Relation between GB virus C/hepatitis G virus and fulminant hepatic failure may be secondary to treatment with contaminated blood and/or blood products

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Abstract

Background—The role of the recently discovered GB virus C (GBV-C)/hepatitis G virus in fulminant hepatic failure (FHF) has been debated. Although GBV-C RNA has been detected in many cases of FHF, recent data suggest that the relation between GBV-C and FHF may be accidental.

Aims—To retrospectively investigate the possible relation between the presence of GBV-C markers (RNA or antibodies to the GBV-C envelope 2 (E2) glycoprotein) and FHF.

Methods—The presence of GBV-C RNA was determined in serum samples from 58 patients diagnosed with FHF using a reverse transcriptase polymerase chain reaction. Amplified genetic fragments were directly sequenced by the dideoxy chain termination method. Antibodies to GBV-C in serum samples were detected by enzyme immunoassay based on a recombinant GBV-C E2 protein.

Results—Nine (16%) patients with FHF had GBV-C RNA and 14 (24%) had GBV-C E2 antibodies, which are higher frequencies than in healthy subjects ($p < 0.01$ and $p < 0.05$ respectively). Seven of ten patients with GBV-C markers during FHF tested negative for these markers before therapy with blood and/or blood products. Sequence analysis of the GBV-C NS3 region fragments of six FHF patients showed no common sequence pattern or motif.

Conclusions—The frequencies of both GBV-C RNA and antibodies are higher in patients with FHF than in healthy subjects. However, these increased frequencies may in many cases be explained by the use of contaminated blood and/or blood products given as therapy.

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Keywords: fulminant hepatitis; GB virus C; hepatitis G virus; RNA; antibodies; liver

The newly discovered parenterally transmitted GB virus C (GBV-C)¹ or hepatitis G virus² has been implicated in cases of fulminant hepatic failure (FHF) of unknown origin.³⁻⁷ For simplicity we will refer to this virus as GBV-C. The possible association between FHF and GBV-C is surprising, as infections with GBV-C seem to occur at a high frequency in both the

healthy blood donor/normal populations and in groups with an increased risk of blood borne viral infections.^{1,2,8} However, recent data suggest that the relation between FHF and the presence of GBV-C RNA may be accidental.⁹⁻¹¹ Apart from the documented detection of GBV-C RNA in serum, saliva, and liver, its tissue distribution in the infected host is largely unknown.^{1,2,12-14} Whether GBV-C actually replicates in the liver has been debated.¹⁵ Chronic GBV-C infections did not seem to occur through social contacts despite the presence of GBV-C RNA in the serum and saliva of two members within a household.¹⁴ Therefore the transmission routes of GBV-C are not completely known.

Today, no definite evidence for the involvement of GBV-C in liver disease has been documented. GBV-C co-infections do not in most cases seem to enhance the pathology of chronic hepatitis C virus (HCV) infection as determined by either liver enzymes or histological examination.¹⁶ It is still unclear which types of disease may be caused by infection with GBV-C. However, recent reports have suggested that particularly aggressive variants of GBV-C may be involved in FHF.^{7,17}

In this study our aim was to characterise further the possible relations between infection with GBV-C and FHF.

Materials and methods

PATIENTS WITH FHF

Serum samples from a total of 58 patients with acute or subacute FHF who were admitted to Huddinge University Hospital during the period 1986 to 1995 were analysed retrospectively (mean (SD) age 36 (17) years; range 2-67 years). Of these, one was caused by hepatitis A virus, four by hepatitis B virus (HBV), and one had a co-infection with HBV, HCV and hepatitis D virus. In 14 patients the FHF was caused by drug intoxication (mainly paracetamol), in five by Budd-Chiari syndrome, in four by veno-occlusive disease after bone marrow transplantation, in one by Wilson's disease, and in one by mushroom intoxication (the latter four groups consisting of 11 patients were collectively termed "miscellaneous"). No cause for the FHF could be determined in the

Abbreviations used in this paper: FHF, fulminant hepatic failure; GBV-C, GB virus C; HCV, hepatitis C virus; HBV, hepatitis B virus; NS3, non-structural 3; ALT, alanine aminotransferase.

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remaining 27. In all 58 cases, samples were taken after admission to the hospital after initiation of treatment often consisting of administration of blood and/or blood products. However, for 10 of the 58 patients that had GBV-C RNA or antibodies during the illness, additional samples were identified which had been taken before the start of any treatment with blood and/or blood products.

As a control material for the sequence analysis, biochemical data and GBV-C non-structural 3 (NS3) region sequences were obtained from seven previously described pregnant intravenous drug users.¹⁴ All except one (IVD30) were co-infected with HCV.

DETECTION OF GBV-C RNA BY POLYMERASE CHAIN REACTION (PCR) AND SEQUENCE ANALYSIS

Total RNA was extracted from 100 µl serum or from liver tissue (2 × 2 × 2 mm) by guanidinium extraction as described.¹³ cDNA synthesis was initiated using reverse transcriptase (Boehringer, Mannheim, Germany). GBV-C DNA was amplified by PCR using primers from the 5'-non-coding region. The outer pair were as follows (locations are as described by Linnen *et al.*); sense HGV-1X nucleotides 205–222 (5'-TTGTGCCTGCG GCGAGAC-3') and antisense HGV-2X nucleotides 533–516 (5'-AATGCCACCCGCC TCAC-3').

The inner primer pair has been described previously.^{13, 14} The nested PCR amplifies a product of about 245 bp. All samples found positive by 5'-non-coding PCR were subjected to a further RNA extraction and were run with primers for the NS3 region for GBV-C as previously described.^{3, 13, 14} The expected size of the PCR product was 140 bp. For sequence analysis, the product from a first round PCR (5 µl) was amplified with biotinylated primers from the NS3 region.³ The sequencing reaction was carried out using the Cy5 AutoRead Sequencing kit and an ALFexpress DNA Sequencer (Pharmacia Biotech, Uppsala, Sweden). Alignment and analysis of the NS3 region sequences was performed using the GeneWorks 2.3 software package (Intelligenetics, Mountain View, CA, USA), and simple dendrograms were constructed using the unweighted pair group method with arithmetic averages

(UPGMA)¹⁸ included in the GeneWorks software package.

GBV-C ENVELOPE 2 (E2) ANTIBODY ENZYME IMMUNOASSAY

Antibodies to a recombinant GBV-C E2 protein^{19, 20} were obtained essentially as previously described in detail.²⁰ In brief, GBV-C E2 protein (kindly provided by Dr I K Mushahwar, Abbott Laboratories, N Chicago, Illinois, USA) was passively adsorbed to microplates at a concentration of 1 µg/ml in phosphate buffered saline at 37°C for two hours. Serum samples were diluted 1:100 or 1:300 in phosphate buffered saline containing 2% goat serum, 1% bovine serum albumin, and 0.05% Tween 20. Before use, the plates were blocked by incubation with dilution buffer for one hour at 37°C. Diluted serum samples were then incubated on the plates for one hour at 37°C followed by an additional incubation of one hour at 37°C with alkaline phosphatase-conjugated goat anti-human IgG (Sigma Chemicals, St Louis, Missouri, USA) diluted 1:1500 in dilution buffer. The presence of enzyme was then detected by adding p-nitrophenyl phosphate and recording the absorbance at 405 nm. Eight samples negative for HBV and HCV by commercial assays (Abbott Laboratories) and which had previously been repeatedly negative—that is, low absorbance—for GBV-C E2 antibodies during initial testing were used as negative controls. The cut off point was set at the mean of the negative controls plus seven times their standard deviation. To simplify graphical comparisons between different runs we also used a cut off point of three times the mean of the negative sera, which showed perfect correlation with the cut off point based on the mean + 7SD.

STATISTICAL METHODS

Frequency comparisons between groups were analysed using Fisher's exact test.

Results

PREVALENCE OF GBV-C RNA DURING FHF

Of the 58 patients with FHF, nine (16%) had GBV-C RNA in the serum after admission to hospital and initiation of treatment (table 1). There were no differences in the prevalence of GBV-C RNA according to aetiology, although none of the patients with FHF of known viral origin were positive for GBV-C RNA (table 1). The 16% frequency of GBV-C RNA during FHF was higher than the previously reported 3% frequency²¹ in 100 healthy Swedish subjects ($p < 0.01$). Also the 19% frequency of GBV-C RNA in the FHF cases of an unknown aetiology was higher than in healthy subjects ($p < 0.05$). The frequency of GBV-C RNA did not differ between the 31 FHF cases of known aetiology and the healthy subjects.

PREVALENCE OF GBV-C E2 ANTIBODIES DURING FHF

Of the 58 patients with FHF, 14 (24%) had GBV-C E2 antibodies in the serum after initiation of treatment (table 1). There were no dif-

Table 1 Presence of GBV-C RNA and E2 antibodies in 58 patients with fulminant hepatic failure sampled after admission to hospital and initiation of treatment. In addition, samples were obtained from 10 patients (six with GBV-C RNA and four with GBV-C E2 antibodies) on first admission to hospital before treatment. Also given is the analysis of GBV-C E2 antibodies in 43 healthy subjects

Aetiology	No of patients	No of patients (%) with GBV-C			No of patients out of those tested with known GBV-C seroconversion after admission	
		RNA	Antibodies	Markers	RNA	Antibodies
Unknown	27	5 (19)*	4 (15)	8 (30)†	3/4	1/1
Viral	6	0	2 (33)	2 (33)	—	—
Drug related	14	2 (14)	3 (21)	5 (36)	1/1	—
Miscellaneous	11	2 (18)	5 (45)*	6 (55)†	0/1	2/3
Total	58	9 (16)**	14 (24)*	21 (36)	4/6	3/4
Healthy subjects	43	††	2 (5)	—	—	—

* $p < 0.05$ compared with the healthy subjects (Fisher's exact test).

** $p < 0.01$ compared with the healthy subjects (Fisher's exact test).

†One patient had both GBV-C RNA and GBV-C E2 antibodies.

††Frequency of GBV-C RNA (3 out of 100; 3%) in healthy Swedish subjects.²¹

Figure 1 Analysis of GBV-C RNA in serum and liver by reverse transcriptase PCR and GBV-C E2 antibodies by enzyme immunoassay in five of the patients with FHF (A to E). Zero on the x axis indicates the date of admission to hospital. Dotted zones indicate periods when blood and/or blood products were given as therapy. GBV-C E2 antibody levels are given as the sample to negative ratio (S:N) in GBV-C E2 enzyme immunoassay. The horizontal line indicates the 3.0 S:N cut off point used in the GBV-C E2 enzyme immunoassay, and the vertical broken line indicates the date of liver transplantation (LTx). Also given is the phylogenetic analysis (F) using the UPGMA algorithm of 82 bp GBV-C NS3 region sequences obtained from six patients with FHF (boxed) and seven pregnant Swedish intravenous drug users (IVD12, IVD15, IVD24, IVD27, IVD30, IVD33, and IVD36). Also included in the analysis are NS3 sequences from two full length GBV-C genomes (GBV-C and HGV45966) and, as an outgroup, an NS3 sequence from a full length hepatitis C virus genome (HCVJ-NS3). ALT, serum alanine aminotransferase. BMT, bone marrow transplantation.

ferences in the prevalence of GBV-C E2 antibodies according to aetiology (table 1). The frequency of GBV-C E2 antibodies during FHF was higher than the 5% frequency in healthy Swedish subjects ($p < 0.05$). The frequency of GBV-C E2 antibodies was not higher in the FHF cases (4/27; table 1) of unknown origin compared with the 43 healthy subjects. In contrast, GBV-C E2 antibodies were more common in the FHF cases of known aetiology (10/31; table 1) than in the healthy subjects ($p < 0.01$).

PREVALENCE OF GBV-C MARKERS ON FIRST ADMISSION TO HOSPITAL

Most of the patients with FHF in this study were admitted to Huddinge University Hospital for further treatment and evaluation for liver transplantation after treatment had been initiated at another hospital. Therefore, in only six of the nine patients in whom serum GBV-C RNA was detected during FHF could samples be identified that were taken on first admission to hospital before any treatment with blood and/or blood products had started. Of these six patients, four were negative for serum GBV-C RNA before treatment with blood and/or blood products (FH1, FH4, FH5, and FH8; table 1 and fig 1). These four patients subsequently tested positive for GBV-C RNA in the serum within 3–18 days of receiving multiple transfusions with blood and/or blood products. Only two samples were available from FH1, a 48 year old man with FHF of an unknown aetiology (data not shown in fig 1). The first sample, taken on admission to hospital before any treatment, was negative for GBV-C RNA and GBV-C E2 antibodies. Later the same day and during the following two days, the patient received a total of 21 units of fresh frozen plasma, erythrocytes, and thrombocytes. He subsequently tested positive for GBV-C RNA in serum taken three days after admission. He recovered spontaneously without liver transplantation. A 14 year old boy (FH4) with FHF of unknown origin had diffuse symptoms for one week and became icteric three days before admission. He was negative for GBV-C RNA and antibodies at admission but became GBV-C RNA positive four days later after receiving a total of six units of fresh frozen plasma. FH5, a 39 year old man with FHF of unknown aetiology, tested negative for GBV-C RNA but positive for GBV-C E2 antibodies on admission; a sample taken eight days later, after he had received at least 23 units of fresh frozen plasma, erythrocytes, and thrombocytes, was GBV-C RNA positive (fig 1). His GBV-C viraemia during the liver failure was probably due to the transfusions although we cannot completely rule out the possibility that, in this case, the GBV-C RNA detected was derived from re-activated endogenous replication. Finally, FH8, a 47 year old man with drug related FHF, had diffuse symptoms for four weeks and had icteric sclerae and elevated bilirubin levels three days before admission to Huddinge University Hospital. He tested negative for GBV-C RNA and GBV-C E2 antibodies on admission but a sample taken 13 days later, after he had

received 17 units of fresh frozen plasma, was shown to be GBV-C RNA positive (fig 1).

Of the two patients who had serum GBV-C RNA on first admission to hospital, one 17 year old girl (FH3) had no previous history of liver disease and no history of drug or alcohol abuse. She had only taken low doses of paracetamol for a period before admission. She underwent orthotopic liver transplantation, and the graft was subsequently infected by GBV-C as evidenced by the presence of GBV-C RNA in a liver biopsy taken four months after the transplantation (fig 1). In this case, a role for GBV-C infection in the aetiology of FHF cannot be excluded, as the cause was not known. A 36 year old man (FH7) diagnosed with chronic myeloid leukaemia in 1992 tested negative for GBV-C RNA in January 1993 (fig 1). He was treated in June 1993 by bone marrow transplantation. Thirteen days later he displayed symptoms of FHF, resulting from veno-occlusive disease, and tested positive for GBV-C RNA in serum taken four days later (fig 1). He subsequently received multiple transfusions and displayed signs of acute graft versus host disease. He died from a brain oedema two days after orthotopic liver transplantation. The role of GBV-C in this case of FHF is unclear.

For four of the 14 FHF cases in which GBV-C E2 antibodies were found during the FHF, samples taken before the start of any treatment were available (table 1). Three of these four patients were negative for GBV-C E2 antibodies on first admission to hospital. This also supports the possibility that the increased frequency of GBV-C E2 antibodies in patients with FHF were caused by the treatment with blood and/or blood products.

SEQUENCE ANALYSIS OF AMPLIFIED NS3 REGION FRAGMENTS

Six of the nine patients assessed to be positive for GBV-C RNA by 5'-non-coding PCR were also found to be positive by NS3 region PCR. The amplified 140 bp NS3 region fragment was partially sequenced. As a control, the NS3 region sequences from these six FHF cases were compared with previously reported corresponding sequences from seven GBV-C infected intravenous drug users with known serum levels of alanine aminotransferase (ALT).¹⁴

In contrast with previous reports,⁷ no separate grouping of the GBV-C sequences derived from patients with FHF and intravenous drug users was observed (fig 1). None of our analysed sequences included all six mutations. However, four mutations were present in three of the four subjects which formed a separate cluster in the dendrogram (IVD15, FH3, and FH4; fig 1F). Patient FH3 with the nucleotide motif 21 G, 93 A, 100 T, and 102 C was known to be GBV-C RNA positive before admission to hospital and the start of any therapy. Patient FH4, who had exactly the same four nucleotide motif, became GBV-C RNA positive after admission to hospital after receiving multiple transfusions, whereas IVD15 did not have any clinical signs

of hepatitis and had ALT levels within the normal range. Although IVD27 had elevated transaminase levels, these may have been due to the concomitant HCV infection. These data suggest that no specific NS3 region sequence motif or GBV-C strain was present in these six cases of FHF.

Discussion

The relation between infection with GBV-C and FHF has been widely debated.^{3-6 9-11 22} In this study we found that serum GBV-C markers were present in about 36% of patients with FHF, regardless of its cause.

A complicating factor in this analysis of the correlation between GBV-C infection and FHF is the fact that many of the 58 patients analysed were initially admitted to other hospitals. In many cases, treatment with blood and/or blood products had already been initiated when the first serum sample was taken on admission to Huddinge University Hospital. Thus, for three of the nine cases of FHF with serum GBV-C RNA, no sample taken before the start of therapy could be obtained despite an extensive search. FHF was caused by Budd-Chiari syndrome in one of these patients, by paracetamol intoxication in one, and for one the cause was not known. Therefore we cannot speculate on the possible role of GBV-C in the aetiology of FHF in these three patients.

Samples taken on first admission to hospital before the start of therapy were available for the remaining six patients. On analysis, four gave negative results for serum GBV-C RNA after the start of symptoms and before the start of treatment. After therapy with blood and/or blood products all four developed GBV-C RNA in the serum within 3-18 days. However, in one of these four cases, GBV-C E2 antibodies were found before therapy. This suggests that, in at least three and probably four of the six patients with FHF, the presence of GBV-C RNA in the serum was secondary to treatment with contaminated blood and/or blood products. Therefore a role for GBV-C as the cause of FHF in these four patients seems unlikely. However, in patient FH3, who had serum GBV-C RNA before treatment with blood and/or blood products, GBV-C infection cannot be excluded as the cause of FHF, and in patient FH4 the role of GBV-C is unclear.

To elucidate further any possible relation between GBV-C and FHF, the sequences of the amplified NS3 region fragments were determined and analysed in six patients with FHF. Interestingly, the GBV-C strain in patient FH3 formed a separate cluster with patient FH4, IVD27 and IVD15. From a previous analysis of FH4, we knew that this patient became infected with GBV-C after initiation of therapy, and that the onset of symptoms preceded the presence of serum GBV-C RNA. In patient IVD15, no clinical signs of hepatitis or increase in serum ALT were present, whereas patient IVD27 had increased ALT at the time of sampling. Both IVD15 and IVD27 were co-infected with HCV. This would suggest that the genetically similar GBV-C

strains infecting subjects FH3, FH4, IVD15, and IVD27 may not be an unusual or aggressive variant of GBV-C.

We conclude that the presence of GBV-C RNA and antibodies in patients with FHF may in many cases be explained by the administration of blood and/or blood products given as therapy. This extends and supports the recent observations made by other groups.⁹⁻¹¹ However, GBV-C cannot be completely ruled out in a minority of cases as contributing to the aetiology of FHF.

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