MINIREVIEW

THE NEWLY DISCOVERED NON A-E HEPATITIS VIRUSES

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Abstract. Two biotechnology companies have recently announced the discovery of 4 new hepatitis viruses, provisionally named HGV and GBV agents (GBV-A, GBV-B, and GBV-C). Using a molecular biological approach, the genomes of these viruses were identified from non-A-E hepatients patients who had no markers to any previously known hepatitis viruses. The new viruses are members of family *Flaviviridae*, and are closely related to hepatitis C virus (HCV). Preliminary studies show that the prevalence of GBV agents and HGV are alarmingly high in blood donors in the United States, Europe, Africa and Japan. The viruses are transmitted parenterally, similar to HCV and hepatitis B virus (HBV), Chronic infection is common and can lead to cirrhosis. Some chronic hepatitis cases caused by these viruses respond to interferon treatment. The viruses can coinfect with HCV and/or HBV. A number of questions about these new viruses remain to be answered, including the magnitude of the problems, clinical significance, mode of transmission and populations at risk, as well as the appropriate treatment.

INTRODUCTION

Viral hepatitis is one of the most important infectious diseases worldwide, particularly in the Southeast Asian region. Two hepatitis viruses, hepatitis A virus (HAV) and hepatitis B virus (HBV) have been isolated more than two decades ago, and are well characterized. Another agent, the delta agent or hepatitis D virus (HDV) which can coinfect some patients with HBV infection and can be co-transmitted with HBV, is uncommon in the general population. However, significant numbers of acute and chronic viral hepatitis cases still occur with no serological and viral markers for either HAV or HBV and are therefore diagnosed as non-A, non-B hepatits (NANBH). Identification of the elusive non-A, non-B agent(s) has been the focus of investigation for the past 20 years.

The application of molecular biology technology has led to the recent identification of two additional hepatitis viruses. Hepatitis E virus (HEV), identified by differential hybridization approach, was found to be responsible in causing most, if not all, cases of enterically-transmitted NANBH. South Asia and Myanmar are known endemic areas of HEV, where HEV epidemics commonly occur following flooding, with high mortality rate among infected pregnant women. Hepatitis C virus (HCV) has been identified to be the etiologic agent in the majority of post-transfusion NANBH cases (Choo *et al*, 1989, 1990). The complete genome of the virus has been sequenced and serological and molecular biological assays for the detection of HCV have been developed and are already used in many blood transfusion centers and in clinical practice.

Prior to the discovery of HCV, about 10% of blood transfusion recipients would develop acute post-transfusion hepatitis (PTH) and many would develop chronic hepatitis. It was proposed that at least 2 transfusion-related NANB hepatitis viruses existed as demonstrated by animal cross-challenge and other experiments (Tabor *et al*, 1979; Bradley *et al*, 1983). The implementation of anti-HCV screening in blood donors reduces the incidence of PTH to less than 1% and it seems that PTH may have been conquered. However, there are still some patients with hepatitis of viral origin who have no markers to any known viruses, suggesting the presence of additional unknown hepatitis virus(es). Such virus(es) are named as non-A, non-B, non-C, non-D, non-E hepatitis virus (non-ABCDE), non-A-E

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virus (non-A-E) or hepatitis X virus (HXV). A recent study at the United States' National Institutes of Health demonstrated that 18% of community-acquired hepatitis and 12% of post-transfusion hepatitis fell into the non-A-E group (Alter, 1994). Another large scale study of chronic liver diseases also found that HCV was responsible for 72.4% of viral hepatitis cases, HBV for 19.5% of cases, and about 8.1% of viral hepatitis patients are non-A-E (Kodali *et al*, 1994). Several attempts were made to isolate these elusive non-A-E agents. In the middle of 1995, 2 major biotechnology companies reported the discovery of 4 new hepatitis viruses. A group of GB agents (GBV-A, GBV-B and GBV-C viruses) was identified by Abbott Laboratories. Another agent, provisionally named hepatitis G virus or HGV, was identified by a collaborative group led by Genelabs Technologies, Inc.

GB AGENTS (GBV-A, GBV-B AND GBV-C)

In 1967, Deinhardt and colleagues described a case of hepatitis which can be transmitted intravenously to inoculated monkeys. A 34-year-old surgeon, with the initials "GB", developed acute hepatitis and became icteric for about 4 weeks, then recovered from the disease (Deinhardt *et al*, 1967). He was seronegative for HBsAg and anti-HBs, and was also anti-HCV antibody-negative. The latter was tested recently when the assay became available. Acute phase serum (3rd day after jaundice) from patient GB was inoculated into 4 marmosets, all of which developed elevated serum alanine aminotransferase (ALT), suggesting an infectious origin. This agent was serially passaged in tamarins for more than 10 passages, without success in isolating the GB agent.

Recently, scientists at Abbott Laboratories have succeeded in isolating parts of the genomes of 2 new viruses from sera of tamarins inoculated with the 11th passage of GB agents, using a novel subtractive molecular biological technique known as Representational Difference Analysis or RDA (Simons *et al*, 1995b). RDA is a PCR-based technique for identifying the different genetic materials of 2 similar sources, in this case the difference between RNA (cDNA) species in sera of inoculated tamarins and those of the uninoculated animals. The unique sequences were exponentially amplified whereas the common sequences were amplified in a linear fashion. This technique has recently been used for cloning a Herpes-like virus associated with Kaposi sarcoma. Eleven unique cDNA clones were isolated from GB inoculated serum, 7 of which were characterized in detail and found to be parts of 2 unknown viruses. These clones were not detected in plasma or liver tissue of tamarins prior to the inoculation with the GB agents. Oligonucleotide primers derived from the sequences could detect these RNA/cDNA from acute phase plasma of GB-inoculated tamarins. Furthermore, partial nucleotide sequence analysis indicated that most of these clones possessed limited identity of putative amino acid sequences to those of the nonstructural proteins of HCV (Simons *et al*, 1995b). Clone extension approaches allowed the sequencing of complete genomes of these two viruses, tentatively designated as GB virus A (GBV-A) and GB virus B (GBV-B).

Interestingly, nucleotide and amino acid sequence analysis of GBV-A and GBV-B showed that these viruses possessed a positive-strand RNA genome of about 9,100-9,500 nucleotides in length. The genome contains a single large open reading frame (ORF) encoding about 2,900-3,000 amino acids. The genes encoding putative structural proteins are located in the 5' one-third of the ORF and those of nonstructural proteins in the 3' two-thirds of the ORF. Based on these features, including the hydropathicity profile and the possessing of putative serine protease domain in the NS3 region and the putative RNA-dependent RNA polymerase domain in the NS5 region, GBV-A and GBV-B could be classified as new members of family *Flaviviridae* (Zuckerman, 1995). Detailed analysis of the genes encoding putative helicase (NS3) and putative RNA-dependent RNA polymerase (NS5) demonstrated that GBV-A and GBV-B differed significantly from other known genus of this family, and thus have been tentatively classified as 2 new genera in the *Flaviviridae* family.

Phylogenetic analysis of nucleotide and complete amino acid sequences of GBV-A and GBV-B showed that these viruses are distantly related to other members of the *Flaviviridae*, in which HCV is its closest neighbor. However, GBV-A and GBV-B are not new genotypes of HCV since the overall amino acid sequence identity of GBV viruses and HCV is much less than that among each genotype of HCV (Table 1).

Table 1

Extent of amino acid sequence identity among prototype GBV agents and HCV (modified from Simons *et al*, 1995a).

	Amino acid sequence identity (%)			
	HCV-1	GBV-A	GBV-B	GBV-C
HCV	100			
GBV-A	26	100		
GBV-B	32	27	100	
GBV-C	29	45	28	100

The analysis of the virus responsible for clinical hepatitis in patient GB eventually proved that GBV-B caused hepatitis in this patient. Infection in animals with GBV-B alone, not GBV-A, was associated with the increase in serum ALT during which the viremia can be found. The GBV-B RNA genome was found in infected tamarin liver. In addition, prior infection with GBV-B or combined GBV-B/GBV-A conferred protection against re-inoculation with GBV-B but not GBV-A (Simons *et al*, 1995a). Subsequently, GBV-A was found to be an endogenous animal hepatitis virus which was accidentally contaminated with GBV-B during serial passage in tamarins.

The cloning of GBV genomes has led to the development of serological and molecular diagnostic assays for the viruses. Recombinant GBV-A and/or GBV-B proteins were used for detecting antibody and degenerate PCR primers derived from consensus sequences in the NS3 gene of GBV-A, GBV-B and HCV were employed in a PCR test. This, accidentally, led to the discovery of another hepatitis virus. Nucleotide sequencing of a PCR product obtained from serum of a Western African patient with antibody to GBV-A/GBV-B revealed a limited nucleotide sequence identity to GBV-A (59.0% in the putative helicase gene), GBV-B (53.7%) and HCV (47.9%). Because this virus is closely related to GBV-A, it was tentatively named as GB virus C (GBV-C), although it was not derived from GB serum (Simons *et al*, 1995a).

Nucleotide sequence, phylogenetic analysis and genomic organization indicates that GBV-C is also a member of *Flaviviridae* family and is more closely related to GBV-A, than to HCV or GBV-B. It should be noted that GBV-C was identified directly from a human specimen. PCR amplification of sera from acute non-A, non-B hepatitis patients using GBV-C-specific primers showed a correlation between clinical hepatitis and GBV-C viremia, indicated that this virus can cause clinical hepatitis in human. Nucleotide sequences of the putative 5'-noncoding region and the core gene of 27 strains of GBV-C showed that multiple genotypes of GBV-C exist. Futhermore, preliminary result also showed some correlation between genotypes and geographic distribution. Several lines of evidence suggested that GBV-C is not a genotype of HCV and can exist independently of HCV (Simons *et al*, 1995a).

The epidemiological and clinical significance of GBV agents are being investigated. GBV-A is relatively uncommon in humans with a prevalence of 0.3% in US volunteer blood donors (Simons *et al*, 1995a). Surprisingly, the prevalence of GBV-B and GBV-C in various groups of population is higher than expected. About 1-2%, 2-3% of US blood donors, and 5-7% and 5-28% of non A-E patients from various geographical regions, have antibodies to GBV-B and GBV-C, respectively (Schlauder *et al*, 1995). The prevalence is even higher in multiply transfusion recipients, and intravenous drug users. About 20% of Western African populations were tested positive for antibody to GBV-A and/or GBV-B. PCR amplification has also been developed. However, there was no correlation between the antibody detection and PCR assay. Both assays are in their early phase of development and the results obtained from these assays should not be overinterpreted. The discrepancy may be due to low level of virus in blood, inappropriate PCR primer pairs, complete clearance of the virus or nonspecific serological cross-reaction. On the other hand, it may be due to the non-crossreactivity as a result of viral heterogeneity.

HEPATITIS G VIRUS (HGV)

In search for an elusive agent causing non-A-E hepatitis, scientists at Genelabs Technologies Inc and the National Institutes of Health used plasma/serum samples from 2 chronic hepatitis patients as starting materials. One was obtained from a Caucasian male with HBV-negative, HCV-positive

postransfusion hepatitis. Another was from a Caucasian female with history of mildly elevated serum ALT. Samples from the former patient caused acute hepatitis when inoculated in tamarins, but caused mild or no disease in other monkeys including chimpanzees. A new hepatitis virus was identified from these samples and was tentatively named as hepatitis G virus (HGV).

Using a combined subtractive molecular approach involving PCR and immunoscreening known as Sequence Independent Single Primer Amplification (SISPA), unique cDNA clones were isolated from inoculated sera. Nucleotide sequence of these clones showed limited identity with the nonstructural gene of *Flaviviridae* family. Complete nucleotide sequence of this virus confirmed that its genomic structure is typical of that of other flaviviruses, with GBV-A, GBV-B and HCV are the closest neighbors. The sequence comparison with GBV-C is yet to be done and thus the extent of identity between HGV and GBV-C is not known. The nucleotide sequence at the noncoding region of various isolates of HGV is conserved, however there is no identity with that of HCV.

HGV genome contains single-stranded RNA of about 9,400 nucleotides encoding a long open reading frame of about 2,900 amino acids. Comparison of deduced amino acid sequence with other viruses showed that HGV is more closely related to GBV-A (45% overall amino acid sequence homology), as compared to GBV-B and HCV. The identification of the conserved regions of HGV genome allowed the development of molecular diagnostics and immunoassays.

Animal experiments demonstrated that HGV is transmissible and persistent viremia was found in chimpanzees inoculated with HGV. HGV RNA was also detected in liver tissue of HGV-inoculated tamarins. Serological test for HGV is currently not available. PCR-based molecular diagnostic assay has been used for preliminary epidemiological study of this virus and it is anticipated that a molecular probe assay for HGV will become widely available soon. Most of the preliminary assays for HGV are thus currently based on PCR of the putative NS5 region.

Little is currently known regarding this virus. HGV is found to be transmitted through blood transfusion and other parenteral routes such as intravenous blood abuse. Preliminary data showed that most of HGV patient have associated risk factors of parenteral contamination. HGV RNA appeared in serum after blood transfusion and prior to the elevation of ALT. HGV-related hepatitis is generally mild, with minor elevation of ALT. Chronic hepatitis may follow acute infection, but the frequency may be lower to that of HCV. Several of HGV patients were co-infected with HCV and/or HBV, probably because these viruses shared the same risk factors. Serum ALT raised in more than half of HGV-infected patients and thus healthy carrier state of HGV may exist.

A preliminary study at the National Institutes of Health demonstrated that HGV RNA was detected in 17% of sera from patients with non-A-E hepatitis, 14% of blood transfusion recipients with minor ALT elevation, and 8% of patients with HCV RNA. Interestingly, the prevalence of HGV in US volunteer blood donors is high. HGV RNA was detected in 1.5-1.7% of US blood donors, the prevalence exceed that of HCV. Some patients with previous diagnosis of non-A, non-B hepatitis and undergone interferon treatment, later tested positive for serum HGV RNA. This provided evidence that HGV-associated chronic liver disease responds to interferon treatment (Alter, 1995).

ARE GBV-C AND HGV THE SAME VIRUS?

Among the 4 new hepatitis viruses, GBV-C and HGV are the more interesting ones and preliminary data indicated that these viruses are quite common. GBV-C and HGV are new viruses which are distinct from GBV-A, GBV-B and HCV. They are human viruses with possible worldwide distribution, at least in the United States, Japan and Europe. There is evidence that GBV-C and HGV are associated with acute and chronic hepatitis, and that persistent infection exists. The viruses are at least parenterally-transmitted and coexist with other known hepatitis viruses, especially HCV. Based on the information available to date, both GBV-C and HGV share some similar characteristics; especially the genomic structure, hydropathicity profile, the extent of nucleotide and amino acid sequence similarity to HCV and other GB agents, as well as the epidemiological profile. It is therefore possible that these two viruses are the same agent. However, the nucleotide and amino acid sequence informa-

tion of both GBV- C and HGV are not publicly available to date, as both companies are currently developing the assays for their own viruses. The comparison of the complete sequences of these organisms, which are expected to be available soon, will elucidate the identity of these two viruses.

THE CLINICAL IMPORTANCE OF THESE NEWLY DISCOVERED HEPATITIS VIRUSES

The prevalence of GBV agents and HGV, obtained from preliminary serological and/or PCR screening of blood donors are not only surprisingly but also alarmingly high. However, the incidence of posttransfusion hepatitis has been reduced to minimal after the introduction of anti-HCV blood donor screening. The discrepancy of high prevalence of the GBV/HGV viruses and the low incidence of PTH has not been explained. It may be because only a low percentage of GBV/HGV-contaminated blood recipients develop clinical hepatitis. Since coinfection of GBV/HGV agents and HCV is common, the screening of anti-HCV-positive bloods may also serve as surrogate marker for reducing the incidence of GBV/HGV-related posttransfusion hepatitis. A number of questions on these new viruses remain to be answered. The size of the problems, clinical significance, mode of transmission apart from parenteral route and population at risk, significance of coinfection with other agents, the association with other non-hepatitis diseases, as well as the necessity of treatment, remained to be studied.

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