Viral hepatitis

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Foreword by Professor Arie J Zuckerman
The last two decades have witnessed an explosion in knowledge of viral hepatitis, a major public health problem throughout the world affecting several hundreds of millions of people. Viral hepatitis is a cause of considerable morbidity and mortality in the human population from acute infection and the chronic sequelae which include, with at least two types of infection, chronic active hepatitis, cirrhosis, and primary liver cancer. Hepatocellular carcinoma is one of the 10 most common cancers worldwide.

The existing alphabet of viral hepatitis includes a range of totally unrelated and often highly unusual pathogenic human viruses:

HEPATITIS A VIRUS
A small unenveloped symmetrical RNA virus which shares many of the characteristics of the picornaviruses family. This virus is classified as Enterovirus type 72, and is the cause of infectious or epidemic hepatitis transmitted by the faecal-oral route.

HEPATITIS B VIRUS
A member of the hepatadnavirus group, double stranded DNA viruses which replicate by reverse transcription. Hepatitis B virus is endemic in the human population and hyperendemic in many parts of the world. Natural hepatitis B infections also occur in woodchucks, beechy ground squirrels, and ducks.

HEPATITIS C VIRUS
An enveloped single stranded RNA virus which appears to be distantly related (possibly in its evolution) to flaviviruses, although hepatitis C is not transmitted by arthropod vectors. Infection with this newly identified virus appears to be common in many countries, and it is associated with chronic liver disease and apparently also with primary liver cancer in some countries.

HEPATITIS D VIRUS
An unusual single stranded circular RNA virus with a number of similarities to certain plant viral satellites and viroids. This virus requires hepatadnavirus helper functions for propagation in hepatocytes, and is an important cause of acute and severe chronic liver damage in many regions of the world.

HEPATITIS E VIRUS
The cause of enterically-transmitted non-A, non-B hepatitis, is another non-enveloped single stranded RNA virus, which shares many biophysical and biochemical features with calicivirus. Hepatitis E virus is an important cause of large epidemics of acute hepatitis in the subcontinent of India, central and south-east Asia, the Middle East, parts of Africa and elsewhere; and this virus is responsible for high mortality during pregnancy. Much progress is currently being made with this important infection.

Roger Williams and the Institute of Liver Studies at King’s College contributed much original and fundamental knowledge to each of these different viruses.


Many intriguing questions remain: is there a hepatitis F virus as the cause of fulminant hepatitis, and a hepatitis G virus; what are the implications of the hepatitis B surface and core variants, and is non-B, non-C hepatitis awaiting discovery?

4 Lee WM, Reed WD, Osman CG, Vaherman J, Zuckerman AJ,
Viral hepatitis continues to be a major global health problem. It is therefore, not surprising that viral hepatitis has been one of the research domains in the Institute of Liver Studies in King's. With the advancement of many scientific disciplines including molecular biology and immunology, we have witnessed an explosion in knowledge of these viruses in the last two decades. We now know that there are at least five major types of primary hepatotropic hepatitis viruses (Table I). Acute hepatitis caused by other viruses in whom involvement of other organs are more common and prominent than liver involvement notably cytomegalovirus and herpes simplex virus is not described here.

Hepatitis A virus (HAV)

Hepatitis A virus was first discovered in faeces of infected patients by immune electron microscopy in as early as 1973, 4 it is currently still ranked by the World Health Organisation as a prevalent infection worldwide; especially in countries where overcrowding and poor standards of hygiene and sanitation are prevalent as shown by the recent major outbreak involving 1-2 million persons in Mainland China in 1988.

VIROLOGY

The HAV genome is a linear, single stranded ribonucleic acid of messenger-sense (positive) polarity. The single open reading frame gives rise to a precursor polypeptide which is subsequently cleaved into four different polypeptides VP1–4 (Fig 1). Existing information suggests that the molecular sizes of these peptides are VP1 33kd, VP2 27kd, VP3 29kd, VP4 (truncated) 17 amino acids. Around 60 copies of each of these four structural proteins, VP1 to VP4 form the capsid of the typical mature HAV. 2, 5

Although HAV is classified as a picornavirus, 1 increasing evidence supports the view that HAV is different from the other well studied four genera of this virus family as evidenced by the size of its structural proteins, the outstanding overall stability of the virus, and the resolution of only one immunodominant neutralisation site. 2, 4, 7

PATHOGENESIS OF LIVER DAMAGE

The pathogenetic mechanism of liver cell damage in acute HAV infection is still unclear. Existing evidence suggests that a close interplay between the virally controlled and host immunological factors is essential to cause a cytolytic infection with elimination of the virus. 2 The postulation that hepatocyte destruction is mediated by anti-HAV antibody with or without the help of complements is untenable 3, 8 and the interferon system is also unlikely to play a major role in the elimination of the virus in vivo. 10 Two recent studies provide evidence that cytotoxic T cells capable of lysing HAV-infected target cells develop in the course of HAV infection. 11 This effect is also shown to be virus-specific and is functionally restricted by the major histocompatibility complex.

CLINICAL COURSE

Hepatitis A virus infection usually causes a minor or unnoticed illness in children and young adults. On a worldwide scale, less than 5% of the cases are recognised clinically. 11, 13 In a recent outbreak in Shanghai, China, approximately one third of those subjects serologically positive for acute HAV infection were asymptomatic, and less than 20% had overt clinical hepatitis. 11 In a recent study in our institute, an increasing mortality from HAV with increasing age was observed. 16

The persistence of high titre IgM anti-HAV antibodies for up to 400 days and the demonstration of intestinal reinfection with prolonged viral excretion may help to explain the mechanism of viral perpetuation in highly endemic areas. 17 Whether relapse of HAV infection occurs remains controversial but the question of possible chronicity from HAV has been addressed carefully and can be excluded with confidence.

In those rare instances in which severe fulminant HAV infection develop, liver transplantation is the treatment of choice. Interestingly, HAV was detected in the graft livers in two of our patients with fulminant hepatitis A after liver transplantation and one had histological evidence of recurrent acute viral hepatitis and HAV detected in stool.
PASSIVE AND ACTIVE IMMUNISATION

High titre serum immunoglobulin preparations have long been recognised to be of value in both pre-exposure and post-exposure prophylaxis against HAV infection. Not all travellers from developed countries to highly endemic areas require protective immunoglobulins, however, screening of patients for antibodies against HAV before giving immunoglobulins is economically sensible and results in a more effective use of a limited resource.

Although HAV infection does not lead to chronic hepatitis and cirrhosis, it is an important source of morbidity. An effective, safe, and low cost vaccine would be beneficial, especially in the developed countries in which the incidence of HAV infection is low. Killed HAV vaccine has been shown to be safe and effective in animals and clinical studies are ongoing. Attenuated HAV vaccine and recombinant HAV vaccine have also been developed and are being assessed.

Hepatitis B virus (HBV)

After identification of the HBV, research into all aspects of infection accelerated with the development of a sensitive radioimmunoassay in Chicago in 1972 for the hepatitis B antigen (which is now called HBsAg). There had not been significant involvement by our Institute until that year – but the pace of progress in HBV research over a period of just less than 20 years is striking.

Epidemiology

Hepatitis B virus had not been implicated as the major aetiological factor worldwide in chronic liver disease and the associated hepatocellular carcinoma until the early seventies. One of the early studies that drew attention to this association was from our Institute in 1973. In a survey of 264 patients with chronic liver disease, 18% with chronic active hepatitis were found to be seropositive for HBsAg. These patients were generally male and had been born outside the United Kingdom – messages that hold true today. The very low incidence of HBsAg in primary biliary cirrhosis was attributed to trans-fusion – a reminder of the changes in clinical practice over the past 20 years. It is likely that these figures were an underestimate of the contribution of HBV to both conditions because of the sensitivity of the assay at that time. The current estimate is 300 million chronic HBsAg carriers worldwide and 75% of them are Asians. Our recent survey in South East London showed a carriage rate of 1%, more frequent than previously thought. It is depressing that an estimated 40% of them will eventually die of chronic liver disease and/or hepatoma.

Virology

The HBV genome is the smallest of all known animal DNA viruses, being only 3200 base pairs (bp) in length. The genome exists in the virion in a circular conformation, with circularity maintained by complementary termini of 250–300 bp at the 5′ end of each DNA strand (Fig 2). The long (minus, −) strand is the coding strand from which viral mRNA and the viral pregenomic RNA are transcribed. Its end is linked to a protein that probably serves as its transcriptional primer. The 5′ ends of both strands are located near 10–12 bp direct repeats (DR1 and DR2) which serve as the primary sites for replication of the two strands.

The coding organisation of the viral genes is remarkably compact, with several regions of the sequence potentially translatable in more than one frame – clearly an efficient use of a genome of such limited size (Fig 2). Six overlapping open reading frames (ORFs) are identified and four ORFs, designated (surface) S/pre-S, (nucleo-
capsid, core) C/pre-c, (polymerase) P, and X are known to code for viral polypeptide/antigen (Table II). An enhancer, a glucocorticoid responsive element as well as four promoter elements (surface, core, pre-S1, X) have also been identified.

The surface ORF contains three in phase translation start codons encoding three envelope polypeptides. The shortest envelope polypeptide, designated ‘major’ based on its relative abundance, contains the group (a) and subtype (d/y, w/r) determinants of the HBsAg. The middle envelope polypeptide contains the major envelope polypeptide plus an extra 55 N-terminal amino acids containing the pre-S2 antigen. The large envelope polypeptide contains the entire middle polypeptide plus an additional 108–119 amino acids including the pre-S1 antigen. The large polypeptide appears to be an important component of the complete virion and may be involved in host cell binding and entry. Of particular interest is the recent demonstration that there is an antigenic mimicry of IgA epitope by a HBV cell attachment site in pre-S1, indicating that HBV may bind to hepatocyte through IgA receptors. Pre-S1 also exerts important structural effects on virus particle formation and secretion such that overproduction of the large envelope polypeptide relative to the other polypeptides inhibits the secretion of HBsAg and may have significant pathogenetic consequences (ground glass formation, hepatocellular necrosis).

The nucleocapsid ORF contains two in phase translation start codons whereby it encodes two identical polypeptides except for 29 amino acids at the extreme N-terminus of the longer (pre-c) polypeptide. The core (c) polypeptide is a nucleic acid binding protein which encapsulates the viral nucleic acid. The 29 N-terminus amino acids of the pre-c translation product function as a single peptide which directs the nascent pre-c polypeptide to the endoplasmic reticulum where, after proteolytic cleavage of N-terminus and C-terminus amino acids, it is either transported to the Golgi apparatus and secreted as HBeAg or transported into the nucleus. The pre-c polypeptide is not required for viral replication. The importance of pre-c in the transport and formation of serum HBeAg has been emphasized by the recent description of a HBV mutant occurring in patients seropositive for anti-HBe, but seronegative for HBV DNA and have active liver disease. This mutant has been characterised to have a single point mutation in the pre-c region which generates a translational stop codon. The absence of pre-c excludes the production of HBeAg and this explains the persistence of serum HBV DNA in the presence of anti-HBe.

The polymerase ORF overlaps all the others. It encodes the viral polymerase and reverse transcriptase activity as well as a DNA binding protein located at the 5’ end of the DNA (−) strand which is thought to serve as a primer for reverse transcription of the RNA pre-genomes.

The X ORF encodes a polypeptide expressed in some patients with chronic HBV infection and HBV related hepatocellular carcinoma. The X polypeptide has transcriptional transactivating properties which positively regulate transcription from HBV and other viral promoters as well as cellular promoters. Whether X protein/gene is tumorigenic or not is controversial.

The function of the two potential ORFs, termed ORF5 and ORF6 is not known and no translational product has been identified. The most conserved area of the HBV genome, termed U5-like region, has a good homology to the 5’-unique region of retrovirus long terminal repeats, which together with the unique replicative cycle of HBV (with a reverse transcription step) suggest that HBV and retrovirus may have a common evolutionary origin.

The other remarkable feature of HBV is its pronounced hepatotropism. This may be related to its surface proteins which bind directly or indirectly to hepatocytes for its entry. As mentioned above, pre-S1 sequences are involved in the binding of virions to IgA receptor on the surface of hepatocytes which may be the first step in the infectious cycle. Less convincing is the
evidence that pre-S2 protein carries a binding site or receptor for polymerised human serum albumin (pHSA) which in turn binds to hepatocytes.\(^7\) pHSA, however, is an in vitro product (albumin treated with glutaraldehyde) and has never been found circulating in human subjects.\(^1\) It is therefore, unlikely that this is the mechanism of viral entry into the hepatocytes. Interestingly, human monomeric serum albumin was also shown recently to bind irreversibly to cysteine of the small HBsAg protein, suggesting an alternative way of viral entry into the hepatocytes.\(^3\)

**IMMUNOLOGY**

One major sustained thrust of HBV research has been the relationship between HBV and the immune system: the questions that were relevant then remain so now, that is, the basis of chronic carriage and the mechanism of liver damage. In this section some of the institute's work will be highlighted.

**(a) Early studies indicating the involvement of the immune system**

The test of leucocyte migration inhibition as an indicator of cell mediated immunity has been central to much of this work. Unfortunately, the factor(s) responsible for inhibition of migration that was released in response to antigen specific T cell stimulation has not been identified. In one of the earlier studies using this assay, sensitisation to HBsAg was identified in all six patients convalescent after acute HBV infection and seropositive for HBsAb.\(^4\) There was also a response to HBsAg in many chronic HBV carriers. Two control groups were used in that study: factory workers unlikely to have been exposed to HBV and those working within the immunology group at that time. The incidence of sensitisation to HBsAg of 30% in the factory workers was almost certainly a reflection of the assay used at that stage where antigen was probably impure. This was further supported by a high positivity rate in the laboratory personnel.

**(b) An autoimmune disease/component?**

The relation between autoimmune disease and viral infection, including HBV, came under scrutiny at this stage. A cell mediated immune response to HBsAg was found in 62% of patients with HBsAg negative chronic active hepatitis, only a small proportion of whom had serological evidence of previous exposure.\(^5\) This led to the provocative hypothesis that HBV itself might be a trigger for an autoimmune response that would persist after eradication of HBV.\(^5\) This view persists, at least in part, and although HBV has been eliminated from the list of candidate triggers, current alternatives include herpes simplex, measles, Epstein-Barr virus, hepatitis D virus and most recently hepatitis C virus and hepatitis A virus infection. This hypothesis has proved an excellent starting point for many research workers.

There is no question, however, that during active HBV infection an autoimmune response to self antigens is induced at both a T and B cell level; it is simply that after the complete eradication of replicating HBV, these reactions are not sustained. In a further series of HBV carriers, cell mediated immune responses to HBsAg were identified in 25% of asymptomatic patients – and were particularly associated with ongoing liver damage. These reactions correlated tightly with cell mediated responses to liver specific lipoprotein.\(^6\) The theory that such autoimmune reactions were triggered by HBV received support from a serial study of patients with acute infection.\(^6\) At the time of entry to the study, 62% of patients were reactive to HBsAg; those that were negative were those with the highest titre of HBsAg (interpreted as immune interference with T cell function). The severity of liver damage correlated closely with the strength of the cell mediated reaction, suggesting that immune clearance of HBsAg was the mechanism of liver cell injury. In this series, T cell recognition of liver specific lipoprotein was detected transiently in 50% of patients, but never after development of immunity to HBV.

This work was supported by parallel studies at the B cell level with the development of a sensitive and specific radioimmunoassay for antibody to liver specific lipoprotein.\(^7\) Antibody was detected in almost all patients with viral related chronic active hepatitis, including most of those patients positive for HBsAg. Patients with less severe lesions histologically, that is, those with chronic persistent hepatitis, were less frequently positive and when present, these antibodies were present in lower titre. By contrast patients with acute, self-limited infection were positive only transiently (as seen with T cell sensitisation).

**(c) Study of the Dane particles and the reactive antibody system**

Dane particles were described in 1970, in sera, faeces, urine and synovial fluid. Intranuclear particles and occasional cytoplasmic particles were reported in liver tissue with a diameter of 26 and 42 nm.\(^8\) Although the HBeAg/anti-HBe system was recognised as early as 1972, the relation between viral antigen expression in liver tissue, viral replication and serum levels of HBV and Dane particles was not finally resolved until recently with the development of assays for serum HBV-DNA and the recognition of the precore mutant, an important cause of HBe negative HBV related liver disease.\(^9\) That there were different patterns even among patients with active HBV replication was recognised in 1978.\(^9\) This was an electron microscopy based study in which Dane particles with or without DNA polymerase (complete or full particles respectively) were sought in sera and the findings related to clinical, serological and histological parameters. HBeAg positive patients were always positive for complete particles, indicating infectivity; and there was no relation to tissue damage. In patients seropositive for anti-HBe, but with ongoing liver damage, Dane particles were also present, but 98% of these were defective. This may have been an early correlate of the precore mutant and suggests a form of packaging disorder in this group.
In the same study there was also the first description of seroconversion from HBeAg positivity to anti-HBe positivity occurring in the natural course of chronic HBV infection. This serologic event was later shown to be of central importance in the understanding of the natural history of chronic hepatitis B, and in particular its relation to the chronic liver disease. Patients showing seroconversion and remission of liver disease also had termination of virus replication, while those with continuing disease activity developed a detectable type of virus replication.

One of the studies that best stands up to retrospective evaluation was the identification of an antibody system reactive with whole Dane particles which was distinct from HBsAg or HBeAg. These antibodies appeared early in the course of acute infection, long before the appearance of antibody to either HBsAg or HBeAg; carriers were negative. The association between anti-Dane particle and viral elimination indicated that it might be neutralising. Neither pre-S1 nor pre-S2 had been identified then; almost a decade passed before the identification of binding between the pre-S antigens and the plasma membrane of hepatocytes. Recently, it has been shown that anti-Dane particle is in fact a mixture of anti-preS1 and anti-preS2 antibodies, anti-preS1 being the earliest antibody response to appear in acute infection. Its specificity is directed against the hepatocyte binding sites of HBV; being virus neutralising both in vitro and in vivo.

(d) Regulation of the immune system
The presence of circulating antibody with the potential to mediate liver damage triggered the study of suppressor cells. The assay that was used to study the regulation of B cell function used Con A activated T cells to inhibit pokeweed mitogen stimulated B cells; the number of proliferating B cells was then quantified by a reverse haemolytic plaque assay. Although the assay is cumbersome and time consuming, the data obtained were remarkably clear; suppressor cells were defective in HBV related and autoimmune chronic active hepatitis. There were differences, however, between the two groups with respect to the response to corticosteroids; suppressor cell activity was corrected by incubation with prednisolone in autoimmune cases but no effect was seen in patients with HBV related disease. This observation parallels the clinical response. Defective suppressor T cell regulation of antibody production was also shown to correlate with disease activity. In addition transient defects in suppressor cell function were associated with the onset of liver damage in acute infection—

which would be permissive for antibody production.

Two further studies were performed in the 80s that extended and strengthened the contributions on cell mediated immunity to HBV antigens from the 70s. These had the advantages first, of a broader range of antigens being available and second, the technology, which now included those prepared by recombinant technology, were of superior quality. In a study of chronic HBV infection, T cell responses to both HBeAg and HBsAg were compared. Among those with a history of acute infection or those who had been vaccinated, there was consistent recognition of HBsAg; none of the HBV carriers recognised HBsAg and neither did naive controls. This was the clearest difference noted between these respective groups in any of the studies performed. In contrast, chronic HBV carriers always recognised HBeAg, consistent with the view that this antigen is the target for immune mediated lysis in these patients. In further experiments, it was proposed that the failure to respond to HBsAg was actively mediated by T suppressor cells. In the second study, patients who were vulnerable contacts of those with acute HBV infection were followed serially from the very earliest pre-symptomatic phase, 30 to 70 days before the onset of liver damage, through to recovery. Cellular immunity to pre-S was the first detectable response and substantially predated liver damage in every case. T cell sensitisation to HBeAg appeared next, just before the detection of IgM anti-HBc. A cellular response to HBsAg was detected 10 days before the onset of liver damage, indicating that this could be pertinent to viral elimination.

(e) The search of the immune viral target
One of the long standing research interests in our Institute has been T cell cytotoxicity. The method used, the autologous hepatocyte microcytotoxicity assay, placed reliance on the ability of healthy hepatocytes to adhere to plastic (not a physiological function) and the number of cells available was small and adherent cells were counted manually. It was felt when Mario Mondelli was doing the experiment that it should be called the macrocytotoxicity assay! It did, however, overcome the absolute requirement for HLA compatibility between target and effector cell; as with all experimental models it was established to answer specific questions and it has undoubtedly achieved this. A previous attempt to investigate cytotoxicity in HBV infection using red blood cells coated with HBsAg illustrated the difficulties of developing an appropriate assay. Increased cytotoxicity was identified in the convalescent phase of acute infection and a smaller proportion of those with chronic HBV infection. This was probably all NK activity because the T cells would not have seen either class I or II antigens on the red cell and even if they had been present, they were unlikely to have been compatible. With the autologous hepatocyte cytotoxicity assay, about 50% of patients exhibited activity. The figure was higher in patients with active liver disease and in contrast with autoimmune disease in which the

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**TABLE II** Hepatitis B virus polypeptide/antigen

<table>
<thead>
<tr>
<th>HBV Gene</th>
<th>Polypeptide/antigen</th>
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<tr>
<td>S</td>
<td>HBsAg (major protein)</td>
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<tr>
<td>S</td>
<td>pre-S2 peptide (middle protein)</td>
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<tr>
<td>pre-S1 + S</td>
<td>pre-S1 peptide (large protein)</td>
</tr>
<tr>
<td>C</td>
<td>HBeAg and HBeAg</td>
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<tr>
<td>pre-C+C</td>
<td>core peptide</td>
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<tr>
<td>F</td>
<td>DNA polymerase</td>
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<td>X</td>
<td>X protein</td>
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effector cells were non-T, effector cells in HBV were both T and non-T. The non-T component was thought to be directed against normal membrane components mediated by an ADCC type reaction. The striking and important observation from this study was that T cell cytotoxicity was restricted to HBcAg positive cases, indicating a relation to replication.

This finding was taken further in experiments to determine whether HBcAg was the target. The experiments were performed in the presence or absence of antibody to either HBsAg or HBcAg. While it was assumed that these would block the reaction to the relevant antigen, it is now known that T and B cell epitopes are not shared. Experiments in mice, however, indicate that for mice at least the T and B cell epitopes are close and the same is probably true for man. These experiments clearly implicated HBcAg as the target for immune assault; any doubts based on immunological theory were dispelled by a simple study in which it was shown that the hepatocytes which survived exposure to autologous T cells never contained HBcAg, but did contain HBsAg.106

These studies provide evidence that the pattern of hepatic HBV antigen expression is important in determining the host immune response. A more recent study using double staining for viral antigens has shown the complex course of hepatic HBV antigen expression in chronic infection with active HBV replication.107 In this study it was demonstrated that the presence of cytoplasmic HBcAg was closely associated with high levels of HBV replication; in contrast, cytoplasmic expression of HBcAg was associated with liver damage. Another interesting observation in this study was an inverse relationship between serum HBsAg titre and hepatic expression of HBcAg, suggesting a variable export of HBsAg in various phases of chronic HBV infection. This was confirmed by a follow up study which demonstrated the export of HBsAg more directly using a primary hepatocyte culture system.102

(j) Role of cytokines
The possibility that an interferon-alpha deficiency might be associated with chronic HBV infection has been well aired. In a very recent study patients with acute and chronic HBV infection were compared with respect to interferon production in the liver seeking both protein and gene. In acute infection both gene and protein were expressed; in chronic infection both were rarely detected and a negative correlation with HBcAg positive cells was identified – suggesting that HBV might modify interferon gene activation,103 which was in accord with the in vitro studies reported by other workers.104–108 A further step was taken to determine the expression of interferon-α receptor in chronic HBV infection which showed no difference with both normal and liver disease controls, indicating that HBV had no effect on interferon-α receptor expression.103

Previous studies had shown a defect of T cell activation in response to a range of stimuli in chronic infection. In particular interleukin-2 production and interleukin-2 receptor expression were reduced.108,111 Both were shown to be improved by in vitro treatment with interferon alpha112. In the treatment of chronic HBV carriers with interferon alpha, seroconversion was shown to be associated with massive release of a series of cytokine including interleukin 1β and tumour necrosis factor-α, but whether this was a non-specific consequence of inflammation was impossible to say.110 In a further study, the production of interleukin 1 and tumour necrosis factor by peripheral blood leukocytes are also shown to be increased in patients with chronic HBV infection.110,114

TREATMENT
The identification of HBV in a substantial proportion of patients with chronic active hepatitis in the mid 70s, in whom autoimmune mechanisms had long been suspected, soon led to the hypothesis that liver damage may be related to an autoimmune reaction against the virus itself. The concept of virus induced autoimmune reactions in general had been widely disseminated by then. It had also been proposed that persistent HBV infection might be the result of a poor immune response to the virus and that immunosuppression with prednisolone and azathioprine would favour evolution of chronic disease.113 Immunosuppression was the mainstay of therapy then and for a further 10 years before the wisdom of this idea became accepted. The identification of the glucocorticoids responsive element in the late 80s further suggested that corticosteroids may have an additional direct pro-viral effect.114,115 This proviral effect of corticosteroids has also been shown more directly using a primary hepatocyte culture system.118

Given that an immune defect had been postulated to account for persistent HBV infection, the move to therapy with hepatitis B antibody in 1973 was logical119, for 1973 it was also inspired. Plasma from blood donors positive for hepatitis B antibody was pooled and given as a single infusion to a small series of patients seropositive for HBsAg. In two of five cases serum was found to become transiently negative for HBsAg and to remain so for nine days. These two cases had lower titres of HBsAg and were almost certainly anti-HBe positive (although this assay was not available). In almost every case serum C3 concentrations fell indicating complement consumption – and by implication antiviral activity. The conclusion, that without repeated long term infusions clearance of HBV was most unlikely, remains valid, particularly as all these patients were maintained on immunosuppression throughout. The approach may have been more correct than was appreciated at the time, HBV specific immunoglobulin now has an established and important role in prophylaxis after transplantation for HBV related chronic liver disease. Anti-HBs (the main constituent of such pooled plasma) would not be the preferred antibody if these experiments were to be performed today. Instead, antibody against either pre-S1 or pre-S2 polypeptides which contain the binding sites for HBV attachment to hepatocytes would be preferred; with the advent of humanised monoclonal
TABLE III Agents that have been studied in the treatment of chronic HBV infection

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<tr>
<th>Anti-virals</th>
<th>Immunomodulators</th>
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<td>Interferons</td>
<td>BCG vaccination</td>
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<tr>
<td>Alpha</td>
<td>Levamisole</td>
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<tr>
<td>Beta</td>
<td>Interferon-2</td>
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<tr>
<td>Gamma</td>
<td>Interferon-gamma</td>
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<tr>
<td>Tumour necrosis factor</td>
<td>Thymosin</td>
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<tr>
<td>Adenine arabinoside (Ara-A)</td>
<td>Tumour necrosis factor</td>
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<td>Acyclovir</td>
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<tr>
<td>deoxyacyclovir</td>
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<td>Zidovudine</td>
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<td>Suramin</td>
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<td>Ribavirin</td>
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<td>Phosphonoformate</td>
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<td>Quinacrine</td>
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<tr>
<td>(+)-cyanidanol-3</td>
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<td>Phyllanthus amarus</td>
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Table IV shows the determinants of positive and negative responses in chronic HBV infection. The determination of positive response includes factors such as acquisition in adulthood, female sex, and high pretreatment ALT. The negative response is determined by factors like low pretreatment ALT, active histology, HIV antibody positive, and anti-HDV negative. These factors play a crucial role in the response to IFNa therapy.

IMMUNISATION

The first generation plasma derived HBV vaccines were licensed in 1981. The fear of transmission of blood borne diseases as well as their cost had considerable effects in limiting their acceptance and use. With the second generation yeast derived recombinant HBV vaccines launched in 1986, much more rapid progress is being made with vaccination of the high risk groups such as health care personnel and babies born to HBsAg seropositive mothers in most developed countries. In some endemic areas, all newborn babies are routinely vaccinated. The World Health Organisation has recommended routine HBV vaccination for countries that possess the economic capacity to purchase the vaccine and where the HBV carrier rate exceeds 2-5% of the population. Third generation synthetic peptide vaccines have already been developed, their safety, immunogenicity and protectivity against HBV infection are at present being evaluated.

The seroconversion rates of all these vaccines vary from 80 to 95%. The same response rate (82-6% > 50 miU/ml) was shown in our institute in a group of health care personnel. Around 35% of healthy HBV vaccine responders will have their anti-HBs titer dropped to below 10 miU/ml in five years' time. The Immunisation Practices Advisory Committee at present does not recommend booster doses for these subjects. Studies in homosexual men, however, showed that the relapse rate attack rate of chronic HBV infections in vaccinated responders was 1-8% annually. Although most of these new infections were subclinical seroconversions, one clinically apparent HBsAg event has been documented. Hepatitis B virus mutants may also be
Hepatitis C virus (HCV)

After the isolation of HBV in the 1960s and the HAV in 1970s, it became obvious that there was a proportion of patients with clinically viral hepatitis without a defined aetiology. The majority of these occurred after blood transfusion or in intravenous drug users and led to chronic hepatitis in some individuals. The term non-A non-B hepatitis (NANB) was coined to describe these cases. The clinical diagnosis of NANB hepatitis was similarly imprecise, and was based on the clinical exclusion of other causes of hepatocellular inflammation and the serological exclusion of other hepatotropic viruses. It is now known that a proportion of NANB hepatitis represented infections with cryptic forms of HBV.

Approaches used to identify HAV, HBV-antigens and viral particles were all used in the search of NANB virus and multiple reports were published but none stood the test of the well known serum panel in the National Institute of Health. The physicochemical characterised of the major non-A, non-B virus, however, was already well characterised and was consistent with it being a member of a group of small lipid coated RNA viruses.

CURRENTLY AVAILABLE SEROLOGICAL ASSAYS

The first generation test, an ELISA using a fusion protein coated plate, passed the National Institute of Health serum panel. With this assay, a gush of papers appeared in the literature within a short time which showed that most patients with post-transfusion NANB hepatitis were seropositive for this antibody by six months. Interestingly, around half of the patients with sporadic NANB hepatitis were also found to be positive at various intervals after the acute infection suggesting a common aetiology in a proportion of cases.

This is just the beginning of the story. More reports come up claiming that HCV has an important role in various conditions including cryptogenic liver cirrhosis, hepatocellular carcinoma, autoimmune chronic active hepatitis, as well as in alcoholics, haemophiliacs receiving replacement therapy, intravenous drug users and haemodialysis patients (Table VI). Despite all these epidemiological data, the true significance of the anti-HCV antibodies is still unknown – do these antibodies to the non-structural antigen of HCV indicate infectivity or immunity? Are all those patients seropositive for anti-HCV antibodies by these first generation test genuinely positive – the question of specificity?

In those patients with well documented autoimmune chronic active hepatitis, an excellent linear correlation between high levels of the globulin serum and the readings (optical density) of the ELISA assay was demonstrated, indicating that high globulin levels might produce nonspecific reactivity in the ELISA system. Utilizing Western Blotting technique, a second generation test, the recombinant immunoblot assay (RIBA), was established but its specificity remains to be established. Serum HCV RNA was also successfully detected recently using the polymerase chain reaction by various groups. This assay will enable us to test the specificity of the various serological assay and also determine the relationship between HCV viraemia and liver disease.

**TABLE V  Host factors associated with a suboptimal HBV vaccine responsiveness**

<table>
<thead>
<tr>
<th>Host factor</th>
<th>Effect</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age</td>
<td>Higher risk of infection</td>
</tr>
<tr>
<td>Alcoholism</td>
<td>Increased risk of infection</td>
</tr>
<tr>
<td>Obesity</td>
<td>Higher risk of infection</td>
</tr>
<tr>
<td>HIV infection</td>
<td>Increased risk of infection</td>
</tr>
<tr>
<td>Immunosuppression</td>
<td>Increased risk of infection</td>
</tr>
<tr>
<td>Chronic illness</td>
<td>Increased risk of infection</td>
</tr>
</tbody>
</table>

**Virology**

Current understanding is that HCV is an approximally 10 000 nucleotide linear, single stranded, positive polarity RNA virus and shares nucleotide and amino acid sequence homology with pestiviruses and flaviviruses as well as two plant virus superfamilies (alphavirus-like and picornavirus-like), indicating that HCV may be evolutionary related to plant and animal viruses. Only one ORF has been named so far. Presumably, there is only one large polyprotein produced that is cleaved post-translationally (Fig 3). Further study showed that the putative nucleocapsid protein gene sequence are highly conserved, suggesting that the core protein may play an important regulatory role in the life cycle of HCV.

Clinically, antibody to HCV appears in the circulation between one and three months after the onset of acute illness, but in rare cases not for up to a year. Antigenaemia is so limited that circulating viral antigen is beyond the limit of detection with most conventional assay technique.
TABLE VI Anti-HCV antibodies in different geographic areas according to risk of hepatitis and presence of liver disease*

<table>
<thead>
<tr>
<th>Groups</th>
<th>Anti-HCV positive (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Italy</td>
</tr>
<tr>
<td>Group I (Normal population)</td>
<td></td>
</tr>
<tr>
<td>Random blood donors</td>
<td>1-8</td>
</tr>
<tr>
<td>Healthy pregnant women</td>
<td></td>
</tr>
<tr>
<td>Community populations</td>
<td>4</td>
</tr>
<tr>
<td>Group II (High risk population)</td>
<td></td>
</tr>
<tr>
<td>Intravenous drug users</td>
<td>75</td>
</tr>
<tr>
<td>Haemophiliacs</td>
<td>82</td>
</tr>
<tr>
<td>Haemodialysis patients</td>
<td>34</td>
</tr>
<tr>
<td>Homosexual men</td>
<td>8</td>
</tr>
<tr>
<td>Female contacts of drug users</td>
<td>6</td>
</tr>
<tr>
<td>Group III (High risk patients with liver disease)</td>
<td></td>
</tr>
<tr>
<td>Post-transfusion NANBH</td>
<td>84</td>
</tr>
<tr>
<td>Sporadic NANBH</td>
<td>74</td>
</tr>
<tr>
<td>Chronic NANBH</td>
<td>74</td>
</tr>
<tr>
<td>Cryptogenic cirrhosis</td>
<td>78</td>
</tr>
<tr>
<td>Alcoholic cirrhosis</td>
<td>39</td>
</tr>
<tr>
<td>Hepatocellular carcinoma</td>
<td>65</td>
</tr>
<tr>
<td>HBeAg+</td>
<td>54</td>
</tr>
<tr>
<td>Alcoholic cirrhosis</td>
<td>38</td>
</tr>
<tr>
<td>Group IV (Autoimmune liver disease)</td>
<td></td>
</tr>
<tr>
<td>Autoimmune chronic active hepatitis†</td>
<td>78</td>
</tr>
<tr>
<td>Primary biliary cirrhosis</td>
<td>38</td>
</tr>
</tbody>
</table>

NANBH = Non-A, non-B hepatitis.

*Extracted from references 162, 166-172; these data should be interpreted with caution as most of these positives were not confirmed with serum HCV RNA assay using polymerase chain reaction.
†Note that a positive anti-HCV test is associated with a high globulin level, suggesting that this may be a false positive. See reference 173.

ANTIVIRAL THERAPY

The use of acyclovir in non-A, non-B hepatitis has produced no apparent benefit and corticosteroids has yielded disputed results.178 The only treatment proven of value is again interferon-alpha. A dose of 3 million units thrice weekly has been shown to be effective in reducing serum transaminases and controlling liver inflammation in chronic hepatitis caused by parenteral non-A, non-B virus infection.179-181 Serum transaminases was normalised in around half of these patients and this was associated with histological improvement. This improvement was, however, only sustained in about 50% of the patients after cessation of treatment and hence treatment may have to be lifelong. Predictors of a favourable response seem to be short duration of illness and high transaminases. A positive HCV test however, did not predict a favourable outcome.179

Hepatitis D virus (HDV)

In 1977 a new viral antigen was found in the nuclei of hepatocytes in patients with chronic HBV infection.182 Subsequent studies revealed that this antigen was related to a new virus, now termed HDV. Hepatitis D virus infection is endemic in Italy, Middle East, some areas of Africa and South America, but is relatively rare in Western Europe, North America and Asia, despite the existence of susceptible HBV carriers in many of these areas (Table VII).183 Family clustering in endemic areas suggests viral transmission by close contacts, whereas intravenous drug use is likely to be the major route of transmission in non-endemic regions of the Western world.184

VIROLOGY

Hepatitis D virus is an incomplete single stranded circular RNA virus that depends on the helper function of HBV to replicate.185 It is encoiled in HBsAg and the RNA genome, which is around 1700 nucleotides long of minus polarity, contains a very high intramolecular base pairing, similar to the genomes of plant viroids.186-188 Although several open reading frames (ORFs) have been identified, only one (ORF5) has been demonstrated to code for protein (HDAg). Replication of viral RNA has been shown to proceed by a rolling circle mechanism, specific self-cleavage and self-ligation of genomic and anti-genomic HDV RNA strands has also been shown in vivo.187-193

Even though HDV appears to require HBsAg for its hepatotropism and propagation, experience in transplanted patients with chronic HDV infection who had HBV recurrence in the liver grafts without evidence of HBV recurrence suggest that HDV does not necessarily rely on HBV for replication.194

PATHOGENESIS OF LIVER DISEASE

The presence of replicating HDV seems to be invariably associated with liver damage suggesting that HDV may be directly hepatotoxic.184 High level expression of HDAg has also been shown to be directly cytotoxic to both HeLa and HepG2 cells.195 Recent studies, however, have shown that immune mediated mechanism may also be involved.196-199 In a study of 98 liver biopsies from 68 patients seen in seven years, the extent of HDAg expression in the liver had no correlation with the activity of liver disease, indicating that host factor, possibly the immune system, was also important in the pathogenesis of the liver disease.196 The similarity in the pattern of T cell infiltration in chronic HBV and HDV infection provide further support that immune mediated mechanisms are important.197 Interestingly, a form of liver kidney microsomal autoantibody similar to that found in a subset of autoimmune chronic active hepatitis has been described in patients with chronic HDV infection, but its significance is unclear.198

CLINICAL FEATURES AND LABORATORY DIAGNOSIS

Infection with HDV may occur simultaneously with HBV infection (coinfection), or as a superinfection in chronic HBsAg carriers. These two conditions have different clinical courses and outcomes. Coinfection is usually self limiting as acute HBV infection, although morbidity may be higher. A massive infecting dose is sometimes associated with a more severe outcome, either in the form of fulminant hepatitis, or as a biphasic illness with initial improvement followed by relapse. In contrast, in a collaborative study with Italy and France, a higher morbidity was noted in HDV superinfection of HBsAg carriers and this was associated with severe acute hepatitis (sometimes fulminant).200 Other studies have also shown that over 70% of the patients with HDV superinfection who had no history of the severe fulminant form of hepatitis developed chronic active hepatitis and cirrhosis. What favours chronicity in HDV superinfection is not well understood but HDV superinfection is
Viral hepatitis

### TABLE VII Prevalence of HDV infection in different geographical areas as evidenced by a high anti-HDV antibody titre

<table>
<thead>
<tr>
<th>Country</th>
<th>HBsAg carriers + liver disease</th>
<th>Healthy HBsAg carriers</th>
<th>HBsAg positive IV drug users</th>
</tr>
</thead>
<tbody>
<tr>
<td>Europe</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Britain</td>
<td></td>
<td>&lt;1%</td>
<td>42%</td>
</tr>
<tr>
<td>Ireland</td>
<td></td>
<td>1%</td>
<td>31%</td>
</tr>
<tr>
<td>Italy</td>
<td>14–28%</td>
<td>2-4%</td>
<td>20–36%</td>
</tr>
<tr>
<td>Greece</td>
<td></td>
<td></td>
<td>35%</td>
</tr>
<tr>
<td>Spain</td>
<td>9%</td>
<td></td>
<td>25–60%</td>
</tr>
<tr>
<td>Portugal</td>
<td>25%</td>
<td></td>
<td>85%</td>
</tr>
<tr>
<td>Belgium</td>
<td>2%</td>
<td></td>
<td>85%</td>
</tr>
<tr>
<td>Yugoslavia</td>
<td>7%</td>
<td></td>
<td>85%</td>
</tr>
<tr>
<td>Hungary</td>
<td>6%</td>
<td></td>
<td>85%</td>
</tr>
<tr>
<td>Romania</td>
<td>83%</td>
<td></td>
<td>85%</td>
</tr>
<tr>
<td>Poland</td>
<td>6–6%</td>
<td>1-6%</td>
<td>25%</td>
</tr>
<tr>
<td>Turkey</td>
<td>25%</td>
<td>17%</td>
<td>25%</td>
</tr>
<tr>
<td>USSR (Europe)</td>
<td>14–28%</td>
<td>3%</td>
<td></td>
</tr>
<tr>
<td>Africa</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Tunisia</td>
<td>18%</td>
<td>10%</td>
<td></td>
</tr>
<tr>
<td>Algeria</td>
<td>15%</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Ethiopia</td>
<td></td>
<td>5-6%</td>
<td></td>
</tr>
<tr>
<td>S Africa</td>
<td></td>
<td>0-6%</td>
<td></td>
</tr>
<tr>
<td>Asia</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>USSR (mid Asia)</td>
<td></td>
<td>15%</td>
<td></td>
</tr>
<tr>
<td>Israel</td>
<td>20%</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Saudi Arabia</td>
<td>53%</td>
<td></td>
<td></td>
</tr>
<tr>
<td>India (Bombay)</td>
<td>25%</td>
<td>11%</td>
<td></td>
</tr>
<tr>
<td>Taiwan</td>
<td>13%</td>
<td>2-2%</td>
<td></td>
</tr>
<tr>
<td>China</td>
<td>1-8%</td>
<td></td>
<td></td>
</tr>
<tr>
<td>America</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>USA</td>
<td>25%</td>
<td>1-4-8%</td>
<td>20–53%</td>
</tr>
<tr>
<td>Amazon basin</td>
<td>85–100%</td>
<td>23-35%</td>
<td></td>
</tr>
<tr>
<td>Venezuela</td>
<td>91%</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Columbia (S Marta)</td>
<td>29-36%</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Columbia (urban)</td>
<td></td>
<td>0%</td>
<td></td>
</tr>
<tr>
<td>Argentina</td>
<td>2-5%</td>
<td>1-2%</td>
<td></td>
</tr>
<tr>
<td>Chile</td>
<td>10–14%</td>
<td>1-8%</td>
<td></td>
</tr>
<tr>
<td>Australia</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Australia</td>
<td></td>
<td>0-5-8%</td>
<td>19%</td>
</tr>
<tr>
<td>W Pacific</td>
<td></td>
<td>0–31%</td>
<td></td>
</tr>
</tbody>
</table>

usually accompanied by a decrease in HBV replication markers.

It is also important to note that in chronic HBsAg carriers with HDV superinfection, the suppression of HBV replication may lead to a transient absence of HBV markers in serum and liver; unless HDV markers are sought, the diagnosis of NANB hepatitis may be made erroneously.

Because HDV RNA can only be detected in a few centres, serum IgM anti-HDV has been suggested as an alternative marker for active HDV replication as early reports showed a good correlation between serum IgM anti-HDV and both intrahepatic HDAg and liver inflammatory activities. A recent study, however, showed that serum HDV RNA may also be detected in up to 32% of patients with chronic HDV infection seronegative for IgM anti-HDV. The role of IgM anti-HDV has been systematically studied in our Institute which showed only a weak correlation between serum IgM anti-HDV and both hepatic HDAg expression and inflammatory activities, indicating that IgM anti-HDV is not a good serum marker for active HDV replication nor active liver disease.

A recent study in our Institute showed that serum IgA anti-HAV was almost exclusively associated with chronic HDV infection and was an independent correlate of moderate/severe histological activity with a sensitivity of 82-6% and a specificity of 90-5%. There was, however, no correlation between serum IgA anti-HDV and expression of hepatic HDAg and serum transaminases. In situ hybridisation and riboprobe assay for the detection of hepatic and serum HDV RNA has also been developed recently and these may prove to be useful tools for detecting active replication and for monitoring treatment.

ANTIVIRAL THERAPY AND IMMUNISATION

Interferon-alpha has been shown to have an inhibitory effect on HDV replication but beneficial effects appear to be only transient in most cases. Control of HDV infections is by vaccination against HBV.

Hepatitis E virus (HEV)

The first massive outbreak of enteric non-A, non-B epidemic was reported in 1955 when drinking water was contaminated by the overflow of an open sewer in Delhi. A total of 29,300 residents developed acute hepatitis, which ran a benign course in the majority and was self limiting. Ten per cent of women affected in their third trimester of pregnancy, however, died of fulminant hepatic failure. The disease displayed an unusual histological pattern with no chronic implications in subsequent follow up studies. These reports confirmed the existence of a faecal-oralily transmitted type non-A non-B hepatitis (HEV) with completely different characteristics to blood borne NANB hepatitis.

Epidemiology

Of 10 Indian hepatitis epidemics subsequently investigated after the first major outbreak, nine were found to be caused by epidemic/enteric NANB hepatitis. Similar epidemics were reported in various Asian, African, Central and South American countries. Sporadic imported cases of HEV have also been reported in the USA and it is likely that HEV is responsible for a small proportion of patients with acute sporadic NANB hepatitis seen in the United Kingdom and Europe.

The attack rate (clinically apparent) has been estimated to be around 2% but is up to 19% in pregnant women, with the majority of attacks occurring in young adults. The pattern of disease is compatible with an infection which is endemic but produces a sustained period of immunity. A reservoir of HEV is therefore required to sustain the infection in the absence of a carrier state. Research workers in the USSR have recently been able to transmit HEV to pigs with induction of an hepatitis illness. In addition, they have also found anti-HEV antibody in rats from areas where the disease is endemic.

Identification of HEV

In 1983, a 27–32 nm virus like particles were demonstrated by immune electron microscopy in the stools of three of nine cases of HEV in Tashkent, USSR. A volunteer then ingested a dilute suspension of stools pooled from the patients and he developed an acute hepatitis, with antibodies detectable to the virus like particle by immune electron microscopy.
subsequently, HEV was also transmitted to a number of primates. In the majority of studies, 27–34 nm virus like particles aggregated by autologous acute phase serum have been found in stool samples. Using immune electron microscopy, it was found that sera and virus like particles obtained from patients from different regions of the world cross-reacted, indicating that HEV in various parts of the world is associated with the same non-enveloped 27–34 nm virus like particles. An antigen related to HEV (HEV-Ag) was detected recently in the liver of experimentally infected macaques. It has a granular distribution in up to 90% of hepatocytes in the early phase of experimental infection.

The recent successful molecular cloning of part of the HEV genome represents a giant leap forward. The translated nucleic acid sequence of the isolated clone contains a consensus amino acid sequence consistent with a RNA directed RNA polymerase, an enzyme that is present in all positive strand RNA viruses.

**CLINICAL FEATURES AND LABORATORY DIAGNOSIS**

The majority of clinical data came from the well characterised Indian epidemics. The incubation period was between three and nine weeks. The attack rate was higher in young adults (2.9%) than those older than 40 (2.0%). A brief prodrome illness with anorexia, nausea, vomiting, and abdominal pain is followed by jaundice. The disease is usually benign and self limiting with no chronic sequelae. Fulminant hepatic failure, however, has been reported to occur in up to 2% of men and 22% of pregnant women with clinical symptoms, in whom it is usually fatal. This severe form of hepatitis in pregnancy, particularly in the third trimester, is a feature of all HEV epidemics.

Histologically, cholestasis was prominent in around 50% of the patients with marked canalicul and intracellular bile stasis in glandular channels. Moderate inflammatory infiltrates of mononuclear and polymorphonuclear leucocytes occurred in the portal and intralobular regions with prominent lipofuscin pigment in Kupffer cells.

With the recent success in identification of HEV-Ag in liver sections of experimentally infected primates and the development of fluorescent linked anti-HEV-Ag, an antibody blocking assay was developed as a prototype test for the identification and titration of specific anti-HEV-Ag antibody in the serum samples. Recently, two additional test for HEV was established: solid phase immune electron microscopy to detect the virus and polymerase chain reaction to detect the HEV genome (and Dr D Bradley, personnel communication). With these tools, the myth of HEV is going to be revealed in the near future.

**TREATMENT AND CONTROL**

As is the case with HAV, treatment is purely supportive. Control of the disease relies on the well established primary health objectives of clean water and adequate sanitation.

**Hepatitis X virus (X=F, G, . . .)**

There are probably some more as yet undiscovered hepatotropic viruses as determined by epidemiology and electronmicroscopic studies. One is the short incubation blood borne non-A, non-B hepatitis virus. It is quite uncommon and may not be epidemiologically important. Based upon the limited data available, this second or minor non-A, non-B or non-A, non-B, non-C virus is thought to be smaller than HCV (around 25–30 nm), naked rather than enveloped, and incapable of producing the characteristic cytoplasmic tubular structures found by electron microscopy in the hepatocytes of chimpanzees experimentally infected with HCV. To date, no antibody specific for this second non-A, non-B hepatitis has been detected. A toga-like virus has been recently found in liver tissue from patients with fulminant hepatitis attributed to sporadic non-A non-B. Syncytial giant-cell hepatitis has also been reported to associate with paramyxo viral features (HGV)23.

All the advancements in the last two decades make us a little wiser – in knowing how ignorant we are. Important questions like the basis of chronic carriage, the mechanism of liver damage, the regulation of viral genes in various phase of the chronic infection, the mechanism by which hepatocellular carcinoma are induced and the precise mode of action of interferon-α are still not clearly answered. Specific therapy for a complete cure of the chronic infections (HBV, HCV, HDV) is still far from scope. Perhaps, the most appropriate way to finish this is by citing Sir Issac Newton:

"I seem to have been only a boy playing on the seashore, and diverting myself in now and then finding a smoother pebble or a prettier shell than ordinary, whilst the great ocean of truth lay all undiscovered before me."

Sir Issac Newton, 1642–1727 AD. 
_Brewster's Memoirs of Newton, Vol 2, Ch 27._

Viral hepatitis


43 Tsu JY, Chou K, Robinson WS. Hepatitis B virus X gene


Viral hepatitis


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Viral hepatitis.

J Y Lau, G J Alexander and A Alberti

Gut 1991 32: S47-S62
doi: 10.1136/gut.32.Suppl.S47

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