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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 (5×10^5/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^{-2}. 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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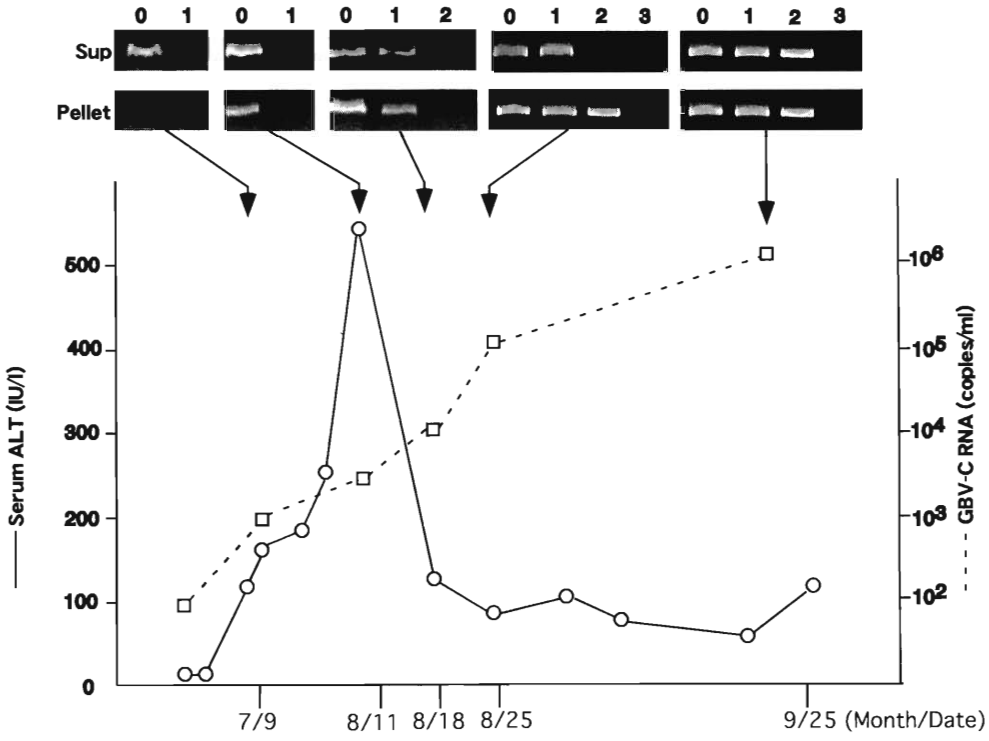

Figure. Agarose gel electrophoresis of PCR products after immunoprecipitation with anti-human IgG. Nucleic acids extracted from supernatants or pellets were diluted serially 10-fold and subjected to RT-PCR (0, undiluted; 1, 10-fold diluted; 2, 10^2-fold diluted; 3, 10^3-diluted). Arrows indicate serum-sampling points. Sup: supernatant, ALT: alanine aminotransferase.

Table, both immune and pre-immune sera did not inhibit detection of GBV-C RNA during culture. Rather, viral RNA tended to persist longer in the culture treated with immune serum (27 days vs. 13 days in the supernatant).

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