 days after the feeding, however, echovirus 14 was isolated in six of the nine tested. Poliovirus type 1, which probably was the vaccine virus, was isolated only in two babies. No viruses were recovered in one case (no. N-7). Significant rise (fourfold) of polio 1 antibody was observed in only three cases, in spite of the absence of polio-antibodies in all babies before vaccine was given. On the other hand, a significant rise of echovirus 14 antibody was found in eight babies. Seven of them had no detectable antibody against echovirus 14 before feeding vaccine. However, one (no. N-7) showed a higher titre of echovirus 14 antibody in the first serum, suggesting a recent infection with echovirus 14 before poliovaccine was given. Another (no. N-1) showed echovirus 14 antibody to a titre of 1/4 in the first serum. This might have been maternal antibody, as no virus was isolated from the faecal specimen obtained on the day when the blood sample was taken, but echovirus 14 was recovered from the faeces 13 days later. No symptoms due to the alimentary infections were observed in any of the infants.

From the results of virological and serological examinations, it can be postulated

Table 1. *Virus isolation and neutralizing antibody titre in nine infants fed Sabin type* 1 *poliovaccine*

			Virus isolation		Neutralizing antibody		
						Titre against	
Baby no.	Age (weeks)	TCD50 of fed vaccine (log)	Sampling time (days)*	Results	Bleeding time (days)*	Polio 1	Echo 14
			−3	Neg.	−3	0†	4
N-1	4	5	10	Echo 14	49	0	16
			−3	Neg.	−3	0	0
N-2	4	5	10	Polio 1	49	0	256
			−3	Neg.	−3	0	0
N-3	4	6	10	Echo 14	49	0	16
			−3	Neg.	−3	0	0
N-4	4	6	10	Echo 14	49	0	256
			−3	Neg.	−3	0	0
N-5	12	5	10	Echo 14	49	0	256
			−3	Neg.	−3	0	0
N-6	13	6	10	Echo 14	49	16	256
			−3	Neg.	−3	0	1024
N-7	13	6	10	Neg.	49	0	256
			−3	Neg.	−3	0	0
N-8	26	5	10	Polio 1	49	16	256
			−3	Neg.	−3	0	0
N-9	27	5	10	Echo 14	49	16	16

* Days from the feeding vaccine.
† Less than 4.

Table 2. *Effectiveness of vaccination with Sabin's attenuated poliovirus* 1 *on infants at the out-patient clinic of National Hospital of Sendai*

			Virus isolation		Neutralizing antibody against poliovirus 1	
Baby no.	Age (weeks after birth)	TCD50 of fed vaccine (log)	Sampling time (days)*	Result	Bleeding time (days)*	Titre
			−3	Neg.	−3	0†
K-2	3	5	10	Neg.	49	64
			−3	Neg.	−3	0
K-13	4	5	10	Neg.	49	0
			−3	Neg.	−3	0
K-22	13	5	10	Polio 1	49	64
			−3	Neg.	−3	0
K-23	14	6	10	Polio 1	49	16
			−3	Neg.	−3	0
K-30	24	5	10	Polio 1	49	64
			−3	Neg.	−3	0
K-31	26	6	10	Neg.	49	0

* Days from the feeding vaccine.
† Less than 4.

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as follows: case no. N-7 had been infected with echovirus 14 before vaccine was fed and then the virus spread to other infants. The time of infection with echovirus 14 was apparently very close to that of the poliovirus vaccine feeding. Thus, the alimentary infection with echovirus 14 in infants possibly interfered with the establishment of infection with the oral poliovirus, and resulted in a failure of the vaccination in six out of nine babies.

In the same programme of this field trial, thirteen babies in the out-patient clinic of the National Hospital of Sendai were also given the same vaccine in the same manner and were examined by the same procedure. Six of them had no detectable antibody against poliovirus type 1 before vaccination. As shown in Table 2, four out of the six showed a significant rise of antibody after vaccination.

Table 3. *Monthly incidence of aseptic meningitis associated with enteroviruses, Aomori,* 1963

Month	No. of patients tested	No. of patients of positive isolation of following virus type: Poliovirus 1	Poliovirus 3	Coxsackievirus A9	Coxsackievirus B2	Coxsackievirus B4	Echovirus 6	Echovirus 14	Un-identified	Total
January	2	—	—	—	1	—	—	—	—	1
February	1	—	—	—	—	—	—	—	—	0
March	2	—	—	—	1	—	—	—	—	1
April	1	—	—	—	—	—	—	—	—	0
May	4	1	—	—	1	1	—	—	—	3
June	8	—	—	—	1	1	—	—	1	3
July	17	1	2	1	1	—	—	—	4	9
August	10	—	—	—	—	—	—	4	—	4
September	6	—	—	—	—	—	—	2	—	2
October	5	—	—	—	—	—	—	1	—	1
November	2	—	—	—	—	—	—	—	—	0
December	1	—	—	—	—	—	1	—	—	1
Total	59	2	2	1	5	2	1	7	5	25

(2) *An outbreak of aseptic meningitis associated with echovirus* 14

During the longitudinal studies on virus etiology of the involvement of the central nervous system in Aomori district, it was found that echovirus 14 was a main causative agent of aseptic meningitis which occurred in the summer and fall of 1963. Virus isolation was attempted from fifty nine children and infants with aseptic meningitis at the Pediatric Clinic of Aomori Prefectural Central Hospital, Aomori City, during 1963. Results are summarized in Table 3. From the beginning of January to the end of July 1963, poliovirus types 1 and 3, coxsackie A9, B2 and B4 viruses, and unidentified viruses which were probably coxsackieviruses since they caused paralysis in unweaned mice, were isolated from the faecal or cerebrospinal fluid specimens of seventeen patients. On the other hand, during the following 3 months (August–October), only type 14 echovirus was recovered from seven patients, and echovirus 6 was isolated from one patient in December. In one case (AM-53-63 in Table 4) echovirus 14 was recovered from both faeces and CSF.

The results suggested that the main causative virus of aseptic meningitis from August to October 1963 was echovirus 14.

Paired sera from twenty-one patients with aseptic meningitis were examined for neutralizing antibody against echovirus 14. As shown in Table 4, antibody titres against echovirus 14 were detected in all seven cases excreting echovirus 14 and in four of the virus-negative patients. Thus over 50 % of the aseptic meningitis patients found from August to October had been infected with echovirus 14. Although paired sera of fourteen patients with aseptic meningitis found in June and July, from whom no virus was recovered, were examined for neutralizing antibody against echovirus 14, no significant rise of antibody was found in any.

Table 4. *Virological and serological evidence on patients with aseptic meningitis caused by echovirus* 14

Patient no.	Age (year-month)	Onset date	Echovirus 14 isolation			Echovirus 14 Neutralizing antibody	
			Specimen	D.D.*	Results	D.D.*	Titre
AM-36-63	4–6	Aug. 12	Faeces	2	+	2	0†
			CSF	2	–	16	64
AM-37-63	1–10	Aug. 13	Faeces	2	+	2	0
			CSF	2	–	14	256
AM-38-63	2–6	Aug. 16	Faeces	2	+	2	0
			CSF	4	–	36	64
AM-49-63	3–3	Aug. 30	Faeces	2	+	2	0
			CSF	2	–	18	256
AM-41-63	2–6	Aug. 30	Faeces	2	–	4	0
			CSF	2	–	17	16
AM-42-63	6–9	Sept. 11	Faeces	1	–	2	0
			CSF	1	–	15	16
AM-43-63	1–10	Sept. 12	Faeces	2	+	2	0
			CSF	2	–	17	16
AM-45-63	2–6	Sept. 13	Faeces	4	+	4	0
			CSF	4	–	18	16
AM-55-63	2–9	Oct. 3	Faeces	3	–	9	64
			CSF	Not available		29	256
AM-53-63	2–9	Oct. 18	Faeces	2	+	2	0
			CSF	1	+	30	16
AM-52-63	1–6	Nov. 18	Faeces	2	–	1	16
			CSF	2	–	27	64

*Days after onset of illness.
† Less than 4.

These facts strongly suggest that a small epidemic of aseptic meningitis caused by echovirus 14 occurred among children and infants in Aomori in the middle summer and fall of 1963.

Symptoms and signs of most of the patients with echovirus 14 infection were typical of aseptic meningitis due to many other enteroviruses. High fever, nausea,

vomiting, and abnormal findings on examination of CSF, were common in these patients, except in one case (no. AM-55-63 in Table 4) who exhibited transient gait disturbance and a pleocytosis in the CSF but no fever.

DISCUSSION

Isolation of echovirus 14 from patients with aseptic meningitis has been reported from many parts of the world. Especially, isolation of the virus from the CSF confirmed that the virus was associated with asceptic meningitis (Henley, Berger & Hodes, 1958; McLean, McQueen & McNaughton, 1962; Lepow *et al.* 1962; Kelen, Delbin, Lesiak & Labzoffsky, 1963; Miyamoto *et al.* 1964). However, no outbreak of aseptic meningitis caused by this virus has been reported so far. In Japan, the first isolation of echovirus 14 from a case of aseptic meningitis was reported from this laboratory by Miyamoto *et al.* (1964). The virus was recovered from both CSF and faeces of one case which occurred in Sendai in the fall of 1962.

The results described in the present paper suggest that the incidence of aseptic meningitis from August to October 1963 in Aomori was an outbreak, though not on a large scale, due to echovirus 14, because frequent isolation of echovirus 14 from the patients was observed in a limited area and over a short period of time. This is also supported by the fact that echovirus 14 was isolated from the CSF of one patient and by the significant rise of antibody titre in many patients. An epidemic of gastro-enteritis caused by echovirus 14 has been reported by Lépine *et al.* (1960). It was also confirmed that the virus, spread widely in a population, did not cause any detectable illness among infants or children, as reported in the paper by Ingram *et al.* (1962) and in the present paper.

Since 1961, viruses causing aseptic meningitis occurring in Aomori have been studied in this laboratory. A large epidemic associated with Coxsackie B5 virus, and to a lesser extent with Coxsackie A9 virus was observed in 1961, as reported previously by Hinuma *et al.* (1964). In 1962, a prevalent virus was Coxsackie A9 virus, and the incidence pattern of the illness suggested that it was a small outbreak due to the A9 virus (unpublished data). In 1963, as noted in this paper, Coxsackie B2 virus was the principal strain isolated from January to June but echovirus 14 from August to October. Although the study is still in progress, echovirus 4 has been isolated from a fairly large number of patients in the summer of 1964 (unpublished data). Thus, the dominant virus associated with aseptic meningitis in Aomori has apparently changed year by year or even season by season within a year.

Another effect of echovirus 14 infection described in this paper is a possible interference with a vaccine strain of poliovirus 1. Several investigators have reported that naturally occurring infections with enteroviruses may interfere with the intestinal infection with attenuated polioviruses, as cited in the paper by Ingram *et al.* (1962). In contrast to our observations, Ingram *et al.* (1962) reported that echovirus 14, which was prevalent in an infants' institution in Cleveland, U.S.A., appeared to have little suppressive effect upon an attenuated strain of poliovirus 1. Possible interference by the echovirus 14 was suggested in only two

of twenty-five vaccinated babies. In our study poliovirus type 1 was recovered from only two of nine babies who were fed 10^5 or 10^6 TCD 50 of type 1 strain of Sabin's poliovaccine, but echovirus 14 was isolated from six babies, 10 days after feeding. Serological examination gave evidence that only three out of nine vaccinated babies showed a rise of poliovirus 1 antibody whereas a significant rise of echovirus 14 antibody was observed in eight babies 7 weeks after the vaccination. This serological evaluation of effectiveness of the vaccine indicates that the vaccination in the infants' institution reported here was less effective than that in the National Hospital of Sendai as shown in Table 2. This failure of vaccination might be caused by the interference with echovirus 14 infection which was evident by virus isolation and serological examination.

In the studies by Ingram *et al.* (1962), antibody response to echovirus 14 was observed in only 12% of the infected infants. In this report, all the serum-negative infants clearly showed the echovirus 14-antibody response. As discussed by Ingram *et al.* (1962) the difference between these two observations may be explained by a variation in antigenicity among echovirus 14 strains distributed in many parts of the world.

SUMMARY

Two events concerned with echovirus 14 infection were reported.

The first, which occurred in Sendai in 1962, was that the intestinal infection with the attenuated poliovirus 1 vaccine was possibly suppressed by naturally occurring infection with echovirus 14. Echovirus 14 was isolated 10 days after the vaccine feeding in six out of nine cases, but poliovirus in only two cases. Six of the vaccinated babies did not exhibit antibody response to the poliovirus 1, while a significant rise of antibody titre against echovirus 14 was seen in as many as eight babies 7 weeks after the vaccination.

The second was a small outbreak of aseptic meningitis due to echovirus 14 in Aomori in 1963. During August to October, echovirus 14 was isolated from the faeces of seven patients with aseptic meningitis. The virus was also isolated from the CSF in one case. The antibody titre against echovirus 14 was detected in all the patients from whom the virus was isolated. In addition, echovirus 14 antibody was detected in four out of fourteen patients with aseptic meningitis from whom no virus was recovered. The onset of serologically positive patients was limited to the months of August to October.

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