New Hepatitis Viruses: Are They Important?

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January 2, 1997

Introduction

Following the discovery of the hepatitis C virus (HCV, 1) and the hepatitis E virus (HEV, 2) and development of sensitive and specific diagnostic tests for HCV and HEV infections, the major role that these agents play in parenterally transmitted acute and chronic hepatitis and enterically transmitted acute hepatitis, respectively, became apparent. However, it also has become evident that a fraction of cases of post-transfusion (3-7) and sporadic, community acquired non-A, non-B hepatitis (8-10) cannot be attributed to infection by hepatitis A, B, C, D or E viruses. Moreover, non-A, non-B hepatitis associated with aplastic anemia (11,12) and fulminant or subfulminant non-A, non-B hepatitis (10,13-17) have been found to rarely be associated with HCV or HEV infections in the U.S. or Europe. For these reasons, the search for additional hepatitis viruses has continued.

A number of candidate agents associated with human hepatitis have been identified. A 20 kb double stranded DNA virus has been reported to be associated with sporadic, enteric non-A, non-B hepatitis cases first identified in France and was named hepatitis F ("French") by the team of Indian investigators that reported this finding (18). However, this report has yet to be substantiated and classification of this DNA isolate as viral in origin has been termed premature by other investigators in the field (19,20). More recently, two groups of investigators have independently reported the discovery of a new RNA virus of the Flaviviridae family that they named GB virus C (GBV-C, 21,22) and hepatitis G (HGV, 23), respectively. It is clear that this agent is a parenterally transmitted virus that infects humans. Because of the high prevalence of HGV/GBV-C infection among blood donors and the phylogenetic relationship with HCV, HGV/GBV-C has become the subject of multiple clinical studies seeking to determine the spectrum of disease, if any, that is associated with this infection.

The GB Hepatitis Viruses

The characterization of each of the major hepatitis viruses has been facilitated by studies utilizing non-human primates (24). Following the characterization of HCV, it became apparent that all or nearly all non-A, non-B viral agents that had been serially passaged in chimpanzees were indeed HCV isolates. However, another putative non-A, non-B viral agent, termed the GB agent, proved not to be associated with evidence of hepatitis A, B, C or E infection (24,25). The "GB agent" was derived in the mid-1960's by Deinhardt, Popper and colleagues (26) who inoculated 4 tamarins (*Sanguinus* sp., incorrectly called marmosets in early reports) with serum collected three days after onset of jaundice from a 34 year old Chicago surgeon (initials G.B.) with acute hepatitis. All 4 tamarins developed biochemical evidence of hepatitis. This agent was then serially passed by infusion of acute phase serum collected from one or more tamarins into new groups of tamarins. As early as 1969, Parks and Melnick reported sporadic cases of hepatitis in tamarins, found some tamarins to be resistant to infection by the GB agent and hypothesized that later

passages of the GB agent did not contain the original virus that infected surgeon G.B. but rather contained a latent tamarin virus (27).

Despite some controversy surrounding the nature of the GB agent, it seemed likely by the early 1990's that this inoculum contained a parenterally transmitted virus unrelated to any of the 5 well characterized human hepatitis viruses and a group of investigators at Abbott Laboratories, North Chicago, IL set out to better characterize this agent. A pool of known infectious sera from passage 11 of the GB agent was inoculated into a tamarin after pre-inoculation plasma had been collected (28). Within 7 days after inoculation, serum alanine aminotransferase (ALT) elevations were noted and on day 12, plasma, liver and kidneys were harvested. The acute phase hepatitis plasma from this animal was subsequently shown to be infectious when inoculated into additional tamarins (25).

Total nucleic acids were isolated from both pre-inoculation and acute phase hepatitis plasma and were converted to cDNA by random-primed reverse transcription and then to double stranded DNA by random-primer second strand synthesis. These double-stranded DNA preparations were used to generate "driver" and "tester" amplicons for application of a previously described representational differential analysis (RDA) technique (29) that permits selective polymerase chain reaction (PCR) mediated exponential amplification of nucleic acid sequences present in the "tester" but not in the "driver" source. Three rounds of RDA of infectious plasma (tester) and preinoculation plasma (driver) from the GB agent inoculated tamarin yielded 76 clones that contained 11 unique sequences. Seven of these sequences were found by genomic PCR to not be present in human, tamarin or *E. coli* DNA. Moreover, each of these sequences could be detected in the infectious tamarin plasma as well as the original GB agent passage 11 serum pool but not in preinoculation plasma. When the sequences of these clones were analyzed, amino acid translations of 5 of the clones were found to have 27.3 to 40.2% identity to HCV nonstructural proteins suggesting that they came from a virus phylogenetically related to HCV. Extension of the sequences from these seven RDA clones revealed that they derived from two distinct flavivirus-like genomes that were named GB virus A and GB virus B (28,30).

During subsequent serial passages of the GB agent passage 11 serum pool, investigators at Abbott Laboratories were able to separately pass GB virus A and GB virus B (25). GB virus B inoculation in tamarins is associated with biochemical and histologic evidence of hepatitis, the development of antibodies reactive with GB virus B encoded antigenic epitopes and the presence of $\geq$ 8.3 kb liver RNA that hybridizes on Northern blot analysis with GB virus B derived probes (25). Thus, GB virus B appears to be a "hepatitis" virus. In contrast, GB virus A inoculation in tamarins is not associated with ALT abnormalities or the development of GB virus A epitope reactive antibodies. In addition, while GB virus A can be readily detected in serum of infected tamarins, it is not detected on Northern blot analysis of liver RNA (25,28). Subsequently, GB virus A related genomes have been isolated from the serum of

tamarins (*Sanguinus labiatus*), mystax (*Sanguinus mystax*) and owl monkeys (*Aotus trivirgatus*) not known to have been inoculated with an infectious agent or to have elevated ALT levels (31). These findings suggest that GB virus A is a nonhuman primate virus not associated with hepatitis. The organ or tissue tropism of this virus has yet to be described.

In a manner similar to the techniques used to establish anti-HCV assays, the GB virus A and GB virus B genomes were used to produce recombinant proteins and ELISA assays were developed (21,32). A small fraction of volunteer blood donors (1.5%) and a larger fraction of intravenous drug users (14%) were found to have antibodies reactive to one or more GB virus A or GB virus B recombinant proteins. However, initial attempts to isolate GB virus A or B RNA from the serum or plasma of these ELISA assay positive individuals were unsuccessful. In light of the high mutation rates and sequence variability in many regions of flavivirus genomes, PCR primers with predicted broader specificity were designed to amplify any GB virus variants. The observation that two regions of the nonstructural protein 3 (NS3) gene are highly conserved between GB virus A, GB virus B and HCV permitted the development of a set of degenerate oligonucleotide primers capable of amplifying the NS3 gene from any of these three viruses. With these primers, a product was amplified from the serum of a West African donor (21). However, this product, when sequenced was only 59%, 53.7% and 47.9% identical at the nucleotide level and 64.2%, 57.3% and 50.4% identical at the amino acid levels with GB virus A, HCV-1 and GB virus B sequences, respectively. Similar sequences were subsequently isolated from serum of additional African and North American subjects (including a Dallas,TX resident with acute non-ABCDE hepatitis). The oligonucleotide sequences of these PCR products were 88-99% identical to that obtained from the first African isolate but were similarly disparate from the sequences of GB virus A, GB virus B or HCV NS3 gene sequences leading the investigators to postulate that they had identified a sequence from yet another new virus that was named GB virus C (21).

The initial GB virus C NS3 region nucleotide sequences were subsequently extended (22,33) to reveal the genome of a positive strand flavivirus with predicted amino acid sequence 48% identical to that of GB virus A and ~30% identical to those of GB virus B and various HCV isolates. Thus, as detailed in Table I, GB virus C is, like GB virus A and GB virus B, a new member of the *Flaviviridae* family.

Discovery of "Hepatitis G"

Concurrent with the extensive analysis of the GB agent(s) by investigators at Abbott Laboratories, another group of investigators at Genelabs Technologies, Redwoods City, CA used a somewhat different strategy (23) to isolate and characterize a viral genome present in the plasma of a patient identified as having acute non-A, non-B hepatitis during the Centers for Disease Control Sentinel Counties Study of Viral Hepatitis (8). RNA isolated from 1 ml of this patient's plasma was

Table I

Representative Members of the *Flaviviridae*

	Viruses	% Identity with HCV-1	Diversity within family
Hepatitis C Viruses			
	HCV-1 (genotype 1a)		
	HCV 1b	85%	
	HCV 2a	72%	
	HCV 3a	74%	
	HCV 3b	74%	
GB Viruses			
	GBV-A	26%	27% identical to GBV-B, 48% identical to HGV/GBV-C
	GBV-B	32%	
	Hepatitis G / GBV-C (HGV/GBV-C)	~30%	~95% identity among HGV/GBV-C genotypes
Pestiviruses			
	Bovine Viral Diarrhea	21%	72% identical to Hog Cholera
	Hog Cholera	21%	
Flaviviruses			
	Yellow Fever	20%	46% identical to West Nile
	Dengue		
	West Nile	19%	
	Kunjin		
	St. Louis Encephalitis		
	Tick-borne encephalitis		

Data obtained from references 23, 28, 30 and 33.

reverse transcribed and amplified. A λgt11 library was created and screened with convalescent plasma for immunoreactive clones. Although originally found to be anti-HCV negative on first generation ELISA assays, the plasma used to create this library was subsequently found to be HCV RNA positive and anti-HCV reactive in second generation assays. Although initial immunoscreening revealed HCV-related sequences,

several novel sequences were identified including one clone with sequence that was found to be present in RNA from plasma from the original hepatitis patient but not in plasma from healthy controls. Starting with this initial sequence, an anchored PCR approach was used to generate multiple overlapping cDNA clones whose sequences were determined and combined to create a 9392 nucleotide sequence. Using sequences from this initial isolate, overlapping cDNAs were generated from the plasma of a second patient with "intermittently abnormal ALTs" to determine a second 9103 nucleotide sequence that was 90.5% identical to the first (97.5% amino acid identity). Analysis of the polyprotein encoded by these sequences revealed that they were 43.8% identical to that of GB virus A, 28.4% identical to that of GB virus B, 26.8% identical to that of hepatitis C and 100% identical within the NS3 region to that of GB virus C (23). Subsequent analysis of the Genelabs isolate that was named Hepatitis G and the full length Abbott Laboratories isolate named GB virus C revealed that they were 86% identical at the nucleotide level and >95% identical in predicted amino acid sequence and thus represented different isolates of the same virus (33).

Characterization of Hepatitis G / GB Virus C (HGV/GBV-C)

As detailed in Table I, HGV/GBV-C and the other GB viruses represent new genera within the *Flaviviridae* family. Unlike HCV and other previously characterized members of this viral family, HGV/GBV-C and GB virus A appear to lack nucleocapsid or core-like proteins at the N-termini of their putative polyproteins (figure 1,33,34). However, as detailed in figure 1, the remainder of the HGV/GBV-C genome is organized in a manner similar to that of hepatitis C (22,30).

When recombinant proteins have been produced from HGV/GBV-C gene sequences and expressed in an *E. coli* expression system identical to that used to develop antigenic epitopes for anti-HCV assays (32), success in identifying humans with active HGV/GBV-C infection has been disappointing with only about 30% of HGV/GBV-C RNA positive individuals displaying immunoreactivity to any of the recombinant epitopes produced in *E. coli* (35). Thus, the human immune response to nonstructural proteins of HGV/GBV-C appears similar to the tamarin immune response to GB virus A and distinct from human immune responses to nonstructural proteins of HCV.

However, when the HGV/GBV-C E2 encoded envelope protein is expressed in mammalian cell lines, an antigenic glycoprotein is produced that appears to identify antibodies that appear in humans concurrent with clearance of HGV/GBV-C infection (36). Early findings indicate that the anti-E2 response in HGV/GBV-C infected humans is similar to the anti-envelope protein response during infection with flaviviruses such as dengue or yellow fever in which anti-envelope responses correlate with clearance of viral infection and development of protective immunity (36,37). In this regard, the anti-E2 response to HGV/GBV-C appears different from the human anti-E2 response during HCV infection which is not associated with viral clearance (38).

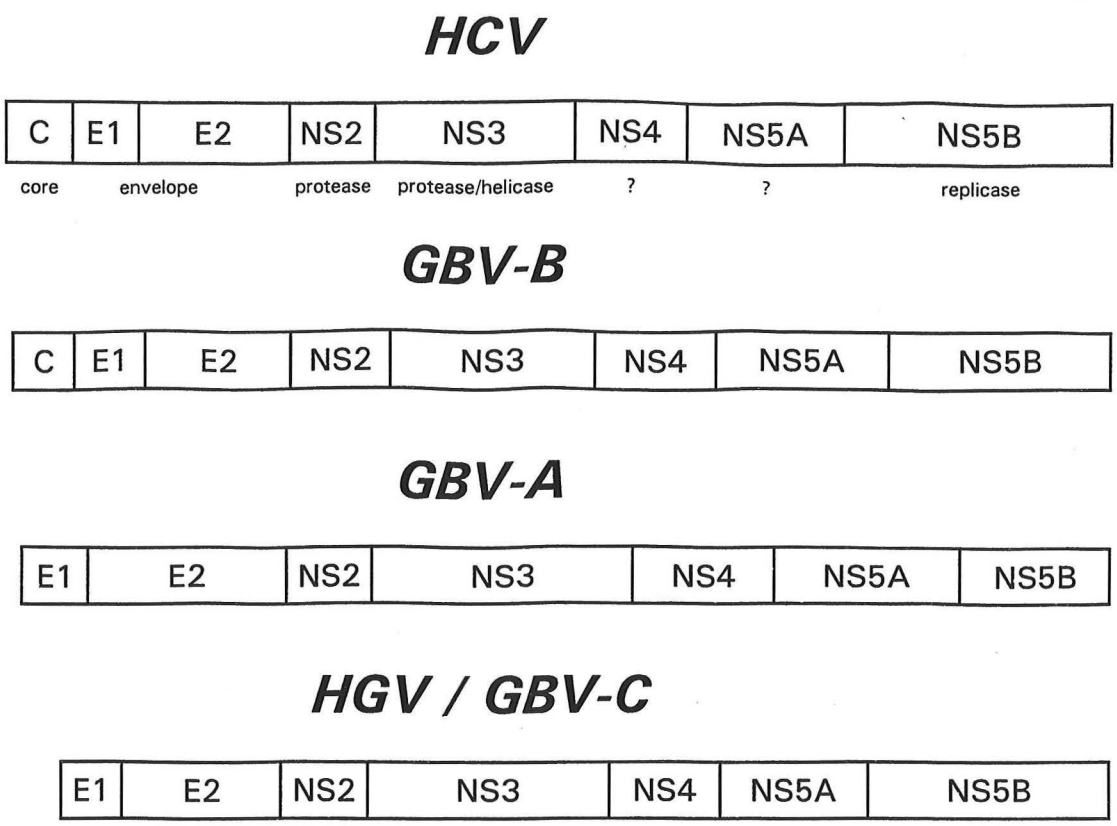

Figure 1. Schematic representation of the genomic organization of HCV, GB virus B, GB virus A, and HGV/GBV-C. Representation based on data from references 22, 23, 28, 30, 33 and 34.

When the HGV isolate has been inoculated into tamarins, ALT elevations and necroinflammatory lesions in the liver have been observed (39). Thus, unlike its closest phylogenetic relative, GB virus A, HGV/GBV-C appears to be associated with some evidence of hepatitis in this species. However, chimpanzees inoculated with HGV/GBV-C exhibit evidence of infection with prolonged appearance of HGV/GBV-C RNA in serum, but do not exhibit ALT abnormalities or hepatic histologic abnormalities (39,40). Nevertheless, primate models of HGV/GBV-C transmission have been used to demonstrate that this virus was present in Factor VIII concentrates prepared in the 1970's and was transmitted along with HCV to chimpanzees inoculated with Factor VIII concentrates in the late 1970's (41). Unfortunately, sera collected from the original patient G.B. during the acute phase of his illness have not been preserved under conditions that leave RNA viruses intact. Thus, the precise cause of hepatitis in G.B. remains unclear. However, a serum specimen collected from G.B. 8 weeks after initial presentation has been found to be strongly reactive in a radioimmunoprecipitation test for antibodies against the HGV/GBV-C E2 protein and weak anti-HGV/GBV-C E2 reactivity was still detected in serum collected > 30 years later (36).

With sequencing of multiple HGV/GBV-C isolates collected from patients from different geographic locales, different viral subtypes (genotypes) have been identified (see figure 2, Table I). However, the degree of genetic diversity among various HGV/GBV-C genotypes is much less than has been described for HCV genotypes. Thus, HGV/GBV-C genotypes retain approximately 95% identity in amino acid sequence whereas different HCV genotypes are only 72-85% identical.

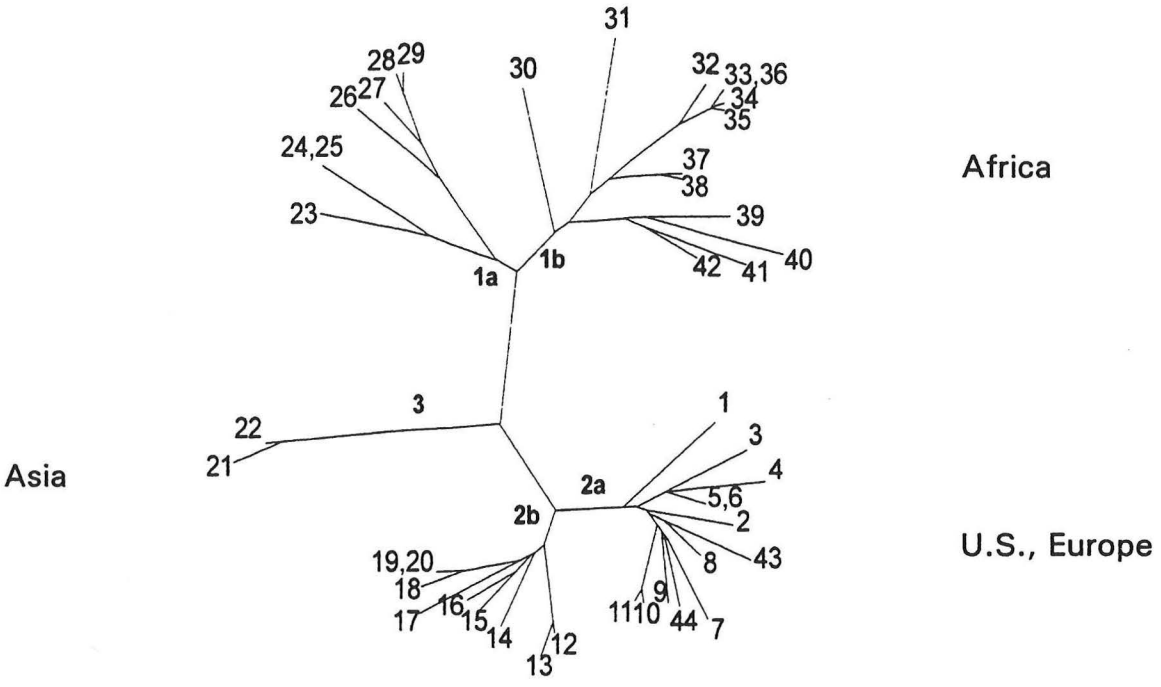

Figure 2. Phylogenetic tree showing evolutionary distance relationships between different HGV/GBV-C isolates and grouping into subtypes (genotypes). Modified from reference 41b.

HGV/GBV-C Infection in Humans

Several reports have provided convincing evidence that HGV/GBV-C is transmitted by blood transfusions (19,23,36,42). In each of the reports summarized in Table II, prospectively followed transfusion recipients who were HGV/GBV-C RNA negative before receiving blood products were observed to develop HGV/GBV-C viremia within 7 to 89 days post-transfusion (23,36,42). When donor units were available for testing, a HGV/GBV-C RNA positive donor was identified in each case (23,36). Moreover, in some analyses (43) complete or near complete sequence identity has been noted between HGV/GBV-C genomes isolated from recipients and implicated donors. In contrast to acute HCV infections, which lead to chronic (> 1 year) viremia in >95% of cases (8,44-47), approximately 2/3 of prospectively followed post-transfusion HGV/GBV-C infections in immunocompetent recipients

resolve (23,36,42) although viremia typically does not clear until 6-18 months after initial infection. In the one study where anti-E2 responses were also followed, development of antibodies to the HGV/GBV-C E2 protein correlated with and persisted after clearance of HGV/GBV-C viremia (36). In addition to evidence implicating blood transfusion and blood exposure during injection drug use as routes of HGV/GBV-C transmission, one report (48) suggests that HGV/GBV-C is transmitted vertically from mother to newborn infant at a rate (33%) higher than observed for HIV (11.8%) or HCV (6.7%).

Table II

Hepatitis G / GB Virus C is Transmitted by Blood Transfusion

Study	# of Initially HGV/GBV-C RNA (-) Recipients	# with HGV/GBV-C RNA Acquired after Transfusion	Donor Units Implicated	Persistent Infection
Linnen, et al (23)	Not Stated	3	2/2 cases	1/3 (33%)
Pilot-Matias, et al (36)	Not Stated	7	7/7 cases	2/7 (29%)
Wang, et al (42)	394	34 (8.6%)	Not Tested	8/22 (36%)

The high rate of post-transfusion HGV-GBV-C observed in the study by Wang, et al that is summarized in Table II is not surprising as the prevalence of HGV/GBV-C infection is much higher among blood donors than is HBV or HCV infection (see Table III and references 19,23,36,42). HGV/GBV-C RNA has been detected in 1-5% of donors from different centers as summarized in Table III. Rates of HGV/GBV-C infection are higher among injection drug users (IDUs) than among blood donors although not as high as HCV infection rates. The fact that HGV/GBV-C viremia rates are lower than HCV viremia rates among IDUs and multi-transfused hemophiliacs is likely related to the significantly higher rate of HGV/GBV-C clearance by these usually immunocompetent individuals. Thus, as detailed in Table III, in one cohort of IDUs who were 99% anti-HCV (+) and 71% anti-HBc (+), only 16% were HGV/GBV-C RNA (+) but 100% of HGV/GBV-C RNA (-) IDUs who were tested had antibodies to the HGV/GBV-C E2 protein suggesting resolved, remote HGV/GBV-C infection (35-36). However, in multi-transfused organ transplant recipients who also receive immunosuppressive agents likely to decrease host immune responses to viral infections, rates of HGV/GBV-C infection may exceed 50% (54,56,57, see Table III).

Table III
Prevalence of HGV/GBV-C Infection Among Adults with Low or High Risk of Parenterally Transmitted Viral Infection

Population	Study	Prevalence of HGV/GBV-C		
		RNA (+)	If RNA(-), Anti-E2(+) ?	RNA(+) &/or anti-E2(+)
Volunteer Blood Donors	nl ALT, US (23)	13/769 (1.7%)	N.T.	N.T.
	↑ ALT, US (23)	11/709 (1.6%)	N.T.	N.T.
	nl ALT, US (35,36)	1/127 (0.8%)	2/59 (3.4%)	4.2%
	↑ ALT, US (35,36)	8/204 (3.9%)	N.T.	N.T.
	Donors, Japan (49)	4/448 (0.9%	N.T.	N.T.
	nl ALT, Australia (50)	4/100 (4%)	N.T.	N.T.
	↑ ALT, Australia (50)	1/20 (5%)	N.T.	N.T.
	Donors, Dallas, TX (51)	10/236 (4.2%)	10/52 (19%)	23%
	Donors, UK (52)[1]	4/125 (3.2%)	N.T.	N.T.
	Donors, Taiwan (42)	4/200 (2%)	N.T.	N.T.
Paid Plasma Donors	US (35,36)	12/93 (13%)	12/24 (50%)	57%
Injection Drug Users	Chicago, IL (35,36)[2]	15/95 (16%)	27/27 (100%)	100%
	Europe, (23)	20/60 (33%)	N.T.	N.T.
Multi-Transfused, Immunocompetent	Hemophilia, Anemia, Europe (23)	27/149 (18%)	N.T.	N.T.
	Hemophilia, Japan (53)[3]	4/33 (12%)	N.T.	N.T.
Multi-Transfused, Immunocompromised	Bone Marrow Transplant, UK (54)	15/18 (83%)	N.T.	N.T.
	Liver Transplant, Germany (55)	35/132 (27%)[4]	N.T.	N.T.
	Liver Transplant, US (56)	51/83 (61%)[5]	N.T.	N.T.
	Renal Transplant, UK (57)	6/11 (55%)	N.T.	N.T.

[1] Only 137/180,658 (0.076%) of this volunteer donor population reported to be anti-HCV (+).
[2] Of these individuals, 99% were anti-HCV (+) and 71% anti-HBc positive.
[3] Anti-HCV detected in 88% of these patients.
[4] Includes 7% who were HGV/GBV-C RNA (+) before liver transplant.
[5] Includes 16% who were HGV/GBV-C RNA positive before liver transplant.

Table IV

Prevalence of HGV/GBV-C Infection Among Patients with Liver Disease

Liver Disease		Study	Fraction HGV/GBV-C RNA (+)
Suspected non-ABCDE origin:	Post-Transfusion non-ABCDE	US (23)	2/12 (17%)
		Taiwan (42)	1/8 (12.5%)
		Japan, (59)	4/15 (27%)
	Sporadic Acute non-ABCDE	US, CDC (23)	5/38 (13%)
		Dallas, TX (35)	4/37 (11%)
		Italy (60)	11/31 (35%)
	Chronic non-ABCDE	Europe, S. Amer. (23)	15/158 (9.5%)
		Italy (60)	7/18 (39%)
	Cryptogenic Cirrhosis	US (35)	5/34 (15.7%)
Known etiology:	Acute HCV	US, CDC (23)	19/107 (18%)
	Acute HCV	Dallas, TX (35)	6/27 (22%)
	Chronic HCV	Europe (23)	18/96 (19%)
	Chronic HCV	Japan (61)	21/189 (11%)
	Chronic HCV	Germany (62)	18/115 (16%)
	Chronic HBV	Europe (23)	7/72 (10%)
	Alcoholic Liver Disease	Europe (23)	5/49 (10%)
	Autoimmune Hepatitis	Europe (23)	5/53 (9%)
	Primary Biliary Cirrhosis	Europe (23)	1/58 (2%)

When rates of HGV/GBV-C infection have been assessed in patients with liver disease thought potentially related to a non-ABCDE virus, only 10-20% of non-ABCDE hepatitis patients in most series have been found to have evidence of active infection with this agent (see Table IV). While this rate of HGV/GBV-C infection is invariably higher than noted among blood donor "control" groups, it has not been found to be significantly higher than the prevalence of HGV/GBV-C viremia among other liver disease control groups as detailed in Table IV.

This finding is quite different than the sequence of events that followed discovery of HCV. Even with early first generation anti-HCV tests, it was readily apparent that the majority of patients with parenterally transmitted non-A, non-B hepatitis had evidence of HCV infection while only a minority of those with other defined etiologies of liver disease had evidence of HCV infection (reviewed in ref.19). Thus, it was easy to demonstrate a role for HCV in development of a well defined liver disease syndrome whereas the nature of liver disease related to HGV/GBV-C has been difficult to define based on seroepidemiologic surveys such as those summarized in Table IV.

Of note, the observed ~ 10% prevalence of HGV/GBV-C infection among patients with chronic hepatitis B, alcoholic liver disease or even autoimmune hepatitis is not unexpected as such patients often either have a history of IDU or have received blood products to manage complications of their primary disease. Indeed, as HGV/GBV-C appears to resemble HCV in ease of transmission, the rates of HGV/GBV-C infection in the liver disease control groups detailed in Table IV are similar to those that would be predicted based on prior reports of HCV co-infection in these patient groups (58). Based on this observation some reviewers have suggested that in the ~10-20% of cases of non-ABCDE hepatitis where HGV/GBV-C RNA is found this too may represent a chance association between liver disease(s) of unknown etiology and coincidental infection with a parenterally transmitted virus (19).

Table V

Frequency of ALT Abnormalities During Post-Transfusion Acute HGV/GBV-C Infections

Study	Total # of HGV/GBV-C[1] PTH Cases	ALT Levels During Acute Infection:		
		All nl	"Minimal" ↑[2]	> 2X ↑
Alter, et al (19,63)	Not Stated	72%	22%	6%
Wang, et al (42)	25	20/25 (80%)	3/25 (12%)	2/25 (8%)

[1] Only cases without HCV or HBV co-infection are included.

Most disappointing for investigators who were seeking to find the cause of non-ABCDE post-transfusion hepatitis has been the observation that no more than a small fraction of such cases can be attributed to this agent (see Table IV). Indeed, in two more extensive analyses (19,42,63) of prospectively followed post-transfusion HGV/GBV-C infections, it was found that only 20-28% of individuals infected with

HGV alone (i.e. no concurrent HCV or HBV) ever developed ALT abnormalities (see Table V). Moreover, more than half of individuals with ALT elevations associated with prospectively observed HGV infections have only mild (< 2 X upper limit normal), transient ALT elevations and none have been observed to develop jaundice or symptomatic liver disease although in some individuals, chronic ALT abnormalities persisting for several years have been observed (23). No information is available regarding liver histology in cases of prospectively observed acute HGV/GBV-C infection.

In contrast to the benign nature of post-transfusion HGV/GBV-C infections, when the subset of HGV/GBV-C RNA (+) patients with sporadic non-ABCDE has been examined, a somewhat different picture has been suggested by some analyses. Among patients in the CDC Sentinel Counties Study of viral hepatitis, the subset of patients with non-ABCDE hepatitis who were found to have HGV/GBV-C RNA viremia had higher mean ALT levels and serum bilirubin levels than previously reported for the non-ABCDE group as a whole (8,64). In a series of prospectively collected and analyzed patients with acute non-ABCDE hepatitis evaluated at UT Southwestern Medical Center in Dallas (10,35), 3 of 7 patients with a fulminant or subfulminant course proved to be HGV/GBV-C RNA (+) whereas HGV/GBV-C RNA was only found in 1/30 patients with an uncomplicated course (35). As summarized in Table VI, varying frequencies of HGV/GBV-C infection have been documented in other centers among patients with acute liver failure due to acute non-ABCDE hepatitis. In many but not all cases, serum HGV/GBV-C RNA was present prior to administration of any blood products.

However, in both the CDC Sentinel Counties Study (64) and in the study underway at this institution (additional unpublished data), prevalence of HGV/GBV-C RNA in patients with acute hepatitis A, acute hepatitis B or acute hepatitis C has been found to be slightly higher than among patients with acute non-ABCDE. In addition, in both the CDC Sentinel Counties Study and the local experience, all patients with HGV/GBV-C RNA detected in the setting of acute non-ABCDE exhibited chronic viremia over long periods of follow-up with none exhibiting the self-limited course noted in 2/3 of prospectively observed acute post-transfusion HGV/GBV-C infections. These observations suggest that HGV/GBV-C infection in such patients is more likely to be a coincidental pre-existing condition rather than the cause of acute hepatitis. The unusually severe course in some presumably chronic HGV/GBV-C infected patients with superimposed acute non-ABCDE hepatitis may relate either to the co-morbidity of two diseases (chronic HGV/GBV-C + acute hepatitis "X") or to the fact that HGV/GBV-C infection is a surrogate marker for life-style risk factors for development of severe acute non-ABCDE hepatitis.

Table VI

Prevalence of HGV/GBV-C RNA in Patients with
Fulminant or Subfulminant Acute non-ABCDE Hepatitis

Site	Reference	Fraction with HGV/GBV-C RNA
Japan	65, 66	3/6 (50%)
Japan	67	0/7 (0%)
UK	68	0/20 (0%)[1]
India	69	3/20 (15%)
US	35	3/7 (43%)
Germany	70	11/22 (50%)[2]

[1] Only liver explant RNA analyzed.
[2] Many of the HGV/GBV-C infected patients in this series appeared to be infected with a common genotype distinct from that found in other liver disease controls from the same locale.

In addition to assessing patients with liver disease of unknown etiology for evidence of HGV/GBV-C related disease, there has been considerable interest in determining whether HGV/GBV-C co-infection alters the disease course or response to interferon-α treatment in patients with chronic hepatitis C. In the two studies detailed in Table VII and multiple additional abstract reports, serum collected from patients with chronic HCV infection before, during and after interferon-α therapy has been analyzed for presence of HGV/GBV-C RNA. During the course of interferon-α therapy, loss of HGV/GBV-C RNA has been observed with relapse of HGV/GBV-C RNA observed in most but not all patients after interferon-α therapy is discontinued. This pattern of interferon-α response is quite similar to that seen with HCV infection. Of additional note, in patients with HGV/GBV-C co-infection, rates of response of HCV viremia and serum ALT to therapy have not been found to be significantly different than in patients with HCV infection alone.

These findings seem to indicate that HGV/GBV-C and HCV respond similarly to interferon-α therapy and that co-infection with HGV/GBV-C does not have a dramatic effect on response of HCV infection to this form of therapy. However, because of the small number of co-infected patients followed in each study, a less dramatic effect of HGV/GBV-C co-infection on response to interferon-α has not been excluded. Of additional note, in most studies no significant difference has been noted in histologic severity of liver disease in patients with HGV/GBV-C + HCV infection versus HCV infection alone. However, in one small study of patients with chronic HCV or HBV

referred for liver transplantation, there was a significantly higher rate of hepatocellular carcinoma discovered in those with HGV/GBV-C co-infection prior to orthotopic liver transplantation (55).

Table VII

Effect of HGV/GBV-C Infection on Response to Interferon-α Treatment of Chronic Hepatitis C

Site	Patient Group	Sustained Loss of HCV RNA[1]	Sustained Loss of HGV/GBV-C RNA[1]
Germany (62)	HCV only	18/97 (19%)	--
	HCV + HGV/GBV-C	5/18 (28%)	3/18 (17%)
Japan (61)	HCV only	33/91 (36%)	--
	HCV + HGV/GBV-C	3/10 (30%)	2/9 (22%)[2]

[1] Assessed >6 months after completion of 6 or more months of 3 X 10^6 units t.i.w. of interferon α2a or interferon α2b therapy.

[2] One patient not assessed for HGV/GBV-C response.

Summary

HGV/GBV-C is a newly discovered parenterally transmitted virus that is phylogenetically related to HCV. This agent infects humans and in at least a subset of patients induces mild ALT abnormalities that may be either transient or persistent. Despite the high prevalence of HGV/GBV-C infection, however, this virus has yet to be linked to a distinct clinical disease syndrome. Indeed, in most patients infected with this agent, no effect on ALT levels or liver pathology can be demonstrated. Moreover, even in patients with liver test abnormalities, the very low levels of ALT elevations observed during post-transfusion HGV/GBV-C and the lack of effect of HGV/GBV-C infection on severity of histologic changes observed in patients with chronic hepatitis C seem reassuring with respect to the risk that this infection might pose for transfusion recipients. Thus, at this time, the presence of HGV/GBV-C viremia can not yet be classified as a disease.

However, long term histologic follow-up in patients with prospectively identified HGV/GBV-C infection is lacking and the site of replication of this virus has yet to be clearly defined. Moreover, it should be remembered with caution that other relatively asymptomatic viral infections such as that with HCV were initially noted to be relatively benign (71,72). Thus, both careful assessment of liver histology in HGV/GBV-C infected individuals and some assessment of effects of this virus on other organ systems are warranted before it is discounted as a benign commensal infection.

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