GB virus C/hepatitis G virus replicates in human haematopoietic cells and vascular endothelial cells

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A novel flavivirus, GB virus C (GBV-C)/hepatitis G virus (HGV), has been detected in chronic liver disease patients. It is known that the viral RNA can be detected in ~5% of American blood donors. However, the implications for liver disease and the sites of virus replication remain unknown. Possible sites of virus replication were studied by using cell lines and/or primary cells derived from human lymphoid cells, myeloid cells, hepatocytes and endothelial cells. RNA was detected by virus strand-specific RT–PCR and GBV-C/HGV antigen was detected with a rabbit polyclonal anti-E2 (envelope 2) antibody by Western blot analysis. Negative-strand RNA, representative of replicating virus, was detected in lymphoid and megakaryocytoid cell lines and primary vascular endothelial cells. In addition, an increase in virus titre over time was demonstrated and viral antigen was detected, and virus could be passaged to infect fresh cells. However, viral RNA or antigen could not be detected in any of the hepatocyte lines tested. These results indicate that the replication site of GBV-C/HGV is not primarily in hepatocytes and that detection of replicating virus in hepatic tissue may reflect virus replication in haematopoietic cells and/or vascular endothelial cells present in the liver.

Introduction

GB virus C (GBV-C) and hepatitis G virus (HGV) were recently identified and proposed as possible aetiological agents of non-A, non-B, non-C hepatitis (Simons et al., 1995; Linnen et al., 1996). The two viruses share 85% nucleotide and 95% amino acid sequence identity and are considered to be different isolates of the same virus (Leary et al., 1996). GBV-C/HGV is a positive-stranded RNA virus, closely related to hepatitis C virus (HCV), and a member of the Flaviviridae. Viraemia can be detected in ~5% of American blood donors (Gutierrez et al., 1997) and, like HCV, the virus was thought originally to be transmitted primarily by parenteral routes (Khudyakov et al., 1995; Seipp et al., 1996). However, we have previously shown that viraemia is detectable in 6–7% of non-transfused American children (Handa et al., 2000) and other studies have been unable to confirm an increased risk with parenteral exposure (Cantaloube et al., 1999), suggesting that non-parenteral transmission is important (Feucht et al., 1999; Menendez et al., 1999).

The role of GBV-C/HGV as a hepatitis virus also remains controversial (Mushahwar & Zuckerman, 1998): the evidence for a role in seronegative hepatitis has been reported as either negligible or weak (Wang et al., 1998) and, although several initial reports suggested an association between GBV-C/HGV viraemia and fulminant hepatitis (Yoshiba et al., 1995; Heringlake et al., 1996), others have not confirmed this (Tameda et al., 1996; Kanda et al., 1997). Similarly, although some groups have claimed that this virus is hepatotropic and have shown its replication in human liver (Seipp et al., 1999; Madejon et al., 1997), there are several reports suggesting that human liver is not a primary site of replication (Pessoa et al., 1998; Laras et al., 1999).

Members of the family Flaviviridae, which includes yellow fever virus, HCV and dengue viruses, utilize the synthesis of replicative RNA, a negative-strand RNA complementary to the genomic RNA, as a template for virus replication (Laskus et al., 1997; Lindenbach & Rice, 1997). We therefore developed a strand-specific RT–PCR assay that could distinguish positive- and negative-strand RNA and used this to look for GBV-C/HGV replicative RNA in a variety of different cell lines inoculated with virus. In addition, we looked for evidence of viral antigen production by using a rabbit polyclonal antibody against a viral envelope protein.
Methods

**Generation of synthetic GBV-C RNA templates.** GBV-C/HGV RNA was extracted from viraemic plasma and cDNA was prepared as described previously (Brown et al., 1997). Full-length E2 was amplified by PCR and cloned into pcDNAFlag, a derivative of the pcDNA3 vector (Invitrogen) modified to include a Flag tag (Take et al., 2000). Positive strand amplification was performed with primers GBO and GBOR from the conserved 5' non-coding region of the GBV-C genome (Ikeda et al., 1997). The product was cloned directly into the TA-cloning vector pCR2.1-TOPO (TOPO TA cloning kit, Invitrogen), which has a T7 promoter sequence upstream of the cloning site. Orientation of the GBV-C/HGV sequence was confirmed by sequencing and plasmids capable of producing either sense or antisense transcripts were selected. The GBV-C/HGV DNA templates were linearized with HindIII and were transcribed with T7 RNA polymerase (Life Technologies) for 30 min at 37 °C, producing positive and negative GBV-C/HGV RNA strands.

**Strand-specific RT-PCR.** Total RNA was extracted from CESS cells by using STAT-60 total RNA/mRNA isolation reagent (Tel-Test Inc., Friendswood, TX, USA) with 10 μl tRNA (10 mg/ml) as a carrier. Serial dilutions of RNA templates were prepared in a solution of cellular RNA and preheated to 70 °C for 10 min in order to reduce RNA secondary structure and to maximize the stringency of the cDNA synthesis. cDNA was synthesized by using 50 pmol of the strand-specific primers GBO (for detection of negative strand; Ikeda et al., 1997) or GBOR (positive strand) in a 25 μl RT mixture following the RNA PCR Core kit protocol (Perkin-Elmer). Reverse transcriptase was inactivated by heating the samples for 5 min at 99 °C prior to nested PCR. Amplification was performed (2-5 μl template) for 30 cycles (94 °C for 1 min, 55 °C for 1 min and 72 °C for 2 min) followed by a final extension step at 72 °C for 7 min with primers GBO and GBOR, followed by second-round amplification under the same conditions (5 μl of the PCR product) with specific internal primers GB and GBIR (Ikeda et al., 1997). The final amplicons (207 bp) were analysed by agarose gel electrophoresis.

**Inoculation of cells with GBV-C/HGV.** The same serum used as a source of GBV-C/HGV RNA was used as the source of infectious virus. Cells (10^6 cells/ml) were aliquotted into 24-well tissue culture plates and incubated with the GBV-C/HGV-positive serum (10 μl, equivalent to 10^6 genome copies) at 37 °C in a humidified atmosphere with 5% CO_2_ (day 0). The cells were maintained in culture for up to 2 months with a half-volume change of medium every 3 days. Cell numbers were estimated and harvested cells and supernatant were stored for the extraction of RNA, Western blotting and immunofluorescence assays.

Total RNA was extracted from cells (2 × 10^5) by using STAT-60 total RNA/mRNA isolation reagent (Tel-Test) with 10 μl tRNA (10 mg/ml) as a carrier. Positive- and negative-strand GBV-C/HGV RNA was amplified by using the strand-specific RT–PCR described above. Titres of positive- and negative-strand GBV-C/HGV RNA were calculated by performing strand-specific PCR on serial dilutions of samples that tested positive. In addition, all the PCR products were confirmed by TA cloning (TOPO TA cloning, Invitrogen) and sequencing of the products. To confirm the extraction of RNA, RT–PCR for β-actin was performed as described previously (Hanazono et al., 1999) and PCR was performed in the absence of reverse transcription to check for DNA contamination.

**Expression of E2 protein and production of rabbit polyclonal antibodies.** The complete GBV-C/HGV E2 envelope coding region was cloned into a mammalian expression vector (Fig. 1), expressed in COS-7 cells as a Flag-fusion protein (Handa et al., 2000) and purified by using an anti-Flag affinity gel (Eastman Kodak). The purified E2 fusion protein (molecular mass 47 kDa; 300 μg) was inoculated into a rabbit. The rabbit immune response was boosted five times (days 14, 28, 42, 56 and 70) with further E2 fusion protein inoculations. The rabbit was exsanguinated at day 85 and the serum was used as polyclonal antibody to E2 fusion protein. Anti-E2 reactivity was confirmed by testing the serum in a commercial anti-E2 antibody detection kit (μPLATE Anti-
HGVenv, Boehringer Mannheim) with HRP-conjugated goat anti-rabbit IgG (Biosource International) instead of the attached anti-human-Fc antibody.

- **Western blotting of cell cultures for E2 protein.** Every 3 days, half of the cells were harvested and 2 × 10⁸ cells were denatured in SDS lysis buffer and the proteins were separated by SDS–PAGE and transferred to nitrocellulose membranes. Non-specific binding was blocked by incubation in Blotto (5% milk powder in PBS with 0.05% Tween 20) for 2 h at room temperature prior to incubation with rabbit polyclonal antibody (diluted 1:100 in PBS plus 0.05% Tween 20, 1% milk powder) for 2 h. The membranes were washed and incubated with HRP-conjugated anti-rabbit IgG (diluted 1:10000; Biosource International) for 2 h at room temperature and bands were detected by chemiluminescence (SuperSignal Substrate, Western blotting, Pierce). The specificity of the bands was confirmed by testing with rabbit serum obtained prior to E2 inoculation and its size was calculated from standard mass markers on the Western blot.

- **Indirect fluorescence assay of cell cultures for E2 protein.** Cells (5 × 10⁶) were cytocentrifuged (Shandon; 800 r.p.m. for 2 min) onto glass slides and fixed in acetone–methanol (1:1) for 10 min at 4°C. After air drying, the slides were incubated with rabbit polyclonal antibody (1:100) for 1 h at 37°C, washed twice with PBS and incubated with FITC-conjugated anti-rabbit IgG (diluted 1:100; Biosource International) for 1 h at 37°C. After a further wash, FITC-positive cells were detected by fluorescence microscopy.

**Results**

**Development of a strand-specific RT–PCR assay**

The specificity and sensitivity of the strand-specific RT–PCR assay were determined by testing serial dilutions of positive- and negative-strand templates in a fixed amount of cellular RNA. Both assays had similar sensitivities, with a limit of detection at < 10⁴ copies per PCR (Fig. 2). In addition, the assays were strand specific, with 10⁸ copies of the negative- or positive-strand template RNA not amplified without the corresponding primers (Fig. 2).

**Detection of negative-strand RNA and E2 antigen in haematopoietic cells**

When the plasma from a GBV-C/HGV-viraemic patient used for inoculation was tested in the strand-specific RT–PCR assay, only positive-strand GBV-C RNA could be detected (Fig. 3), with a titre of 10⁶ copies/ml. After 24 h, positive-strand RNA only was demonstrated in CESS cells, primary lymphocytes, HL-60, U937, HEL and Meg-01 cultures, presumably from the original inoculum (Table 1). From day 3 post-infection, both positive- and negative-strand GBV-C/HGV RNA were detected in some cell cultures, in some cases for up to 30 days (lymphoid cells, CESS and megakaryocytoid cells, Meg-01; Table 1, Fig. 3). The virus titres in harvested cells and supernatant were determined by semi-quantitative RT–PCR and the increase in virus titre was calculated. In both the CESS and Meg-01 cell lines, there was a 1 log increase in the virus titre in the supernatant and > 2 log increase in the cell pellet compared with input virus (10⁶ genome copies inoculated) by day 3 (Table 2), not accounting for the dilution resulting from subculturing the cells twice weekly. In addition, > 10⁶ copies of negative-strand RNA were detected in the cultures.

In contrast, negative-strand RNA was not detected in the other lymphoid cell lines tested (Jurkat, C5/MJ and ARH-77) and the myeloid cell lines were all uniformly negative (HL-60, K562, U937, KG1a, HEL and UT-7/Epo). After 40 days of culture, no viral RNA could be detected in any of the cultures, despite over 90% cell viability as estimated by trypan blue dye exclusion.

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![Fig. 2](image_url)

**Fig. 2.** Sensitivity and specificity of strand-specific RT–PCR. Synthetic negative- and positive-strand RNA templates were produced by T7 RNA polymerase and serial dilutions were prepared in total cellular RNA (from 2 × 10⁸ CESS cells) and tested by strand-specific RT–PCR assay. After electrophoresis, the bands were visualized by ethidium bromide staining. Specificity was confirmed by testing with or without the reverse transcription step. (a) Positive-strand-specific PCR; (b) negative-strand-specific PCR. Controls were water (negative) and DNA template (positive).
Expression of GBV-C/HGV RNA (positive and negative strands) and virus antigen in haematopoietic cells

Cells were infected with GBV-C/HGV-containing serum (10^6 genome copies; day 0) and harvested at different time-points post-inoculation and strand-specific RT–PCR and Western blotting for E2 antigen were performed. Results are given as (1)/(2)/(3): (1) positive-strand RNA, (2) negative-strand RNA, (3) E2 antigen detected by Western blot. Detection is scored as: +, > 10^4 copies per reaction or detection of E2 antigen; –, < 10^4 copies per reaction or E2 antigen not detected. NT, Not tested.

<table>
<thead>
<tr>
<th>Cell line</th>
<th>Time post-inoculation (days)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>1</td>
</tr>
<tr>
<td><strong>Lymphoid</strong></td>
<td></td>
</tr>
<tr>
<td>CESS</td>
<td>+/−/−</td>
</tr>
<tr>
<td>Jurkat</td>
<td>−/−/−</td>
</tr>
<tr>
<td>C5/MJ</td>
<td>−/−/−</td>
</tr>
<tr>
<td>ARH-77</td>
<td>−/−/−</td>
</tr>
<tr>
<td>Primary lymphocytes</td>
<td>+/−/−</td>
</tr>
<tr>
<td><strong>Myeloid</strong></td>
<td></td>
</tr>
<tr>
<td>HL-60</td>
<td>+/−/−</td>
</tr>
<tr>
<td>K562</td>
<td>−/−/−</td>
</tr>
<tr>
<td>U937</td>
<td>+/−/−</td>
</tr>
<tr>
<td>KG1a</td>
<td>−/−/−</td>
</tr>
<tr>
<td>HEL</td>
<td>+/−/−</td>
</tr>
<tr>
<td>Meg-01</td>
<td>+/−/−</td>
</tr>
<tr>
<td>UT-7/Epo</td>
<td>−/−/−</td>
</tr>
</tbody>
</table>

All of the cell cultures were also tested for the presence of GBV-C/HGV antigen. No E2 protein was detected on day 0, but E2 protein could be detected thereafter in all the cell cultures that had detectable negative strand RNA (Table 1, Fig. 4), but not in cell cultures that were only positive for positive-strand RNA. These data supported the observation that detection of positive strand represented inoculated virus only.

Cell cultures that were positive for E2 antigen by Western blot (CESS, Meg-01 and primary vascular endothelial cells) were also tested by indirect immunofluorescence. In Meg-01
Table 2. Semiquantitative strand-specific RT–PCR for GBV-C/HGV RNA

A semiquantitative strand-specific RT–PCR was used to detect GBV-C/HGV RNA in supernatant or cells of GBV-C/HGV-sensitive cells (CESS, Meg-01 and primary endothelial cells). Serum (10 µl, 10⁶ genome copies) was inoculated into cell cultures (day 0). Cells and supernatant were harvested every 3 days, cell numbers were estimated and RT–PCR was performed. Titres indicate the number of RNA copies/ml culture. Results are shown as (1)/(2): (1) positive-strand RNA, (2) negative-strand RNA. –, < 10⁶ copies per ml culture. NT, Not tested.

<table>
<thead>
<tr>
<th>Cell line</th>
<th>Time post-inoculation (days) (dilution since inoculation)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>1 (× 1)</td>
</tr>
<tr>
<td>Supernatant</td>
<td></td>
</tr>
<tr>
<td>Primary endothelial cells</td>
<td>– / –</td>
</tr>
<tr>
<td>Cells</td>
<td></td>
</tr>
<tr>
<td>CESS</td>
<td>10⁶ / –</td>
</tr>
<tr>
<td>Meg-01</td>
<td>10⁶ / –</td>
</tr>
<tr>
<td>Primary endothelial cells</td>
<td>10⁶ / –</td>
</tr>
</tbody>
</table>

cells at 3 days post-infection, specific immunofluorescence could be detected, restricted to the cytoplasm (Fig. 5). Viral antigens could not be detected in the other cells by indirect immunofluorescence.

**Passage of GBV-C/HGV infectivity to fresh cells**

In order to confirm the production of infectious virus in CESS and Meg-01 cells, the day 9 supernatant was collected, the GBV-C/HGV titre was calculated and 100 µl was re-inoculated into fresh cells. For both cell lines, the day 9 supernatant contained 10⁸ GBV-C/HGV copies/ml; negative strand could not be detected (< 10⁵ copies/ml). Total samples (cells and supernatant together) were harvested at day 3 and semiquantitative strand-specific RT–PCR was performed. In both cell lines, there was a 3 log increase in positive-strand titre compared with input virus (10⁸ copies per culture compared...
with $10^5$ input virus) and $10^6$ copies of negative-strand GBV-C/HGV RNA were detected.

**Detection of negative-strand RNA and E2 antigen in hepatocytes and endothelial cells**

Primary hepatocyte cultures and cell lines were also tested for evidence of GBV-C replication by strand-specific RT–PCR. As with some of the haematopoietic cell lines, positive-strand RNA could be detected in primary hepatocytes, PLC/PRF/5 and primary vascular endothelial cells after 24 h inoculation (Table 3). However, we were unable to detect negative-strand RNA in any of the hepatocyte cultures or cell lines. In contrast, we could detect both positive- and negative-strand RNA in primary vascular endothelial cells for 30 days post-inoculation (Table 3). The virus titre in primary vascular endothelial cells was determined by semiquantitative RT–PCR (Table 2). Compared with the input virus ($10^6$ genome copies), $10^5$ copies of the positive strand were detected in the supernatant by day 3 and $10^9$ copies of positive strand and $10^6$ copies of negative strand were detected in the cell pellets. In addition, E2 viral antigen could also be directed over this time-period (Fig. 4). As with the haematopoietic cultures, no negative-strand RNA or E2 protein could be detected after 40 days of culture, despite the presence of viable cells (Table 3).

**Discussion**

Despite the identification of GBV-C/HGV in 1995, there is no efficient way of growing the virus in culture and little is known about its pathogenesis, including its site of replication in the body. However, viraemia is not uncommon among healthy adults (Gutierrez et al., 1997) and even in children (Handa et al., 2000) and we hypothesized that haematopoietic blood cells or vascular endothelial cells were good candidates for virus replication sites. To identify the replication site of GBV-C/HGV, we have developed assays to detect negative-strand or replicative RNA transcripts in cells and expressed E2 protein as a fusion protein in order to produce a polyclonal rabbit antiserum capable of detecting viral antigens.

Using these reagents and assays, we were able to detect negative-strand RNA, indicative of replicative RNA, for up to 30 days in some tissue cultures (Meg-01, CESS and primary vascular endothelial cells). In addition, we could demonstrate a significant increase in positive-strand RNA titre in these cell lines and virus could be passaged into fresh cells. We could also detect viral antigens in the cells by Western blotting, although, perhaps due to the relative lack of sensitivity, we could only detect antigens by immunofluorescence in Meg-01 cells. Together, these results indicate that active virus replication was occurring in these cell lines.

GBV-C/HGV is known to be transmitted readily by blood transfusion (Tsuda et al., 1996; Wang et al., 1996) and viral RNA has been detected in peripheral blood mononuclear cells (Sheng et al., 1997; Fogeda et al., 1999). In addition, several reports have detected GBV-C/HGV negative-strand RNA associated with peripheral blood mononuclear cells, suggesting that haematopoietic cells may be one of the sites of GBV-C replication (Laskus et al., 1997; Fogeda et al., 1999; Cabrero et al., 1999). However, other studies have not been able to confirm these findings (Mellor et al., 1998; Laskus et al., 1998; Kao et al., 1999), although these studies concluded that the possibility of very low levels of GBV-C replication in mononuclear cells could not be excluded. In our study, we could detect both negative-strand RNA and E2 antigen in one T cell line (CESS) for 30 days after virus inoculation, although we could detect only the positive-strand RNA from primary lymphocytes, perhaps suggesting that GBV-C infects only a sub-population of lymphocytes.

Some of the discrepancy between the different studies may be due to differences in the inoculum used as a source of virus. Patients with GBV-C/HGV are often also infected with HCV (Sheng et al., 1997; Schleicher et al., 1996) and, in several studies, serum that was co-infected with HCV has been used as
different time-points post-inoculation. Strand-specific RT–PCR and Western blotting for E2 antigen were unsuccessful (Fogeda et al., 1999). Controversial, with some groups detecting strand-specific RNA or liver samples. As with the lymphocyte data, the results are inconsistent: studies have looked for negative-strand RNA in hepatocytes and endothelial cells (human T-cell line) and PH5CH (hepatocyte cell line) cells were used to study GBV-C/HGV (Ikeda et al., 1997; Shimizu et al., 1999).

GBV-C/HGV was originally detected in patients with hepatitis (Simons et al., 1995; Linnen et al., 1996), and several studies have looked for negative-strand RNA in hepatocytes or liver samples. As with the lymphocyte data, the results are controversial, with some groups detecting strand-specific RNA (Seipp et al., 1999; Madejon et al., 1997) and other groups being unsuccessful (Fogeda et al., 1999; Cabrero et al., 1999). However, liver tissue contains not only hepatocytes, but also connective tissue and blood, and detection of negative-strand RNA in liver tissue may represent virus in lymphocytes or other cell types, including vascular endothelial cells.

In our study of both primary hepatocytes and hepatocyte cell lines, we were unable to detect either GBV-C antigen or negative-strand RNA. In a similar study to ours, but using different cell lines, Seipp et al. (1999) inoculated hepatocyte cell lines with a GBV-C/HGV-positive, HCV-negative serum and looked for the presence of negative-strand RNA. They were able to detect positive-strand RNA in 40% of hepatocytes but negative-strand RNA in only 1/500 hepatocytes by fluorescent in situ hybridization analysis. This difference in results may be due to differences in the sensitivity of the assays; in their study, the assay had a 10-fold higher sensitivity, detecting 10^5 copies of positive strand and 10^4 copies of negative strand per PCR. Although they detected negative-strand RNA in four liver samples, three liver samples were positive only at the limit of sensitivity (10^3 copies). Therefore, even these results suggest that the negative-strand titre was at the limit of detection in hepatocytes and that liver tissue was not an efficient site of GBV-C/HGV replication.

We were also able to detect negative-strand RNA in primary vascular endothelial cells and, although some reports have suggested an association of GBV-C/HGV with vasculitis (Tepper et al., 1998; Francesconi et al., 1997), the ability of GBV-C/HGV to infect endothelial cells has not been reported. However, it was recently suggested that members of the Flaviviridae, including GBV-C/HGV, use the low-density lipoprotein receptor (LDL-R) as a receptor for virus entry (Agnello et al., 1999), and LDL-R is known to be present on vascular endothelial cells (Sawamura et al., 1997).

The ability of GBV-C to infect megakaryoblasts was unexpected. Although the presence of LDL-R on a rat megakaryoblast cell line has been described in one report (Budd et al., 1991), we have been unable to find any studies of the expression of LDL-R on human megakaryoblasts. HCV infection can be associated with thrombocytopenia (Hernandez et al., 1997; Emilia et al., 1997); however, no reports have indicated an association of GBV-C with low platelet counts. Thus, the significance of this observation is unknown and further studies are necessary to determine both the relationship between GBV-C and megakaryoblast infection and the true cellular tropism of GBV-C. However, our studies indicate that the virus can replicate in lymphocytes, megakaryocytes and vascular endothelial cells.

Table 3. Expression of GBV-C/HGV RNA (positive and negative strands) and virus antigen in hepatocytes and endothelial cells

<table>
<thead>
<tr>
<th>Cell line</th>
<th>1</th>
<th>3</th>
<th>9</th>
<th>21</th>
<th>30</th>
<th>60</th>
</tr>
</thead>
<tbody>
<tr>
<td>Primary hepatocytes</td>
<td>+/−/−</td>
<td>+/−/−</td>
<td>−/−/−</td>
<td>−/−/−</td>
<td>−/−/−</td>
<td>NT</td>
</tr>
<tr>
<td>Chang liver cells</td>
<td>−/−/−</td>
<td>−/−/−</td>
<td>−/−/−</td>
<td>−/−/−</td>
<td>−/−/−</td>
<td>NT</td>
</tr>
<tr>
<td>SK-HEP</td>
<td>−/−/−</td>
<td>−/−/−</td>
<td>−/−/−</td>
<td>−/−/−</td>
<td>−/−/−</td>
<td>NT</td>
</tr>
<tr>
<td>PLC/PRF</td>
<td>+/−/−</td>
<td>+/−/−</td>
<td>−/−/−</td>
<td>−/−/−</td>
<td>−/−/−</td>
<td>NT</td>
</tr>
<tr>
<td>Primary endothelial cells</td>
<td>+/−/−</td>
<td>+/−/−</td>
<td>NT</td>
<td>+/−/−</td>
<td>+/−/−</td>
<td>−/−/−</td>
</tr>
</tbody>
</table>

A source of GBV-C/HGV. Alternatively, the cell lines chosen for study may have been co-infected with HCV: MT-2C (human T-cell line), interferon-resistant Daudi cells (Burkitt’s lymphoma cell line) and PH5CH (hepatocyte cell line) cells were used to study GBV-C/HGV (Ikeda et al., 1997; Shimizu et al., 1999).


References


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