Leading article

Hepatitis G virus: identification, prevalence and unanswered questions

Following the cloning and sequencing of hepatitis C virus (HCV), it became apparent that this virus accounted for more than 90% of cases of post-transfusion and sporadic non-A, non-B hepatitis.¹ The residual cases of hepatitis are often referred to as the non-A-E hepatitis group and probably have a viral aetiology. Serum from such patients, and other high risk groups, provided the cloning material from which two independent groups of scientists identified a new virus, provisionally designated hepatitis G virus (HGV)² or GBV-C.³

Virus identification

Linnen *et al*² cloned the new virus from the serum of a patient with non-A, non-B hepatitis, later shown to be positive by second, but not first, generation HCV tests. RNA extracted from the serum was reverse transcribed with random primers and the resulting copy DNA (cDNA) was amplified by SISPA (sequence independent single primer amplification). The amplified products were then cloned into an expression library and a single colony reactive with the serum of the same patient was identified. An anchored PCR was then used to generate multiple overlapping cDNA clones, the sequences of which were determined and combined to create the 9392-nucleotide long HGV genome.

Human serum samples that were reactive with recombinant proteins from two other viruses, named GBV-A and GBV-B, were used in the identification of the second isolate, designated GBV-C. GBV-A and GBV-B were cloned and sequenced from the serum of a tamarin inoculated with the GB agent.⁴ This inoculum was established in 1967, following inoculation of marmosets and tamarins with the serum of a surgeon (initials GB) with acute sporadic hepatitis.⁵ The two viruses present in this inoculum seem to be of tamarin origin, as suggested at the time by Parks and Melnick.⁶ Immunoreactive human serum samples to GBV-A and GBV-B recombinant proteins did not contain sequences of these two viruses when amplified using PCR. However, sequences which belonged to a new virus, GBV-C, were amplified by the use of degenerate primers.^{3,7}

Sequence comparisons of the prototypes, HGV² and GBV-C,³ reveal that they have more than 90% and 95% homology at the nucleotide and amino acid level, respectively. The two viruses are therefore isolates of one and the same virus and as the nomenclature of the virus has not yet been decided, for the purposes of this article it will be referred to as HGV. The virus has only 25% homology at the nucleotide level with HCV. Its genome organisation and the presence of characteristic conserved sequence motifs in functional domains of the genome place the virus within the family of flaviviridae.^{2,3}

Detection of HGV in serum relies on PCR amplification of HGV RNA by reverse transcription (RT)-PCR.² Single or nested primers have been used for amplification of sequences from the non-structural proteins NS3 and NS5a, but also from the 5' non-coding region of the genome. Visualisation of the PCR products is by ethidium bromide staining of agarose gels following electrophoresis or by hybridisation to an internal enzyme or radiolabelled probe. To date, there are no serological tests available for the detection of HGV.

Prevalence of HGV infection

HGV has been detected in 1.7% of blood donors from the USA,² a prevalence higher than that of HCV. HGV RNA was rarely found in the serum of patients with sporadic acute non-A-E hepatitis (14%), but was found in 33% of patients with acute or chronic non-A-E hepatitis following blood transfusion.⁸ High frequencies of infection were also found in patients exposed repeatedly to blood or blood products, including haemophiliacs and those with thalassaemia. The prevalence of HGV RNA positivity in these groups was 18%.^{2,8} The high positivity in patients exposed to blood or blood products was underscored by the observation that 33% of intravenous drug misusers were also positive for HGV RNA.⁸

HGV RNA has been reported in 14% of the patients with sporadic acute non-A-E hepatitis but was also found in a similar proportion of patients with documented acute hepatitis A virus infection (11%). Thus, the increased frequency of infection in these groups does not seem to relate to an aetiological association of the virus with acute non-A-E hepatitis.

Ten per cent of patients with HBV infection, 20% with HCV infection, 10% with alcoholic liver disease, and 8% with autoimmune chronic active hepatitis were also HGV RNA positive. The vast majority of these patients (75-100%) had a history of exposure to blood or blood products, or they were current or ex-intravenous drug misusers. Of the five HGV positive patients in the chronic HBV group, four were homosexual men. It is not clear at the moment whether sexual transmission of the virus occurs: covert intravenous drug use in these subjects could not be ruled out.

Recent studies have confirmed and extended the above findings (Table). Apart from transmission of HGV through blood and by drug addiction, HGV transmission has been shown to occur during haemodialysis^{9,10} and from mother to infant.¹¹

The role of HGV in fulminant hepatitis remains controversial. Three of six patients from Japan were reported to harbour HGV sequences.¹² Subsequent studies, however, have not confirmed this finding (Table)^{13,14} with HGV

Prevalence of HGV RNA in different patient groups

Patient group	Country	No of cases	Per cent positive	Reference
Blood donors	Japan	4/448	0.9	9
	Japan	3/6	50	12
	Japan	0/7	0	13
	UK	0/20	0	14
Haemodialysis	Japan	16/519	3.1	9
	France	35/61	57.5	10
Haematological malignancies	UK	18/38	47	15
IVDU	Japan	12/49*	24	16
IVDU/homosexual (HIV+)	Germany	9/100	10	17
Mother to infant:				
Mothers (HCV+)	Germany	6/30	20	11
Mothers (HIV+)		3/17	17.6	
Infants**		3†/9	33.3	
Plasma pools	Germany	30/106‡	7–40§	17

IVDU=intravenous drug user.

*All HCV positive.

**Only those born to HGV positive mothers.

†Two mothers were HIV positive and one HCV positive.

‡Plasma pools from Europe and USA.

§Depending on origin. Higher positive prevalences in American plasma pools.

RNA positivity at levels similar to those in other liver disease groups.

Relation of HGV infection to induction of hepatitis

Acute and chronic hepatitis have been documented following blood transfusion.² However, more recent prospective studies show that 75% of HGV infected transfusion recipients have no biochemical evidence of liver disease, whereas in those with alanine aminotransferase (ALT) abnormalities the HGV RNA and ALT levels are often asynchronous, and in cases of HGV and HCV co-infection ALT activities mirror HCV RNA levels rather than those of HGV RNA.¹⁸

The relation between HGV infection and the presence of biochemical evidence of hepatitis was studied in a group of 33 patients who were HGV RNA positive and had no other aetiological factors for acute or chronic liver disease. Forty five per cent had normal and 55% increased aminotransferase activities. About a third of the latter group had ALT activities just above the upper limit of normal. In this group no viral, toxic or other causes of liver injury could be found and it was assumed that the chronic hepatitis was attributable to HGV infection.¹⁹ However, absence of liver dysfunction was seen in HGV positive patients with haematological malignancies,¹⁵ in all or the majority of patients on haemodialysis^{9 10} and in the majority of liver transplant recipients (Karayiannis *et al*, manuscript submitted).

Persistence of viraemia

Retrospective testing of stored serum samples from HGV positive patients shows that viraemia can persist for many years (up to 17).^{9 19 20} The occurrence of viraemia in the absence of raised liver aminotransferases suggests that a normal carrier state does exist. This is supported by the findings of Linnen *et al*² who detected HGV RNA with more or less equal frequency (1.7%) in blood donors with and without raised ALT activities.

Treatment with interferon

Treatment of HGV positive patients also infected with either HBV or HCV has shown a decline in HGV RNA levels to negative values during treatment and normalisation of ALT activities.¹⁹ These, however, returned to pre-treatment levels soon after treatment ceased. In one patient with a sustained HCV response to treatment,

persistence of HGV RNA was noted in the presence of normal ALT activities. The virus, therefore, seems to be sensitive to interferon but the treatment regimens and duration of therapy may need to be revised.

Detection of HGV in lymphocytes

HGV RNA has been detected in the lymphocytes of all patients tested.¹⁹ The significance of this finding, which concerns detection of the positive sense viral genome and not of the negative replicative strand, is presently unclear. In view of the technical difficulties encountered in similar studies with HCV, it may be some time before this is resolved.

Concluding remarks

High prevalence rates of HGV have been recorded in patients exposed to blood and blood products, drug addicts, and patients on haemodialysis. Initial observations suggest that hepatic damage is mild or absent. These findings raise the question as to whether HGV is a hepatotropic virus or whether it is a virus with other tissue tropism which may cause hepatitis under certain circumstances. The detection of the virus in lymphocytes raises the possibility that HGV may behave like the Epstein-Barr virus or cytomegalovirus. Confirmation of tissue tropism must await detection of the replicative minus strand either in the liver or lymphocytes or in another organ. In addition, more studies are needed to tackle the issue of whether HGV causes cirrhosis and hepatocellular carcinoma. These studies should be extended to cover patients with haematological disorders and particularly lymphomas, currently of unknown aetiology. The variable association of HGV with hepatitis may relate to viral load, to presence of different strains of the virus, or to other factors, including the possible presence of another agent. The latter is of importance as HGV accounts at best for only one third of cases of residual post-transfusion hepatitis.¹⁸ Finally, in the absence of serological tests which would identify those with previous infection and without current viraemia, it is difficult to ascertain what percentage of patients with HGV clear the virus.

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