Review Article

Newer Hepatitis Viruses

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For nearly two decades the only addition to the hepatitis alphabet was the delta agent (hepatitis D virus, HDV), a defective virus requiring hepatitis B virus (HBV) coinfection for its replication. However, recent advances in molecular technology, including immunohybridization, screening and sequence independent single primer amplification led to the discovery of hepatitis C virus (HCV), the major cause of parenteral non A non B hepatitis and hepatitis E virus (HEV), the major cause of enteric non A non B hepatitis. To the surprise of many, analysis of non A non B hepatitis cases showed that a substantial proportion were also non ABCDE suggesting the existence of additional agents. The question of whether the hepatitis alphabet was to expand or not, was answered with successive announcements of the discovery of a further enteric agent, tentatively called hepatitis F virus (HFV), followed by the blood borne hepatitis G virus (HGV) and the GB hepatitis virus group. What factors led to this sudden identification of these newer hepatitis agents? The major factor has been application of the latest molecular technology to the primate model. The primate model has been instrumental in the characterization of all the known hepatitis viruses because it allows *in vivo* biological amplification followed by *in vitro* amplification

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using molecular technology. This communication attempts to summarize the recent relevant information in relation to these newer hepatitis viruses.

Hepatitis F

Besides the epidemics of enterally transmitted HEV hepatitis, isolated cases of non-parenterally acquired non A non B hepatitis are seen occasionally. Such cases have been reported from northwest England(l), northern Italy(2), France(3), United States(4), and India(5). The agent responsible for sporadic non A non B hepatitis in humans was serially transmitted in rhesus monkey by intravenous inoculation of the stool extract from a patient. A novel agent called HFV (hepatitis French (origin) virus) was present as 27 to 37 nm particles in the infectious stool extract (6). Hepatopathic lesions were noticed in infected monkeys during the acute phase of illness. The purified viral 27 to 37 nm particles consist of a double strand DNA of 20 kb and are detected in infected monkeys liver. The 20 kb long viral DNA has been detected in stool samples from infected monkeys and from sporadic enteric non A non B hepatitis patients. These results indicate that 27 to 37 nm virus like particles may be responsible for sporadic non A non B hepatitis. The discovery of hepatitis F virus has yet to be confirmed by others and more epidemiological studies will be required before the existence of HFV can be accepted by the International Committee for the taxonomy of viruses. Its exact role in human disease in not clear and needs further exploration.

GB Agents

This agent that induced hepatitis and that was passaged serially in primates,

originated from the serum of 34 years old surgeon (with the initials GB) obtained during the third day of jaundice(7). Passage of this "GB agent" in tamarins allowed extensive virological characterization. A subtraction PCR methodology known as representational difference analysis was used to clone specific nucleotide sequences present in the infectious plasma from a tamarin infected with the GB hepatitis agent. From the above experiments, two flavi-like viruses were cloned which were designated GB virus A (GBV-A) and GB virus B (GBV-B)(7). In order to determine the prevalence of GBV-A and GBV-B in various populations, scientists at Abott developed antibody assays based on GVB-A and GVB-B recombinant proteins. In a West African population, some serum proteins were found to be dually reactive to GBV- A and B proteins with IgM class antibodies being detected(8) with the indication of recent infection. PCR was used to determine whether any viral sequences could be detected. The PCR primers used were designed to amplify a gene segment from GBV-A, GBV-B or HCV *(i.e.,* degenerate or consensus primers). Analysis of the product of the polymerase chain reaction from one of the immunoreactive samples fortuitously revealed that another related but distinct virus was present, the GB virus C (GBV-C). This nomenclature is confusing because clearly GBV-C was not found in the original infectious tamarin pools produced by passage of the GB agent. Later, further experiments on the GB viruses by Abbott Laboratories have shown that some uninoculated tamarins are infected with viruses very similar to GBV-A, suggesting that GBV-A may have been a latent or indigenous virus adventitiously acquired during the serial passaging and pooling in tamarins. GBV-B is the virus most likely to be responsible for the original hepatic illness in the surgeon, although sequences

corresponding to GBV-B have not been detected in any human samples(9). GBV-A and GBV-B were identified in tamarins and are probably not human viruses.

Hepatitis G Virus

Certain blood borne viruses were independently discovered by two groups of investigators and named GB virus C (GBV-C)(10,11) and hepatitis G virus (HGV)(12). GBV-C and HGV share 86 per cent of their nucleotides and 96% of their amino acid sequences and hence they are perhaps different isolates of the same virus or closely related variants of the same species (13). Nucleotide humology between HGV/GBV-C and HCV is much less (approximately 25%) suggesting that these viruses are probably only distantly related.

The presence of HGV has been reported from United States (14), Western Europe-(15), Australia (8), UK (16), India (17) and Pakistan (18). There are no screening tests yet available for HGV. A polymerase chain reaction test to detect HGV-RNA is available primarily for research purposes. Recently an immunoassay for antibodies to a viral protein E_2 located on the surface of the virus has been developed (19). Thus E_2 specific antibodies might serve as a useful marker for diagnosing recovery from HGV infection. Whether E_2 specific antibodies have a protective role against HGV infection, is yet to be determined. It was also seen that humoral immune response to E_2 is associated with loss of detectable HGV viremia and *vice versa.* Extensive clinical data indicating the prevalence of HGV-RNA in the general population from various countries is not available. Studies of prevalence are restricted to small numbers comprising selected high risk groups like transfusion recipients and voluntary blood donors. HGV-RNA has been detected in 1.4 to 1.7% of randomly selected blood in the

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United States (12). The rate of infection in the general population may be higher as voluntary blood donors comprise a select group of populations.

The blood borne nature of HGV infection has been clearly elucidated (12). HGB infection due to hemodialysis has also been described (20). The largest study of transfusion associated HGB infection showed that only ten per cent of recipients (35 of 357) were found to be infected with HGV after blood transfusion (21). The exact rate of HGV transmission is not known; transmission to at least one per cent of recipients is probable given the one to two per cent prevalence of viremia among donors. There is a high level of HGV contamination of non virus inactivated blood products but their use was not associated with high rates of persistent infection in recipients (16). The infectivity of HGV in blood products is perhaps less than that of HCV or the virus may be less able to establish persistent infection in humans. As compared to patients with acute hepatitis C, subjects with acute non A-E post transfusion hepatitis have only minimal elevation of hepatic enzymes and no clinical jaundice (21). Chronic hepatitis due to HCV in transfusion recipients is not well established as histologically documented chronic liver disease has never been seen. To what extent these non A-E post transfusion hepatitis is solely due to HGV is still far from clear.

As regards acute hepatitis, in a study from four US countries, HGV-RNA was detected in 4 of 45 patients with a diagnosis of acute non A-E hepatits (9%), 23 of 116 patients with hepatitis C (20%) and 32 of 100 patients with hepatitis B (32%)(14). The clinical characteristics of the acute illness were similar for patients with HGV alone and those with hepatitis A, B or C with or without HGV. During a follow up period of one to nine years, chronic hepatitis did not develop in any of these patients with HGV alone but 75% were persistently positive for HGV RNA as were 87% of those with both hepatitis C and HGV. The rates of chronic hepatitis were similar in patients with hepatitis C alone (60%) and those with both hepatitis C and HGV infection (61%). In another study, HGV RNA was detected in 13% of cases of acute hepatitis but none progressed to chronic liver disease (12). As coinfection with HBV and HCV was frequent in this study, interpretation of disease due to HGV alone was difficult. The documented prevalence of HGV RNA in acute hepatitis and in various other groups in Western Europe is reproduced in *Table 1(15)*.

There are few reports claiming an association between HGV infection and fulminant hepatic failure (12, 22). The agent has also been demonstrated in 10% of patients with non A-E chronic liver disease; a causative association with disease can only be assumed and is not yet definitely established (13). HGV infection is frequent among patients with cirrhosis who undergo liver transplantation, also infection with the virus persists after grafting but does not seem to be associated with recurrent liver disease. In many HGV viremia is detectable in a large proportion of patients only after transplantation (23). Whether this finding reflects reactivation or spontaneous acquisition of the infection is unknown.

Hepatitis G and Vertical Transmission

There is preliminary evidence for mother to baby transmission of HGV. A mother tested positive for HGV by reverse transcription polymerase chain reaction (RT-PCR) before and at delivery. Her baby tested HGV negative by RT-PCR at birth but serum samples at four and six weeks of age were HGV positive, suggesting transmission of HGV from mother to baby (24).

TABLE I-*Hepatitis G Virus (HGV) RNA Rates in High Risk Groups in Western Europe.*

Diagnostic Groups	Number Studied	HGV-RNA % Positive
General population	200	0.5
Acute NANBNC	7	14
Chronic NANBNC	100	6
Multiply transfused		
Thalassemia	100	18
Hemophilia	49	18
Drug addicts	54	33
Primary biliary cirrhosis	38	0
Autoimmune hepatitis	52	8
Alcohol induced chronic liver disease	49	10
Chronic hepatitis B	72	10
Chronic hepatitis C	102	21

Adapted from reference 15.

Here transmission from the mother appears unlikely to have occurred in utero because of the negative HGV blood specimen. Transmission of HGV is most likely to have occurred perinatally either during the delivery or early in the post partum period or via breast milk. Lin et al. reported three mothers who had Caesarean section and were HGV positive by RT-PCR but did not transmit infection to their babies (25). However the Caesarean section may have protected these babies from acquiring HGV, as it does with hepatitis B and HIV. More clinical data is still required to definitely establish vertical transmission of HGV.

So where does this leave us? There is no definite data to establish the role of HGV in the causation of liver disease. The exact sites of multiplication of the virus and its various modes of transmission are still far from clear. The high prevalence of HGV infection among patients with hepatitis A or B without history of drug abuse suggests that there are many modes of transmission besides the parenteral route. The clinical spectrum of disease attributed to HGV is still not well defined. A majority (73%) of patients with HGV infection demonstrate no elevation of hepatic enzymes and its presence does not seem to alter the clinical course of liver disease caused by established agents(21). Even in voluntary blood donors, the prevalence rates of HGV RNA were similar in those with and without elevation of ALT levels (1.5 and 1.7% respectively) (12). Transfusion recipients with HGV demonstrate only minimal elevation of hepatic enzymes and progression to chronic liver disease is unknown. Hence in the study of community acquired hepatitis (14), attributing the cause of hepatitis to HGV alone would be just a presumption. May be hitherto unknown agents still exist which are responsible for hepatitis in these patients. Thus it still remains to be determined if HGV is a true virus which has the potential to cause liver disease or merely travels with as yet unknown non A-E viruses.

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