

**Laboratory and Epidemiology Communications**

**Duration of Echovirus 30 Excretion in Stools from Patients with Aseptic Meningitis**

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Aseptic meningitis (AM) is chiefly a childhood disease caused by viruses. Most serotypes of enteroviruses have been isolated from patients with AM (1). Echovirus 30 (E30), which was first isolated in New York State from a patient with AM in 1958 (2), was the most predominant serotype (3). Fecal-oral contamination is well-known as the most important route by which many enterovirus infections are spread (4). This route of transmission is especially common in families with young children or in nursery school. Therefore, it is important to understand the duration of virus excretion in order to prevent the secondary transmission of viruses. We describe here the duration of E30 excretion in the stools of AM patients.

From May to September in 1998, an outbreak of AM occurred in Shoubara City, the northern area of Hiroshima Prefecture. E30 was isolated from the stool and/or cerebrospinal fluid of a total of 162 patients with AM. Most (96.9%; 157/162) patients ranged from 3 months to 14 years of age, with the highest incidence in 5-year-old children. After the onset of illness, 230 stools were collected from the patients several

times in and out of the hospital, and we investigated the duration of virus excretion. Most (82.2%; 189/230) of the stool samples showed E30 for up to 10 days (Table). In one case, E30 was recovered from the stool continuously for 23 days (Figure). These results suggested that a patient with AM due to E30 infection may excrete the virus over days or even few weeks, after recovery from the illness.

An enterovirus infection such as E30 is highly communicable within a community. The viruses generally spread horizontally through preschool children and thus to their families. Therefore, good hygienic practices such as handwashing are necessary for several weeks to prevent secondary transmission of the virus, especially in homes and nursery schools.

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Table. Duration of E30 excretion in stools from patients with AM

Number of patients	Number of stools	E30 isolation	Days after onset of illness																							
			0	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23
162	230	Positive	3	39	38	16	13	14	4	8	9	23	22	8	3	6	2	1	2		1	1				1
		Negative		1						1			5	1	2	2	2	1						1		

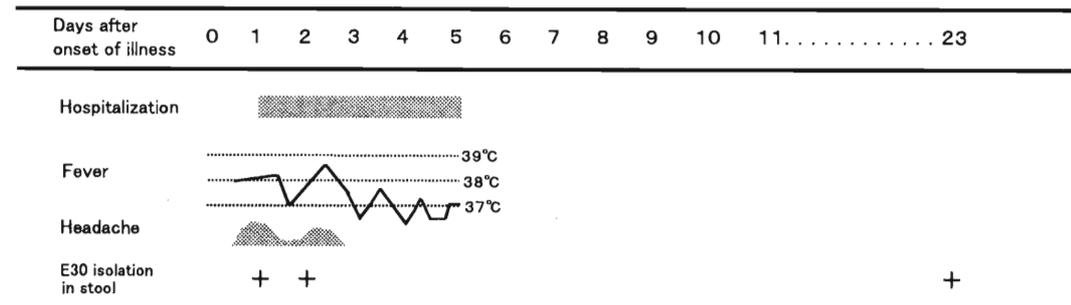


Figure. Clinical and virological observation on one AM patient with E30 (8-year-old, female).

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### Possible Enhancement of GB Virus C Infection in Cultured Cells by Addition of Anti-E1 Antibodies

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Recently, a novel member of the virus family *Flaviviridae* was identified as a causative agent for human non-A-E hepatitis, called GB virus C (GBV-C) or hepatitis G virus (HGV). GBV-C is distantly related to hepatitis C virus (HCV). HCV virions are bound to anti-HCV antibodies as immune complexes in the circulation of chronically infected hosts, and this phenomenon is related to the antibody-escape mutations in the hypervariable region of E2 protein. However, GBV-C has no hypervariable region within envelope proteins. Therefore, the mechanism for GBV-C to establish persistent infection may be different from that for HCV.

We obtained serial sera from a Japanese patient with acute lymphatic leukemia who had developed hepatitis after repeated blood transfusions. The patient was found to be infected with GBV-C. Immunoprecipitation of the sera with anti-human IgG followed by detection of GBV-C RNA by RT-PCR (1) revealed that, in the first sample, GBV-C RNA was present only in the supernatant, while from the second sample on, GBV-C RNA was present in the pellet as well; i.e., from the second point on, the antibody was present. In spite of the presence of the antibody, however, GBV-C RNA continued to increase; i.e., the antibody did not suppress GBV-C replication (Figure). This observation led us to the hypothesis that the antibody was enhancing even the infection, as reported for other flaviviruses (2).

In order to test this possibility, we examined the effect of E1 antibody on GBV-C infection in cultured cells. A rabbit was injected intradermally twice (at a 1-month interval) with 500 µg keyhole limpet hemocyanin-conjugated synthetic

E1 peptide (amino acid number 61-80) in Freund's adjuvant, and a serum sample was obtained 6 weeks after the last injection. Enzyme-linked immunosorbent assay (ELISA) revealed that the immune serum was reactive with the homologous peptide. Double immunoprecipitation using GBV-C and HCV positive plasma #53 (3) revealed that the majority of GBV-C virions were brought by the immune serum to the pellet.

The immune and control pre-immune sera were heated at 56°C for 30 min and diluted 10-fold. One hundred microliters of diluted sera were mixed with an equal volume of 1:100 dilution of #53 serum and incubated at 4°C overnight. They were inoculated into 1 ml of H-903 cell (3) suspension (5 × 10<sup>5</sup>/cells) and incubated for 2 h at 37°C. After being washed once, the cells were cultured further in the continuous presence of immune or pre-immune serum at a final concentration of 10<sup>-2</sup>. Presence of the viral sequence in the cells and in the supernatants was monitored for 32 days by the RT-PCR method as described in a previous report (3). As shown in the

Table. Detection of GBV-C RNA by RT-PCR in cell culture

Days PI	Pre-immune serum		Immune serum	
	Cell	Sup	Cell	Sup
3	+	+	+	+
7	+	+	+	+
13	+	+	+	+
17	+	-	+	+
22	-	-	+	+
27	+	-	-	+
32	-	-	+	-

PI: post inoculation, Sup: supernatant

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