Viral hepatitis

Johnson Y N Lau, Graeme J M Alexander, Alfredo Alberti

Royal Free Hospital School of Medicine, Pond Street, London NW3 2QG
Arie J Zuckerman

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 picornavirus 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 hepadnavirus 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 hepadnavirus 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 hepadnavirus 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 caliciviruses. 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 fundament knowledge to each of these different viruses.

On a more personal note, productive and friendly collaboration and close association between our two laboratories commenced over 20 years ago. Some of the published work which resulted from this very pleasant association included studies on epidemiology (Exposure and immunity to hepatitis virus in a Liver Unit, *Lancet* 1974[1]; Australia antigen among heroin addicts attending a London addiction clinic, *J Hyg Camb* 1971[2]; Hepatitis B virus infection in dental surgical practice, *BMJ* 1976)[3]; immunology of hepatitis B (Immune responses to the surface antigen and liver specific lipoprotein in acute hepatitis B, *Gut* 1977[4]; Cellular and humoral immunity to hepatitis B antigen in chronic active hepatitis, *BMJ* 1975)[5]; fulminant hepatitis (Fulminant hepatic failure in leukaemia and choriocarcinoma related to withdrawal of cytotoxic therapy, *Lancet* 1975[6]; Enhanced HBsAg production in pathogenesis of fulminant viral hepatitis B, *BMJ* 1976[7]; Fulminant hepatitis. An ultrastructural study, *J Hepatol* 1986)[8]; primary liver cancer (Detection of hepatitis B antigen by radioimmunoassay in chronic liver disease and hepatocellular carcinoma in Great Britain, *Lancet* 1973)[9]; non-A, non-B hepatitis (non-A, non-B hepatitis associated with chronic liver disease in a haemodialysis unit, *Lancet* 1979[10]; Transmission by factor IX concentrate, *Lancet* 1979[11]; Cellular changes associated with non-A, non-B hepatitis, *J Med Virol* 1980[12] and 1987[13]; Virus-like particles in liver in sporadic non-A, non-B hepatitis, *J Med Virol* 1989),[14] and many others.

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?

1 Reed WD, Eddleston ALWF, Mitchell CG, Lee WM, Williams R, Zuckerman AJ. Exposure and immunity to hepatitis B virus in a Liver Unit. *Lancet* 1974; i: 581–3.
2 Hunter J, Carella M, Williams R, Taylor PE, Zuckerman AJ. The Australia (hepatitis-associated) antigen amongst heroin addicts attending a London addiction clinic. *J Hyg Camb* 1971; **69**: 565–70.
3 Goubran GF, Cullens H, Zuckerman AJ, Eddleston ALWF, Williams R. Hepatitis B virus infection in dental surgical practice. *BMJ* 1976; ii: 559–60.
4 Lee WM, Reed WD, Osman CG, Vahrman J, Zuckerman A J,

Eddleston ALWF, *et al.* Immune responses to hepatitis B surface antigen and liver specific lipoprotein in acute type B hepatitis. *Gut* 1977; **18**: 250–7.
5 Lee W, Reed WD, Mitchell CG, Galbraith RM, Eddleston ALWF, Williams R, *et al.* Cellular and humoral immunity to hepatitis B antigen in active chronic hepatitis. *BMJ* 1975; i: 705–8.
6 Galbraith RM, Eddleston ALWF, Williams R, Zuckerman AJ, Bagshawe KD. Fulminant hepatic failure in leukaemia and choriocarcinoma related to withdrawal of cytotoxic drug therapy. *Lancet* 1975; ii: 528–30.
7 Woolf IL, Sheikh N El, Cullens H, Lee WM, Eddleston ALWF, Williams R, *et al.* Enhanced HBsAg production in pathogenesis of fulminant viral hepatitis type B. *BMJ* 1976; ii: 669–71.
8 McCaul TF, Fagan EA, Tovey G, Portmann B, Williams R, Zuckerman AJ. Fulminant hepatitis. An ultrastructural study. *J Hepatol* 1986; **2**: 276–90.
9 Reed WD, Eddleston ALWF, Stern RB, Williams R, Zuckerman AJ, Bowes A, *et al.* Detection of hepatitis B antigen by radioimmunoassay in chronic liver disease and hepatocellular carcinoma in Great Britain. *Lancet* 1973; ii: 690–3.
10 Galbraith RM, Dienstag JL, Purcell RH, Gower RH, Zuckerman AJ, Williams R. Non-A, non-B hepatitis associated with chronic liver disease in a haemodialysis unit. *Lancet* 1979; i: 951–3.
11 Wyke RJ, Tsiquaye KN, Thornton A, White Y, Portmann B, Das PK, *et al.* Transmission of non-A, non-B hepatitis to chimpanzees by factor IX concentrate after fatal complications in patients with chronic liver disease. *Lancet* 1979; i: 520–4.
12 Tsiquaye KN, Bird RG, Tovey RJ, Wyke J, Williams R, Zuckerman AJ. Further evidence of cellular changes associated with non-A, non-B hepatitis. *J Med Virol* 1980; **5**: 63–71.
13 Fagan EA, Ellis DS, Portmann B, Tovey GM, Williams R, Zuckerman AJ. Microbial structures in a patient with sporadic non-A, non-B fulminant hepatitis treated by liver transplantation. *J Med Virol* 1987; **22**: 189–98.
14 Fagan EA, Ellis DS, Tovey GM, Portmann B, Williams R, Zuckerman AJ. Virus-like particles in liver in sporadic non-A, non-B fulminant hepatitis. *J Med Virol* 1989; **27**: 76–80.

Institute of Liver Studies, King's College School of Medicine and Dentistry, London
J Y N Lau

Addenbrookes Hospital, Hills Road, Cambridge
G J M Alexander

Instituto di Medicina Clinica, University of Padova, Padova, Italy
A Alberti

Correspondence to:
Dr J Y N Lau, Institute of Liver Studies, King's College School of Medicine and Dentistry, Bessemer Road, London SE5 9PJ.

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 hepatotrophic 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,[1] 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,[2] 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 6 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[2 8 9] 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 12] 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.[13 14] 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.[15] 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.

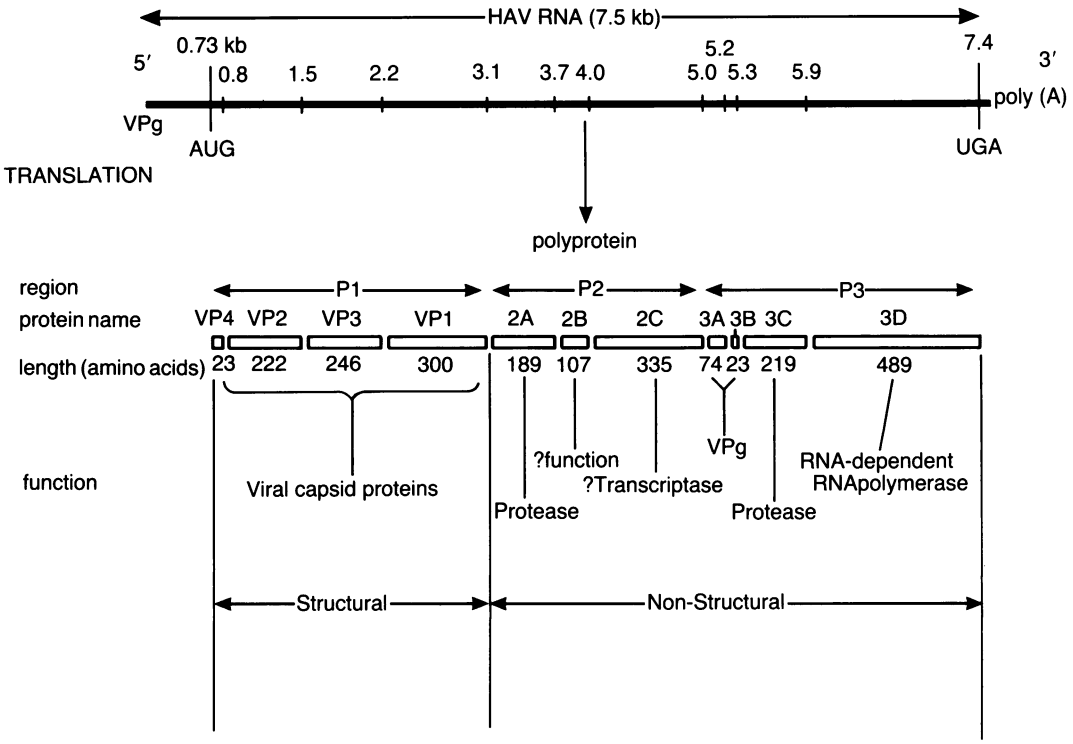

Figure 1: Structure of the hepatitis A virus genome and the tentative protein cleavage sites. VPg – genome linked viral protein.

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.[18] 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.[19]

Although HAV infection does not lead to chronic hepatitis and cirrhosis, it is an important source of morbidity.[14] 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.[20] 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.[21] 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 transfusion – 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.[22] Our recent survey in South East London showed a carriage rate of 1%, more frequent than previously thought.[23] It is depressing that an estimated 40% of them will eventually die of chronic liver disease and/or hepatoma.[24]

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.[25,26] 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.[27]

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-

TABLE I *Classification of hepatotrophic and other viruses responsible for acute viral hepatitis*

Hepatitis type	*Previously named/description*
Hepatotrophic viruses	
Hepatitis A (HAV)	Infectious hepatitis
Hepatitis B (HBV)	Serum hepatitis
Hepatitis C (HCV)	Parenteral non-A, non-B hepatitis
Hepatitis D (HDV)	Delta hepatitis (agent)
Hepatitis E (HEV)	Enteral non-A, non-B hepatitis
Others	
Cytomegalovirus (CMV)	
Epstein-Barr virus (EBV)	
Herpes Simplex virus	
Coxsackie B virus	
Echovirus	
Yellow fever virus	

capsid, core) C/pre-c, (polymerase) P, and X are known to code for viral polypeptide/antigen (Table II).[28] An enhancer, a glucocorticoid responsive element as well as four promoter elements (surface, core, pre-S1, X) have also been identified.[29]

The surface ORF contains three in phase translation start codons encoding three envelope polypeptides.[30]. 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[30 31] and may be involved in host cell binding and entry.[32 33] 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.[34 35] 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[36–41] and may have significant pathogenetic consequences (ground glass formation, hepatocellular necrosis).[42]

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.[29] The core (c) polypeptide is a nucleic acid binding protein which encapsulates the viral nucleic acid.[43] 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[44–48] or transport into the nucleus.[49] The pre-c polypeptide is not required for viral replication.[50–52] The importance of pre-c in the transport and formation of serum HBeAg has been emphasised by the recent description of a HBV mutant occurring in patients seropositive for anti-HBe, but seropositive for HBV DNA and have active liver disease.[53 54] 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.

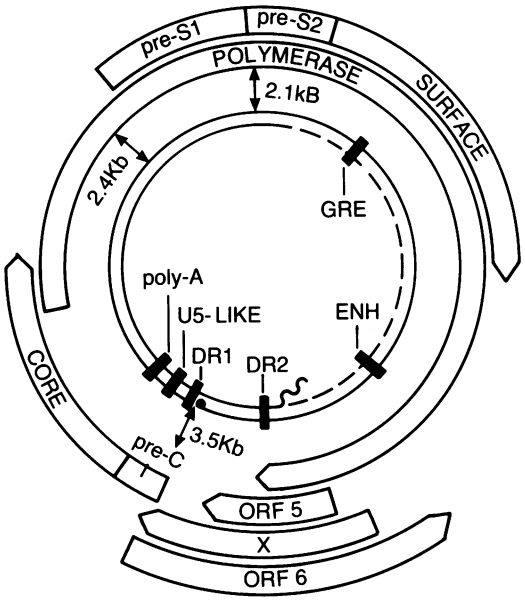

Figure 2: Structure of the HBV genome. The positions of the four open reading frames (ORFs) known to produce HBV proteins are shown in open boxes. The relative positions of the enhancer element (ENH), glucocorticoid responsive element (GRE), and the direct repeats (DR1 and DR2) are also shown. (+) and (−) denotes the positive and negative strands. Small, solid arrows show the 5′ ends of the three major virus RNA transcripts of 2·1, 2·4, 3·5 kb.

The polymerase ORF overlaps all the others. It encodes the viral polymerase and reverse transcriptase activity[55–57] 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 pregenomes.[58–60]

The X ORF encodes a polypeptide expressed in some patients with chronic HBV infection and HBV related hepatocellular carcinoma.[61–66] The X polypeptide has transcriptional transactivating properties which positively regulate transcription from HBV and other viral promoters as well as cellular promoters.[67–72] Whether X protein/gene is tumourigenic or not is controversial.[73 73a]

The function of the two potential ORFs, termed ORF5 and ORF6 is not known and no translational product has been identified.[29] 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.[74]

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.[75] 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.[32–36] 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.[77-80] pHSA, however, is an in vitro product (albumin treated with glutaraldehyde) and has never been found circulating in human subjects.[81-82] 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.[83]

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 indicant 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.[84] 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.[85] This led to the provocative hypothesis that HBV itself might be a trigger for an autoimmune response that would persist after eradication of HBV.[86] 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.[87] The theory that such autoimmune reactions were triggered by HBV received support from a serial study of patients with acute infection.[58] 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.[89] 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.[90] 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.[53,54] That there were different patterns even among patients with active HBV replication was recognised in 1978.[91] 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 longterm outcome.[91a] Patients showing seroconversion and remission of liver disease also had termination of virus replication, while those with continuing disease activity developed a detective 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.[92] 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.[32-35] 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[92a]; 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.[93] 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.[94] In addition transient defects in suppressor cell fuction 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 antigens, which now included those prepared by recombinant technology, were of superior quality. In a study of chronic HBV infection, T cell responses to both HBcAg and HBsAg were compared[95] 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 HBcAg, 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 presymptomatic phase, 30 to 70 days before the onset of liver damage, through to recovery.[96] Cellular immunity to pre-S was the first detectable response and substantially predated liver damage in every case. T cell sensitisation to HBcAg 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[97] 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.[98] The figure was higher in patients with active liver disease and in contrast with autoimmune disease in which the

TABLE II *Hepatitis B virus polypeptide/antigens*

HBV Gene	*Polypeptide/antigen*
S	HBsAg (major protein)
pre-S2+S	pre-S2 peptide (middle protein)
pre-S1+pre-S2+S	pre-S1 peptide (large protein)
C	HBcAg and HBeAg
pre-C+C	pre-core peptide
P	DNA polymerase
X	X protein

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 HBeAg positive cases, indicating a relation to replication.

This finding was taken further in experiments to determine whether HBcAg was the target.[99] 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 autologus T cells never contained HBcAg, but did contain HBsAg.[100]

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.[102] In this study it was demonstrated that the presence of cytoplasmic HBeAg 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 HBsAg, suggesting a variable export of HBsAg in various phase 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]

(f) Role of cytokines

The possibility that an interferon-alpha deficiency might be associated with chronic HBV infecton 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.[109]

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.[110 111] Both were shown to be improved by in vitro treatment with interferon alpha[112]. 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.[113] In a further study, the production of interleukin 1 and tumour necrosis factor by peripheral blood leucocytes 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.[115] 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 proviral effect.[116 117] 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 logical[119]; 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*

Anti-virals	*Immunosuppressive*
Interferons	Corticosteroids
Alpha	
Beta	
Gamma	
Tumour necrosis factor	
Adenine arabinoside (Ara-A)	*Immunostimulators*
Acyclovir, deoxyacyclovir	BCG vaccination
Zidovudine	Levamisole
Suramin	Interleukin-2
Ribavirin	Interferon-gamma
Phosphonoformate	Thymosin
Quinacrine	Tumour necrosis factor
(+)−cyanidanol-3	
Phyllanthus amarus	

antibodies, such an approach may well be resurrected in the coming decade, with application not just to transplantation, but to therapy of carriers.

The development of an assay for HBV DNA polymerase paved the way within and without our institute for the study of antiviral compounds.[120] Using this method, it was shown in the mid 70s that leucocyte interferon had antiviral activity in chronic HBV infection.[121] Insufficient interferon meant that controlled trials did not materialise either in our institute or elsewhere. A series of trials were conducted in the search for alternative therapeutic agents (Table III),[122-125] which had two things in common: they were dull to perform and provided a clear negative result (reviewed in 126).

With the advancement in cell culture and recombinant DNA technology, sufficient interferon did eventually become available and a series of controlled trials were performed.[126] These proved that interferon-α (more than 5 MU/m^2 thrice weekly for more than three months) was an effective therapy for a proportion of patients with chronic HBV infection. Determinants of a likely positive response were also identified (Table IV) – in particular, those with a short history and those with active liver disease.[127] It wasn't the answer, but it proved a good start; the fascinating feature was that some patients also cleared HBsAg, a most unusual feature that led to a series of papers investigating how interferon might modulate recognition of HBsAg.[128 129] It also provided a valuable line of research: if interferon can reverse the carrier state, does this tell us anything about the mechanism of chronic HBV carriage? Another major but still unanswered question is: what can we offer to those interferon-α non-responders?

For those with HBV related end stage liver disease, liver transplantation offers the only hope. On a theoretical basis, according to the T cell cytotoxicity story, liver grafts without a class I or II match between donor and recipient should be safe from immune assault. Additionally, there was no evidence that HBV was cytopathic and liver transplantation should have been an excellent operation for HBV carriers even if complicated by recurrence. Practice and theory were a gulf apart; HBV infection in grafts was associated with a novel histological pattern termed fibrosing cholestatic hepatitis, complicated by a fulminant course.[130 131] The extraordinary high level of HBV antigen expression, together with the relatively mild degree of inflammatory infiltrate in the liver, suggest that HBV may be cytopathic to the graft livers in this condition.[132]

TABLE IV *Patient related determinants in chronic HBV infection that influence the response to IFNα therapy*

Determinants of positive response	*Determinants of negative response*
Acquisition in adulthood	Acquisition in infancy/childhood
Female sex	Male sex
High pretreatment ALT	Low pretreatment ALT
Low pretreatment HBV-DNA	High pretreatment HBV-DNA
Active histology	Inactive histology
HIV antibody negative	HIV antibody positive
Heterosexual	Homosexual
Anti-HDV negative	Anti-HDV positive

ALT-serum alanine aminotransferase; HIV-human immunodeficiency virus; HDV-hepatitis D virus.

The observation that a proportion of patients with chronic HBV infection developed severe and sometimes fulminant hepatitis upon withdrawal of chemotherapy was first reported in 1975.[133] Similar observations were also observed by other groups.[134-137] It is likely that intense chemotherapy/immunosuppression enhances HBV replication and the immune rebound upon cessation of chemotherapy induce a massive destruction of the infected cells. On a theoretical basis, a rapid reintroduction of corticosteroids followed by slow withdrawal may alleviate the severe life threatening reactivation and save the patient from a liver transplantation. This has been shown to be applicable recently.[138]

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.[139 140] In some endemic areas, all newborn babies are routinely vaccinated. The World Health Organisation has recommened 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.[141] Around 35% of healthy HBV vaccine responders will have their anti-HBs titre 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 life table attack rate of new HBV infections in vaccinated responders was 1·8% annually.[140 142] Although most of these new infections were subclinical seroconversions, one clinically apparent HBsAg event has been documented.[142] Hepatitis B virus mutants may also be

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

- Age
- Alcoholism
- Obesity
- HIV infection
- Immunosuppression
- chronic illness – for example chronic renal failure

HIV – human immunodeficiency virus.

responsible for some of the patients who developed HBV infection after successful vaccination.[143]

Host factors associated with suboptimal HBV vaccine responsiveness have also been identified (Table V). A supplementary dose may help a small proportion of these non-hyporesponders. New HBV vaccines which include additional antigens such as pre-S1, pre-S2 and possibly HBc are being developed.[144]

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.[145 146] The term non-A non-B hepatitis (NANB) was coined to describe these cases.[145 146] 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 hepatotrophic viruses. It is now known that a proportion of NANB hepatitis represented infections with cryptic forms of HBV.[147]

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.[148] 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 virus.[149–154]

IDENTIFICATION OF HCV

The recent identification of the major non-A, non-B virus, which has been termed HCV, was the result of the major advances in molecular cloning techniques.[155] At around the same time, HCV was also cloned in Japan.[156] Two more HCV genomes from Japanese patients were recently reported and was found to have 70–80% homology with the American HCV nucleotide sequence.[157 158]

VIROLOGY

Current understanding is that HCV is an approximately 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 supergroups (alphavirus-like and picornavirus-like), indicating that HCV may be evolutionary related to plant and animal viruses.[159] Only one ORF has been identified so far. Presumably, there is only one large polyprotein produced that is cleaved post-translationally (Fig 3).[160] Further study showed that the putative nucleocapsid protein gene sequence are highy conserved, suggesting that the core protein may play an important regulatory role in the life cycle of HCV.[161]

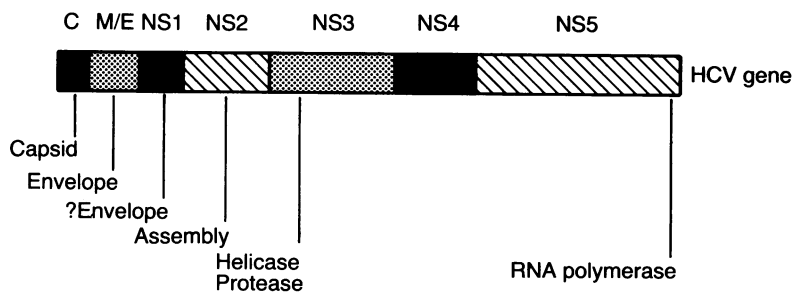

Figure 3: Schematic alignment of the polyproteins encoded by the putative domains of the hepatitis C virus.

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.[162] Antigenaemia is so limited that circulating viral antigen is beyond the limit of detection with most conventional assay technique.[160]

CURRENTLY AVAILABLE SEROLOGICAL ASSAYS

The first generation test, an ELISA using a fusion protein coated plate, passed the National Institute of Health serum panel.[163 164] 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.[165]

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).[166–172] 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 non-specific reactivity in the ELISA system.[173] Utilising 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.[174–177] This assay will enable us to test the specificity of the various serological assays and also determine the relationship between HCV viraemia and liver disease.

TABLE VI *Anti-HCV antibodies in different geographic areas according to risk of hepatitis and presence of liver disease**

Groups	Anti-HCV positive (%)			
	Italy	*Spain*	*United Kingdom*	*USA*
Group I (Normal population)				
Random blood donors		0	0·3–0·6	1–2
Healthy pregnant women	1·8			
Community populations		4		
Group II (High risk population)				
Intravenous drug users	75	92	50–81	76
Haemophiliacs	82	64	85	79
Haemodialysis patients		20	1–3	8
Homosexual men		8	0–26	24
Female contacts of drug users		6		
Group III (High risk patients with liver disease)				
Post-transfusion NANBH	84	85		85
Sporadic NANBH	74	69	29	73
Chronic NANBH	74	62	50	79
Cryptogenic cirrhosis		78		42
Alcoholic cirrhosis		39		
Hepatocellular carcinoma	65	75	60	26
HBsAg+	54	56		
Alcoholic		77		
Group IV (Autoimmune liver disease)				
Autoimmune chronic active hepatitis†	78	44	70	43
Primary biliary cirrhosis		38	0	9

NANBH=Non-A, non-B hepatis.
*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.[184] It is encoated 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.[185 186] 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 HDV 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 hepatocytotoxic.[184 195] High level expression of HDAg has also been shown to be directly cytotoxic to both HeLa and HepG2 cells.[195a] Recent studies, however, have shown that immune mediated mechanism may also be involved.[196 197] 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.[198] 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.[199]

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

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

Country	*HBsAg carriers + liver disease*	*Healthy HBsAg carriers*	*HBsAg positive IV drug users*
Europe			
Britain		<1%	42%
Ireland			31%
S Italy	14–28%		20–36%
Greece		2·4%	35%
Spain	9%		25–60%
Portugal	25%		85%
Belgium		2·8%	
Yugoslavia	7%		
Hungary	6%		
Romania	83%		
Poland	6·6%	1·6%	
Turkey	25%	17%	
USSR (Europe)	14–28%	3%	
Africa			
Tunisia	18%	10%	
Algeria	15%		
Ethiopia		5·8%	
S Africa		0·6%	
Asia			
USSR (mid Asia)	41%	15%	
Israel	20%		
Saudi Arabia	53%	11%	
India (Bombay)	25%		
Taiwan	13%	2·2%	
China		1·8%	
America			
USA	25%	1·4–8%	20–53%
Amazon basin	85–100%	23–35%	
Venezuela	91%	33%	
Columbia (S Marta)		29–36%	
Columbia (urban)		0%	
Argentina	2–5%	1·2%	
Chile	10–14%	1·8%	
Australasia			
Australia	19%	0·5–8%	19%
W Pacific		0–31%	

usually accompanied by a decrease in HBV replication markers.[201]

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[200 201]; 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.[202 203] 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.[204] 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.[205]

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%.[206 207] 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.[208 209]

ANTI-VIRAL 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.[210–212] Control of HDV infections is by vaccination against HBV.[213]

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.[214] Ten per cent of women affected in their third trimester of pregnancy, however, died of fulminant hepatic failure.[215] The disease displayed an unusual histological pattern with no chronic implications in subsequent follow up studies.[216] These reports confirmed the existence of a faecal-orally 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.[217 218] Similar epidemics were reported in various Asian, African, Central and South American countries.[219] 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.[219 220]

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.[218] 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.[221] 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.[221] Sub-

sequently, 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.[222-227] An antigen related to HEV (HEV-Ag) was detected recently in the liver of experimentally infected macaques.[225] 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.[228] 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.[218] 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 2·8% of men and 22% of pregnant women with clinical symptoms, in whom it is usually fatal.[229] 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 canalicular and intracellular bile stasis in glandular channels.[230] Moderate inflammatory infiltrates of mononuclear and polymorphonuclear leucocytes occurred in the portal and intralobular regions with prominent lipofuscin pigment in Küpffer cells.[230]

With the recent success in identification of HEV-Ag in liver sections of experimental 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.[225] 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[231] (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 hepatotrophic 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 epidemiological 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.[150] 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.[232 233] Syncytial giant-cell hepatitis has also been reported to associate with paramyxoviral features[234] (? HGV).[235]

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 Isaac Newton, 1642–1727 AD.
Brewster's Memoirs of Newton, Vol 2, Ch 27.)

1 Feinstone SM, Kapikian AZ, Purcell RH. Hepatitis A: Detection by immune electron microscopy of a virus like antigen associated with acute illness. *Science* 1973; **182**: 1026–8.
2 Siegel G. Virology of hepatitis A. In: Zuckerman AJ, ed. *Viral hepatitis and liver disease*, New York: Alan R Liss, 1988: 3–7.
3 Cohen JL, Ticehurst JR, Purcell RH, Buckler-White A, Baroudy BM. Complete nucleotide sequence of wild-type hepatitis A virus: comparison with different strains of hepatitis A virus and other picrornaviruses. *J Virol* 1987; **61**: 50–9.
4 Anderson DA, Locarnini SA, Ross BC, Coulepis AG, Anderson BN, Gust ID. Single-cycle growth kinetics of hepatitis A virus in BSC-1 cells. In: Briton WA, Rueckert RR, eds. *Positive strand RNA virus*. New York: Alan R Liss, 495–507.
5 Ticehurst JR. Hepatitis A virus: clones, culture and vaccines. *Semin Liver Dis* 1986; **6**: 46–55.
6 Huges JV, Stanton LW, Tomassini JE, Long WJ, Scolnick EM. Neutralising monoclonal antibodies to hepatitis A virus: partial purification of a neutralising antigenic site. *J Virol* 1984; **52**: 465–73.
7 Stapleton JT, Lemon SM. Neutralisation escape mutants define a dominant immunogenic neutralisation site on hepatitis A virus. *J Virol* 1987; **61**: 491–8.
8 Slusarczyk J, Hannson BG, Nordenfelt E, Krawczynski K, Karwowska S, Knap J. Etiopathogenic aspects of hepatitis

A. I. Excretion of hepatitis A virus, biochemistry of liver function, and humoral immune response in patients with hepatitis A on admission to hospital. *J Med Virol* 1985; **15**: 105–16.
9 Gabriel P, Vallbracht A, Flehmig B. Lack of complement dependent cytolytic antibodies in hepatitis A virus. *J Med Virol* 1986; **20**: 23–31.
10 Vallbracht A, Gabriel P, Zahn J, Flehmig B. Hepatitis A virus infection and the interferon system. *J Infect Dis* 1985; **152**: 211–3.
11 Kurane I, Binn LN, Bankroft WH, Ennis FA. Human lymphocyte response to hepatitis A virus-infected cells: interferon production and lysis of infected cells. *J Immunol* 1985; **135**: 2140–4.
12 Vallbracht A, Grabiel P, Maier K, *et al.* Cell-mediated cytotoxicity in hepatitis A virus infection. *Hepatology* 1986; **6**: 1308–14.
13 Gust ID, Feinstone SM. Epidemiology. In: *Hepatitis A*, Florida: CRC Press, 1988: 163–91.
14 Lemon SM. Type A viral hepatitis. New developments in an old disease. *N Engl J Med* 1985; **313**: 1059–67.
15 Forbes A, Williams R. Changing epidemiology and clinical aspects of hepatitis A. In: Zuckerman AJ, ed. *British medical bulletin: viral hepatitis.* London: Churchill Livingstone, 1990: 303–18.
16 Yang NY, Yu PH, Mao ZX, Chen NL, Chai SA, Mai JL. Inapparant infection of hepatitis A virus. *Am J Epidemiol* 1988; **127**: 599–604.
17 Sjogren MH, Tanno H, Fay O, *et al.* Hepatitis A virus in stool during clinical relapse. *Ann Intern Med* 1987; **106**: 221–6.
18 Gust ID, Feinstone SM. General preventive measures. In: *Hepatitis A.* Florida: CRP Press, 1988: 163–91.
19 Cossar JH, Reid D. Not all travellers need immunoglobulin for hepatitis A. *BMJ* 1987; **294**: 1503.
20 Gust ID. Design of hepatitis A vaccines. In: Zuckerman AJ, ed. *British medical bulletin: viral hepatitis.* London: Churchill Livingstone, 1990; 319–28.
21 Reed WD, Eddleston ALWF, Stern RB, Williams R, Zuckerman AJ, Bowes A, Earl PM. Detection of hepatitis-B antigen by radioimmunoassay in chronic liver disease and hepatocellular carcinoma in Great Britain. *Lancet* 1973; ii: 690–4.
22 King R, Johnson PJ, White YS, Smith HM, Williams R. Frequency of asymptomatic hepatitis type B and C in an inner city community and relation to possible risk factors. *Q J Med* 1991 (in press).
23 Maynard JE, Kane MA, Alter MJ, Hadler SC. Control of hepatitis B by immunization: global prospective. In: Zuckerman AJ, ed. *Viral hepatitis and liver diseases.* New York: Alan R. Liss, 1988: 967.
24 *Bulletin of the World Health Organisation.* 1988; **66**: 443.
25 Gerlich WH, Robinson WS. Hepatitis B virus contains protein attached to the 5′ terminus of its complete DNA strand. *Cell* 1980; **21**: 801.
26 Molnar-Kimber KL, Summers J, Taylor JM, Mason WS. Protein covalently bound to minus-strand DNA intermediates of duck hepatitis B virus. *J Virol* 1983; **45**: 165.
27 Seeger C, Ganem D, Varmus HE. Biochemical and genetic evidence for the hepatitis B virus replication strategy. *Science* 1986; **232**: 477–84.
28 Tiollais P, Pourcel C, Dejean A. The hepatitis B virus. *Nature* 1985; **317**: 489–95.
29 Miller RH, Kaneko S, Chung CT, Girones R, Purcell RH. Compact organisation of the hepatitis B virus genome. *Hepatology* 1989; **9**: 322–7.
30 Heermann KH, Goldmann U, Schwartz W, Seiffarth T, Baumgarten H, Gerlich WH. Large surface proteins of hepatitis B virus containing the pre-S sequence. *J Virol* 1984; **52**: 396–402.
31 Takahashi K, Kishimoto S, Ohnuma H, *et al.* Polypeptides coded for by the region pre-S and S of hepatitis B virus infection. *J Immunol* 1986; **136**: 3467–72.
32 Neurath AR, Kent SBH, Strick N, Parker K. Identification and chemical synthesis of a host cell receptor binding site on hepatitis B virus. *Cell* 1986; **46**: 429–36.
33 Pontisso P, Bankowski MJ, Petit MA, Peeples ME, Recombinant HBsAg particles containing pre-S proteins bind to human liver plasma membranes. *Hepadna viruses.* New York: Alan R. Liss, 1988: 205–21.
34 Neurath AR, Strick N. Antigenic mimicry of an immunoglobulin A epitope by a hepatitis B virus cell attachment site. *Virology* 1990; **178**: 631–4.
35 Pontisso P, Rumoletto MG, Chemello L, Tiribelli C, Gerlich WH, Alberti A. Hepatitis B virus binds to IgA receptors of liver cells. *J Hepatol* 1990; **11** (suppl 2): S50.
36 Persing DH, Varmus HE, Ganem D. Inhibition of secretion of hepatitis B surface antigen by a related pre-surface polypeptide. *Science* 1986; **234**: 1388–91.
37 Chisari FV, Filippi P, McLachlan A, *et al.* Expression of hepatitis B virus large envelope polypeptide inhibits hepatitis B surface antigen secretion in transgenic mice. *J Virol* 1986; **60**: 880–7.
38 Standring DN, Ou JH, Rutter WJ. Assembly of viral particles in Xenopus oocytes: Pre-surface-antigens regulate secretion of the hepatitis B surface envelope particle. *Proc Natl Acad Sci USA* 1986; **83**: 9338–42.
39 McLachlan A, Milich DR, Raney AK, *et al.* Expression of hepatitis B virus surface and core antigens: influences of pre-S and precore sequences. *J Virol* 1987; **61**: 683–92.
40 Molnar-Kimber KL, Jarocki-Witek V, Dheer SK, *et al.* Distinctive properties of the hepatitis B virus envelope proteins. *J Virol* 1988; **62**: 407–16.
41 Ou JH, Rutter WJ. Regulation of secretion of the hepatitis B virus major surface antigen by the preS1 protein. *J Virol* 1987; **61**: 782–6.
42 Chisari FV, Filippi P, Buras J, *et al.* Structural and pathological effects of synthesis of hepatitis B virus large envelope polypeptide in transgenic mice. *Proc Natl Acad Sci USA* 1987; **84**: 6909–13.
43 Petit MA, Pillot J. HBc and HBe antigenicity and DNA-binding activity of major core protein P22 in hepatitis B virus core particles isolated from the cytoplasm of human liver cells. *J Virol* 1985; **53**: 543–51.
44 Ou JH, Laub O, Rutter WJ. Hepatitis B gene function: The precore region targets the core antigen to cellular membranes and causes the secretion of e-antigen. *Proc Natl Acad Sci USA* 1986; **83**: 1578–82.
45 Roossinck M, Jameel S, Loukin SH, Siddiqui A. Expression of hepatitis B viral core region in mammalian cells. *Mol Cel Biol* 1986; **6**: 1393–400.
46 Uy A, Bruss V, Gerlich WH, Kochel HG, Thomssen R. Precore sequence of hepatitis B virus inducing e antigen and membrane association of the viral core protein. *Virology* 1986; **155**: 89–96.
47 Weimer T, Salfeld J, Will H. Expression of hepatitis B core antigen in vivo and in vitro. *J Virol* 1987; **61**: 3109–13.
48 Standring DN, Ou JH, Masiarz FR, Rutter WJ. A signal peptide encoded within the precore region of hepatitis B virus directs the secretion of a heterogenous population of e antigens in Xenopus oocytes. *Proc Natl Acad Sci USA* 1988; **85**: 8505–9.
49 Ou JH, Yeh CT, Yen TSB. Transport of hepatitis B virus pre-core proteins into nucleus after cleavage of its signal peptide. *J Virol* 1989; **63**: 5238–43.
50 Schlicht HJ, Salfeld J, Schaller H. The duck hepatitis B virus pre-C region encodes a signal sequence which is essential for synthesis and secretion of processed core proteins but not for virus formation. *J Virol* 1987; **61**: 3701–9.
51 Change C, Enders G, Sprengel R, Peters N, Varmus HE, Ganem D. Expression of the precore region of an avian hepatitis virus is not required for viral replication. *J Virol* 1987; **61**: 3322–5.
52 Junker M, Galle P, Schaller H. Expression and replication of the hepatitis B virus genome under foreign promoter control. *Nucl Acids Res* 1987; **15**: 10117–32.
53 Carman WF, Jacyna MR, Hadziyannis S, *et al.* Mutation preventing formation of hepatitis B e antigen in patients with chronic hepatitis B infection. *Lancet* 1989; ii: 588–91.
54 Brunetto MR, Stemler M, Bonino F, *et al.* A new hepatitis B virus strain in patients with severe anti-HBe positive chronic hepatitis B. *J Hepatol* 1990; **10**: 258–61.
55 Mack DH, Bloch W, Nath N, Sninsky JJ. Hepatitis B virus particles contain a polypeptide encoded by the largest open reading frame: A putative reverse transcriptase. *J Virol* 1988; **62**: 4786–90.
56 Bavand MR, Laub O. Two proteins with reverse transcriptase activities associated with hepatitis B virus-like particles. *J Virol* 1988; **62**: 626–8.
57 Schlicht HJ, Radziwill G, Schaller H. Synthesis and encapsidation of duck hepatitis B virus reverse transcriptase do not require formation of core-polymerase fusion proteins. *Cell* 1989; **56**: 85–92.
58 Bosch V, Bartenschlager R, Radziwell G, Schaller H. The duck hepatitis B virus P-gene codes for protein strongly associated with the 5′ end of the viral DNA minus strand. *Virology* 1988; **166**: 475–85.
59 Bartenschlager R, Schaller H. The amino-terminal domain of the hepadnaviral P-gene encodes the termial protein (genome-linked protein) believed to prime reverse transcription. *EMBO* 1988; **7**: 4185–92.
60 Bartenschlager R, Junker-Niepmann M, Schaller H. The P gene product of hepatitis B virus is required as a structural component for genomic RNA encapsidation. *J Virol* 1990; **64**: 5324–32.
61 Moriarty AM, Alexander H, Lerner RA, Thornton GB. Antibodies to peptides detect new hepatitis B antigen: Serological correlation with hepatocellular carcinoma. *Science* 1985; **227**: 429–33.
62 Meyers ML, Trepo LV, Nath N, Sninsky JJ. Hepatitis B virus polypeptide X: Expression in Escherichia coli and identification of specific antibodies in sera from hepatitis B virus-infected humans. *J Virol* 1986; **57**: 101–9.
63 Persing DH, Varmus HE, Ganem D. Antibodies to pre-S and X determinants arise during natural infection with ground squirrel hepatitis virus. *J Virol* 1986; **60**: 177–84.
64 Elfassi E, Haseltine WA, Dienstag JL. Detection of hepatitis B virus X product using an open reading frame Escherichia coli expression vector. *Proc Natl Acad Sci USA* 1986; **83**: 2219–22.
65 Kaneko S, Miller RH. X-region-specific transcript in mammalian hepatitis B virus-infected liver. *J Virol* 1988; **11**: 3979–84.
66 Katayama K, Hayashi N, Sasaki Y, *et al.* Detection of hepatitis B virus X gene protein and antibody in type B chronic liver disease. *Gastroenterology* 1989; **97**: 990–8.
67 Spandau DF, Lee CH. Trans-activation of viral enhancers by the hepatitis B virus X protein. *J Virol* 1988; **62**: 427–34.
68 Seto E, Yen TSB, Peterlin BM, Ou JH. Trans- activation of the human immunodeficiency virus long terminal repeat by the hepatitis B virus X protein. *Proc Natl Acad Sci USA* 1988; **85**: 8286–90.
69 Feitelson MA. Products of the 'X' gene in hepatitis B and related viruses. *Hepatology* 1986; **6**: 191–8.
70 Hu KQ, Vieling JM, Siddiqui A. Trans-activation of HLA-DR gene of hepatitis B virus X gene product. *Proc Natl Acad Sci USA* 1990; **87**: 7140–4.
71 Twu JS, Robinson WS. Hepatitis B virus X gene can transactivate heterologous viral sequences. *Proc Natl Acad Sci USA* 1989; **86**: 2046–50.
72 Twu JS, Chu K, Robinson WS. Hepatitis B virus X gene

activates kB-like enhancer sequences in the long terminal repeat of human immunodeficiency virus 1. *Proc Natl Acad Sci USA* 1989; **86**: 5168–72.
73 Lee TH, Finegold MJ, Sheen RF, De Mayo JL, Woo SLC, Butel JS. Hepatitis B virus transactivator X protein is not tumorigenic in transgenic mice. *J Virol* 1990; **64**: 5939–47.
73a Kim CM, Koike K, Saito I, Miyamura T, Jay G. HBx gene of hepatitis B virus induces liver cancer in transgenic mice. *Nature* 1991; **351**: 317–20.
74 Miller RH, Robinson WS. Common evolutionary origin of hepatitis B virus and retroviruses. *Proc Natl Acad Sci USA* 1986; **83**: 2531–5.
75 Gerlich WH, Bruss V, Heermann KH, Marquardt O, Seifer M. Presurface and precore products of human hepatitis B virus. In: Robinson WS, ed. *Hepadna viruses*. New York: Alan R. Liss, 1987; 147–60.
76 Imai M, Yanase Y, Nojiri T, Miyakawa Y, Mayumi M. A receptor for polymerised human and champanzee albumins on hepatitis B virus particles co-occurring with HBeAg. *Gastroenterology* 1979; **76**: 242–7.
77 Trevisan A, Gudat F, Guggenheim R, *et al.* Demonstration of albumin receptors on isolated human hepatocytes by light and scanning electron microscopy. *Hepatology* 1982; **2**: 832–5.
78 Machida A, Kishimoto S, Ohnuma H, *et al.* A polypeptide containing 55 amino acid residues coded by the pre-s region of hepatitis B virus DNA, bears the receptor for polymerised human as well as chimpanzee albumins. *Gastroenterology* 1984; **86**: 910–8.
79 Thung SN, Gerber MA. Polyalbumin receptors: their role in the attachment of hepatitis B virus to hepatocytes. *Semin Liver Dis* 1984; **4**: 69–75.
80 Thung SN, Gerber MA. Albumin binding sites of human hepatocytes. *Liver* 1983; **3**: 290–4.
81 Yu MW, Finlayson JS, Shih JWK. Interaction between various polymerised human albumins and HBsAg. *J Virol* 1985; 55: 736–43.
82 Heermann KH, Waldeck F, Gerlich WH. Interaction between native human serum and the pre-S2 domain of HBsAg. In: Zuckerman AJ, ed. *Viral hepatitis and liver disease*. New York: Alan R Liss, 1988: 697.
83 Krone B, Lenz A, Heerman KH, Seifer M, Lu XY, Gerlich WH. Interaction between haptatitis B surface proteins and monomeric serum albumin. *Hepatology* 1990; **11**: 1050–6.
84 Reed WD, Mitchell CG, Eddleston ALWF, Lee WM, Williams R. Exposure and immunity to hepatitis B virus in a liver unit. *Lancet* 1974; i: 581–3.
85 Lee WM, Reed WD, Mitchell CG, Galbraith RM, Eddleston ALWF, Zuckerman AJ, Williams R, Cellular and humoral immunity to hepatitis-B surface antigen in active chronic hepatitis. *BMJ* 1975; i: 705–8.
86 Eddleston ALWF and Williams R. Inadequate antibody response to HBsAg or suppressor-T cell defect in the development of active chronic hepatitis. *Lancet* 1974; ii: 1543–5.
87 Lee WM, Reed WD, Mitchell CG, Woolf IL, Dymock IW, Eddleston ALWF, Williams R. Cell mediated immunity to hepatitis B surface antigen in blood donors with persistent antigenaemia. *Gut* 1975; **16**: 416–20.
88 Lee WM, Reed WD, Osman CG, Vahrman J, Zuckerman AJ, Eddleston ALWF, *et al.* Immune responses to the hepatitis B surface antigen and liver specific lipoprotein in acute type B hepatitis. *Gut* 1977; **18**: 250–7.
89 Jensen DM, McFarlane IG, Portmann BS, Eddleston ALWF, Williams R. Detection of antibodies directed against a liver specific membrane lipoprotein in patients with acute and chronic active hepatitis. *N Engl J Med* 1978; **299**: 1–7.
90 Haynes ME, Haynes DWG, Williams R. Cytoplasmic particles in hepatocytes of patients with Australia antigen positive liver disease. *J Clin Pathol* 1972; **25**: 403–9.
91 Alberti A, Diana A, Scullard GH, Eddleston ALWF, Williams R. Full and empty Dane particles in hepatitis B virus infection: a relation to hepatitis B e antigen and the presence of liver damage. *Gastroenterology* 1978; **75**: 869–75.
91a Realdi G, Alberti A, Rugge M, *et al.* Seroconversion from hepatitis B e antigen to anti-HBe in chronic hepatitis B virus infection. *Gastroenterology* 1980; **79**: 195–9.
92 Alberti A, Diana S, Scullard GH, Eddleston ALWF, Williams R. Detection of a new antibody system reacting with Dane particles in hepatitis B virus infection. *BMJ* 1978; ii: 1056–8.
92a Alberti A, Cavalletto D, Chemello L, *et al.* Fine specificity of human antibody response to the preS1 domain of hepatitis B virus. *Hepatology* 1991; **12**: 199–203.
93 Nouri-Aria KT, Hegarty JE, Alexander GJM, Eddleston ALWF, Williams R. Effect of corticosteroids on suppressor cell activity in autoimmune and viral chronic active hepatitis. *N Engl J Med* 1982; **307**: 1301–4.
94 Nouri-Aria KT, Alexander GJM, Portmann B, Vergani D, Eddleston ALWF, Williams R. In vitro study of IgG production and concanavalin A induced suppressor cell function in acute and chronic hepatitis B virus infection. *Clin Exp Immunol* 1986; **64**: 50–8.
95 Vento S, Hegarty J, Alberti A, O'Brien CJ, Alexander GJM, Eddleston ALWF, Williams R. Evidence of T lymphocyte sensitisation to HBcAg and T cell mediated unresponsiveness to HBsAg in hepatitis B virus related chronic liver disease. *Hepatology* 1985; 5: 192-7.
96 Vento S, Rondanelli EG, Ranieri S, O'Brien CJ, Williams R, Eddleston ALWF. Prospective study of cellular immunity to hepatitis-B-virus antigens from the early incubation phase of acute hepatitis B. *Lancet* 1987; ii: 119–22.
97 El Sheikh N, Osman CG, Cullens H, Eddleston ALWF, Williams R. T lymphocyte-mediated cytotoxicity in HBsAg positive liver disease. *Clin Exp Immunol* 1978; **31**: 158–65.
98 Mieli-Vergani G, Vergani D, Portmann B, White Y, Murray-Lyon IM, Marigold JH, *et al.* Lymphocyte cytotoxicity to autologous hepatocytes in HBsAg positive chronic liver disease. *Gut* 1982; **23**: 1029–36.
99 Mondelli M, Mieli-Vergani G, Alberti A, Vergani D, Portmann B, Eddleston ALWF, *et al.* Specificity of T-lymphocyte cytotoxicity to autologous hepatocytes in chronic hepatitis B virus infection: evidence that T cells are directed against HBV core antigen expressed on hepatocytes. *J Immunol* 1982; **129**: 2773–8.
100 Naumov NV, Mondelli M, Alexander GJM, Tedder RS, Eddleston ALWF, Williams R. Relationship between expression of hepatitis B virus antigens in isolated hepatocytes and autologous lymphocyte cytotoxicity in patients with chronic hepatitis B virus infection. *Hepatology* 1984; **4**: 63–8.
101 Naoumov NV, Portmann BC, Tedder RS, Ferns B, Eddleston ALWF, Alexander GJM, *et al.* Detection of hepatitis B virus antigens in liver tissue: A relation to viral replication and histology in chronic hepatitis B infection. *Gastroenterology* 1990; **99**: 1247–53.
102 Lau JYN, Bain VG, Davies SE, Alexander GJM, Williams R. Export of HBV surface antigen in chronic hepatitis B virus infection is related to viral replication. *Hepatology* 1991 (in press).
103 Nouri-Aria KT, Arnold JC, Davison F, Portmann BC, Meager A, Morris AG, Eddleston ALWF, *et al.* Regulation of interferon alpha gene activation in acute and chronic HBV infection. *Hepatology* 1991 (in press).
104 Kato Y, Nakagawa H, Kobayashi K *et al.* Interferon production by peripheral lymphocytes in HBsAg positive liver disease. *Hepatology* 1982; **2**: 789–90.
105 Ikeda T, Lever AML, Thomas HC. Evidence for a deficiency of interferon production in patients with chronic hepatitis B virus infection acquired in adult life. *Hepatology* 1986; **6**: 962–6.
106 Jilbert AR, Burrell CJ, Gowans EJ, *et al.* Cellular localization of alpha interferon in hepatitis B virus-infected liver tissue. *Hepatology* 1986; **6**: 957–61.
107 Twu JS, Lee CH, Schloemer RH. Interaction between hepatitis B virus and human beta-interferon. In: Robinson WS, ed. *Hepadna viruses* New York: Alan R. Liss, 1987: 47–63.
108 Twu JS, Schloemer RH. Transcriptional trans- activating function of hepatitis B virus. *J Virol* 1987; **61**: 3448–53.
109 Lau JYN, Sheron N, Morris AG, Bomford AB, Alexander GJM, Williams R. Expression and regulation of interferon-alpha receptor in chronic hepatitis B infection. *Hepatology* 1991; **13**: 322–8.
110 Anastassakos Ch, Alexander GJM, Wolstencroft RA, *et al.* Interleukin-1 and interleukin-2 activity in chronic hepatitis B virus infection. *Gastroenterology* 1988; **94**: 999–1005.
111 Nouri-Aria KT, Magrin S, Alexander GJM, Anderson MG, Eddleston ALWF, Williams R. Abnormal T cell activation in chronic hepatitis B virus infection. A consequence of monocyte dysfunction? *Immunology* 1988; **64**: 733–8.
112 Nouri-Aria KT, Alexander GJM, Magrin S, Anderson MG, Eddleston ALWF, Williams R. Differential effect of interferon alpha on CD4 and CD8 positive lymphocytes in chronic hepatitis B virus carriers. *J Hepatol* 1988; 7: 1–6.
113 Daniels HM, Meager A, Eddleston ALWF, Alexander GJM, Williams R. Spontaneous TNF alpha and IL-1 beta production during successful interferon alpha therapy for chronic hepatitis B virus infection. *Lancet* 1990; **335**: 875–7.
114 Sheron N, Lau JYN, Daniels HM, Goka J, Eddleston ALWF, Alexander GJM, *et al.* Increased production of tumour necrosis factor alpha in chronic hepatitis B virus infection. *J Hepatol* 1991; **12**: 241–5.
115 Dudley FJ, Giustino V, Sherlock S. Cell mediated immunity in patients positive for hepatitis-associated antigen. *BMJ* 1972; iv: 754–6.
116 Tur-Kaspa r, Burk RD, Shaul Y, Shafritz DA. Hepatitis B virus contains a glucocorticoid-responsive element. *Proc Natl Acad Sci USA* 1986; **83**: 1627–31.
117 Tur-Kaspa R, Laub O. Coricosteroids stimulate hepatitis B virus DNA, mRNA and protein production in a stable expression system. *J Hepatol* 1990; **11**: 34–6.
118 Lau JYN, Bain VG, Smith HM, Alexander GJM, Williams R. Modulation of hepatitis B viral antigen expression by immunosuppressive drugs in primary hepatocyte culture. *Transplantation* (in press).
119 Reed WD, Eddleston ALWF, Cullens H, Williams R, Zuckerman AJ, Peters DK, *et al.* Infusion of hepatitis-B antibody in antigen-positive active chronic hepatitis. *Lancet* 1973; ii: 1347–51.
120 Alberti A, Diana S, Eddleston ALWF, Williams R. Changes in hepatitis B virus DNA polymerase in relation to outcome in acute hepatitis type B. *Gut* 1979; **10**: 190–5.
121 Scullard GH, Alberti A, Wansbrough-Jones MH, Howard CR, Eddleston ALWF, Zuckerman AJ, *et al.* Effects of human leucocyte interferon on hepatitis B virus replication and immune responses in patients with chronic hepatitis B virus infection. *J Clin Lab Immunol* 1979; **1**: 4–8.
122 Alexander GJM, Fagan EA, Hegarty JE, Yeo J, Eddleston ALWF, Williams R. A controlled trial of acyclovir in chronic hepatitis B virus infection. *J Med Virol* 1987; **21**: 81–7.
123 Bain VG, Daniels HM, Chanas A, Alexander GJM, Williams R. Foscarnet therapy in chronic hepatitis B virus infection. *J Med Virol* 1989; **29**: 152–5.
124 Alexander GJM, Fagan EA, Rolando N, Guarner P, Eddleston ALWF, Williams R. Differential effect of ARA-

AMP on serum DNA-polymerase and HBV-DNA. A possible reason for lack of efficacy. *J Hepatol* 1986; **3**: S81–6.
125 Sheron N, Lau JYN, Daniels HM, Webster J, Eddleston ALWF, Alexander GJM, *et al.* TNF to treat chronic hepatitis B virus infection. *Lancet* 1990; **336**: 321–2.
126 Alexander GJM. Treatment of acute and chronic viral hepatitis. In: Davis M, ed. *Therapy of liver disease.* Bailliere's *Clinical gastroenterology series*, London, Bailliere Tindall, 1989; **3**: 1–20.
127 Alexander GJM, Brahm J, Fagan EA, Smith HM, Daniels HM, Eddleston ALWF, *et al.* Loss of HBsAg with interferon therapy in chronic hepatitis B virus infection. *Lancet* 1987; ii: 66–9.
128 Bain VG, White YS, Naoumov NV, Lau JYN, Alberti A, Cavaletto D, *et al.* Differential effect of interferon-α on hepatitis B viral antigen expression in primary hepatocyte culture. *Hepatology* 1989; **10** (suppl): 110 Abstract 295.
129 Lau JYN, Bain VG, Naoumov NV, Smith HM, Alexander GJM, Williams R. Effect of interferon-gamma on the expression of hepatitis B virus antigen in primary hepatocyte culture. *Hepatology* (in press).
130 Davies SE, Portmann B, O'Grady JG, Aldis PM, Chaggar K, Alexander GJM, Williams R. Hepatic histology following transplantation for chronic hepatitis B virus infection including a unique pattern of fibrosing cholestatic hepatitis. *Hepatology* 1991; **13**: 150–7.
131 O'Grady JG, Smith HM, Davies SE, Daniels HM, Donaldson PT, Cohen AT, Portmann B, Alexander GJM, Williams R. Hepatitis B virus reinfection after orthotopic liver transplantation: serological and clinical implications. *J Hepatol* 1991 (in press).
132 Lau JYN, Bain VG, Davies SE, O'Grady JG, Alberti A, Alexander GJM, Williams R. High level expression of hepatitis B viral antigen in fibrosing cholestatic hepatitis: evidence that HBV may be cytopathic in liver grafts. *Gastroenterology* (in press).
133 Galbraith RM, Eddleston AL, Williams R, Zuckerman AJ, Bagshawe KD. Fulminant hepatic failure in leukaemia and choriocarcinoma related to withdrawal of cytotoxic drug therapy. *Lancet* 1975; ii: 528–30.
134 Hoofnagle JH, Dusheiko GM, Schafter DR, *et al.* Reactivation of chronic hepatitis B virus infection by cancer chemotherapy. *Ann Intern Med* 1982; **96**: 447–9.
135 Thung SN, Gerber MA, Klion F, Gilbert H. Massive hepatic necrosis after chemotherapy withdrawal in a hepatitis B virus carrier. *Arch Intern Med* 1985; **145**: 1313–4.
136 Bird GLA, Smith HM, Portmann BC, Alexander GJM, Williams R. Acute liver decompensation on withdrawal of cytotoxic chemotherapy and immunosuppressive therapy in hepatitis B carriers. *Q J Med* 1987; **73**: 895–902.
137 Lau JYN, Lai CL, Lin HJ, *et al.* Fatal reactivation of chronic hepatitis B virus infection following chemotherapy withdrawal in lymphoma patients. *Q J Med*, 1989; **73**: 911–7.
138 Lau JYN, Bird GLA, Gimson AES, Alexander GJM, Williams R. Treatment of HBV reactivation after withdrawal of immunosuppression. *Lancet* 1991; **337**: 802.
139 Zuckerman AJ. Immunisation against hepatitis B. In: Zuckerman AJ, ed. *British medical bulletin: viral hepatitis.* London: Churchill Livingstone, 1990: 383–98.
140 Stevens CE, Taylor PE, Tong MJ, *et al.* Hepatitis B vaccine: An overview. In: Vyas GN, Dienstag JL, Hoofnagle JH, eds: *Viral hepatitis and liver disease.* Orlando, FL: Grune & Stratton, 1984: 275.
141 Fagan EA, Tolley P, Smith HM, *et al.* Hepatitis B vaccine: immunogenicity and follow-up including two year booster doses in high-risk health care personnel in a London teaching hospital. *J Med Virol* 1987; **21**: 49–56.
142 Hadler SC, Francis DP, Maynard JE, *et al.* Long term immunogenicity and effiacy of hepatitis B vaccine in homosexual men. *N Engl J Med* 1986; **315**: 209.
143 Carman WF, Zanetti AR, Karayiannis P, *et al.* Vaccine-induced escape mutant of hepatitis B virus. *Lancet* 1990; **336**: 325–9.
144 Itoh Y, Takai E, Ohnuma H, *et al.* A synthetic peptide vaccine involving the product of the pre-S2 region of hepatitis B virus DNA: protective effiacy in chimpanzees. *Proc Natl Acad Sci USA* 1986; **83**: 9174–8.
145 Feinstone SM, Kapikian AZ, Purcell RH, Alter HJ, Holland PV. Transfusion-associated hepatitis not due to viral hepatitis type A or B. *N Engl J Med* 1975; **292**: 767–70.
146 Dienstag JL. Non-A, non-B hepatitis: Recognition, epidemiology and clinical features. *Gastroenterology* 1983; **83**: 439–62.
147 Fagan EA, Davison FD, Trowbridge R, *et al.* Detection of hepatitis B virus DNA sequences in liver in HBsAg seronegative patients with liver disease with or without anti-HBc antibodies. *Q J Med* 1991; **78**: 123–34.
148 Shih JWK, Esteban JI, Alter HJ. Non-A, non-B hepatitis: advances and unfulfilled expectations of the first decade. *Prog Liver Dis* 1986; **8**: 433–52.
149 Shimizu YK, Feinstone SM, Purcell RH, Alter HJ, London WT. Non-A, non-B hepatitis: Ultrastructural evidence for two antigens in experimentally infected chimpanzees. *Science* 1979; **205**: 197–200.
150 Bradley DW, Maynard JE, Popper H, *et al.* Post-transfusion non-A, non-B hepatitis: Physicochemical properties of two district agents. *J Infect Dis* 1983; **148**: 254–65.
151 Bradley DW, McCaustland KA, Cook EH, *et al.* Post-transfusion non-A, non-B hepatitis in chimpanzees: Physicochemical evidence that the tubule-forming agent is a small enveloped virus. *Gastroenterology* 1985; **88**: 773–9.
152 He LF, Alling d, Popkin T, Shapiro M, Alter HJ, Purcell RH. Determining the size of Non-A, non-B hepatitis virus by filtration. *J Infect Dis* 1987; **156**: 636–40.
153 Hollinger FB, Mosley JW, Szmuness W, Aach RD, Peter RI, Stevens C. Transfusion-transmitted viruses study: experimental evidence for two non-A, non-B hepatitis agents. *J Infect Dis* 1980; **142**: 400–7.
154 Bradley DW, Maynard JE, Cook EH, *et al.* Non-A, non-B hepatitis in experimental infected chimpanzees: cross challenge and electron microscope studies. *J Med Virol* 1980; 6: 185–201.
155 Choo QL, Kuo G, Weiner AJ, Overby LR, Bradley DW, Houghton M. Isolation of a cDNA clone derived from a blood-borne non-A, non-B viral hepatitis genome. *Science* 1989; **244**: 359.
156 Arima T, Nagashima H, Murakami S, Kaji C, Fujita J, Shimomura H, *et al.* Cloning of a cDNA associated with acute and chronic hepatitis C infection generated from patients serum RNA. *Gastroenterol-Jpn* 1989; **24**: 540–4.
157 Kato N, Hijikata M, Ootsuyama Y, Nakagawa M, Ohkoshi S, Sugimura T, *et al.* Molecular cloning of the human hepatitis C virus genome from Japanese patients with non-A, non-B hepatitis. *Proc Natl Acad Sci USA* 1990; **87**: 9524–8.
158 Takamizawa A, Mori C, Fuke I, *et al.* Structure and organisation of the hepatitis C virus genome isolated from human carriers. *J Virol* 1991; **65**: 1105–13.
159 Miller RH, Purcell RH. Hepatitis C virus shares amino acid sequence similarity with pestiviruses and flaviviruses as well as members of the two plant virus supergroups. *Proc Natl Acad Sci USA* 1990; **87**: 2057–61.
160 Choo QL, Weiner AJ, Overby LR, Kuo G, Houghton M, Bradley DW. Hepatitis C virus: The major causative agent of viral non-A, non-B hepatitis. In: Zuckerman AJ, ed: *British medical bulletin: viral hepatitis.* London: Churchill Livingstone, 1990: 423–41.
161 Takeuchi K, Kubo Y, Boonmar S, *et al.* The putative nucleocapsid and envelope protein genes of hepatitis C virus determined by comparison of the nucleotide sequences of to isolates derived from an experimentally infected chimpanzee and healthy human carriers. *J Gen Virol* 1990; **71**: 3027–33.
162 Alter HJ, Purcell RH, Shih JW, *et al.* Detection of antibody to hepatitis C virus in prospectively followed transfusion recipients with acute and chronic non-A, non-B hepatitis. *N Engl J Med* 1989; **321**: 1494–500.
163 Kuo G, Choo QL, Alter HJ, *et al.* An assay for circulating antibodies to a major etiologic virus of non-A, non-B hepatitis. *Science* 1989; **244**: 362.
164 Miyamura T, Saito I, Katayama T, *et al.* Detection of antibody against antigen expressed by molecularly cloned hepatitis C virus cDNA: application to diagnosis and blood screening for posttransfusion hepatitis. *Proc Natl Acad Sci USA* 1990; **87**: 983–7.
165 Editorial. Will the real hepatitis C stand up. *Lancet* 1989; ii: 307–8.
166 Sirchia G, Bellobuono A, Giovanetti A, Marconi M. Antibodies to hepatitis C virus in Italian blood donors. *Lancet* 1989; ii: 797.
167 Esteban JI, Esterban R, Viladomiu L, *et al.* Hepatitis C virus antibodies among high risk groups in Spain. *Lancet* 1989; ii: 294–6.
168 Bruix J, Barrera JM, Calvet X, *et al.* Prevalence of antibodies to hepatitis C virus in Spanish patients with hepatocellular carcinoma and hepatic cirrhosis. *Lancet* 1989; ii: 1004–6.
169 Lenzi M, Ballardini G, Fusconi M, *et al.* Type 2 autoimmune hepatitis and hepatitis C virus infection. *Lancet* 1990; i: 258–9.
170 Jeffers L, deMedina M, Hasan F, *et al.* Hepatitis C associated idiopathic chronic hepatitis and cryptogenic cirrhosis. [Abstract] *Hepatology* 1989; **1** (suppl): 301.
171 Colombo M, Kuo G, Choo QL, *et al.* Prevalence of antibodies to hepatitis C virus in Italian patients with hepatocellular carcinoma. *Lancet* 1989; ii: 1006–9.
172 Katkov WN, Cody H, Evans AA, *et al.* The role of hepatitis C virus in chronic liver disease. [Abstract] *Hepatology* 1989; **1** (suppl): 302.
173 McFarlane IG, Smith HM, Johnson PJ, Bray G, Vergani D, Williams R. Significance of possible anti-HCV antibodies in autoimmune chronic active hepatitis. *Lancet* 1990; i: 754–7.
174 Weiner AJ, Kuo G, Bradley DW, *et al.* Detection of hepatitis C viral sequences in non-A, non-B hepatitis. *Lancet* 1990; i: 1–5.
175 Garson JA, Tedder RS, Briggs M, *et al.* Detection of hepatitis C viral sequences in blood donations by "nested" polymerase chain reaction and prediction of infectivity. *Lancet* 1990; **335**: 1419–22.
176 Dourakis S, Brown J, Karayiannis P, Kumar U, Monjardino J, Thomas HC. Serological response and detection of viraemia in acute hepatitis C virus infection. [Abstract] *BASL meeting* 1991.
177 Sallie R, Tibbs C, Rayner A, Alexander G, Williams R. Hepatitis "C" in patients with chronic liver disease. [Abstract] *BASL meeting* 1991.
178 Hoofnagle JH, Di Biscaglis AM. Therapy of chronic viral hepatitis: chronic hepatitis D and non-A, non-B hepatitis. In: Zuckerman AJ ed. *Viral hepatitis and liver disease.* New York: Alan R Liss, 1988: 823–30.
179 Davis GL, Balart LA, Schiff ER, *et al.* Treatment of chronic hepatitis C with recombinant interferon alfa – a multicentre randomised controlled trial. *N Engl J Med* 1989 321: 1501–6.
180 Bisceglie AM, Martin P, Kassianides C, *et al.* Recombinant interferon alfa therapy for chronic hepatitis C – a randomised, double-blinded, placebo-controlled trial. *N Engl J Med* 1989; **321**: 1506–10.
181 Marcellin P, Boyer N, Giostra E, *et al.* Recombinant human

α-interferon in patients with chronic non-a, non-B hepatitis: a multicenter randomized controlled trial from France. *Hepatology* 1991; **13:** 393–7.
182 Rizzetto M, Canese MG, Arico S, *et al.* Immunofluoresence detection of a new antigen-antibody system (delta-antidelta) associated with the hepatitis B virus in the liver and in the serum of HBsg carriers. *Gut* 1977; **18:** 997–1003.
183 Rizzetto M, Gerlin JL, Purcell RH. The hepatitis delta virus and its infection. *Prog Clin Biol Res* 1987; **234:** 361–534.
184 Rizzetto M, Verme G. Delta hepatitis. *J Hepatol* 1985; **1:** 187–93.
185 Wand KS, Choo QL, Weiner AJ, *et al.* Structure, sequence and expression of the hepatitis delta (δ) viral genome. *Nature* 986; 323: 508–14.
186 Kos A, Kijkema R, Arnberg AC, van der Meide PH, Schellkens H. The hepatitis delta (δ) virus possesses a circular RNA. *Nature* 1986; **323:** 558–60.
187 Chen PJ, Kalpana g, Goldberg J, *et al.* Structure and replication of the genome of the hepatitis δ virus. *Proc Natl Acad Sci USA* 1986; **83:** 8774–8.
188 Sharmeen L, Kuo MYP, Dinter-Gottieb G, Taylor J. Antigenomic RNA of human hepatitis delta virus can undergo self-cleavage. *J Virol* 1988; **62:** 2674–9.
189 Wu HN, Lin YJ, Lin FP, Makino S, Cheng MF, Lai MMC. Human hepatitis δ virus RNA subfragments contain an autocleavage activity. *Proc Natl Acad Sci USA* 1989; **86:** 1831–5.
190 Sharmeen L, Kuo MYP, Taylor J. Self-ligating RNA sequences on the antigenome of huma hepatitis delta virus. *J Virol* 1989; **63:** 1428–30.
191 Wu HN, Lai MMC. Reversible cleavage and ligation of hepatitis delta RNA. *Science* 1989; **243:** 652–4.
192 Taylor J, Mason W, Summers J, *et al.* Replication of human hepatitis delta virus in primary cultures of woodchuck hepatocytes. *J Virol* 1987; **61:** 2891–5.
193 Kuo MYP, Chao M, Taylor J. Initiation of replication of the human hepatitis delta virus genome from cloned DNA: role of delta antigen. *J Virol* 1989; **63:** 1945–50.
194 Davies SE, Portmann BC, Lau JYN, O'Grady JG, Alexander GJM, Williams R. Does hepatitis D virus require hepatitis B virus for replication: experience from liver transplantation. *Hepatology* 1990; **12:** 399. [Abstract 35.]
195 Monjardino JP, Saldanha JA. Delta hepatitis – the disease and the virus. In Zuckerman AJ ed: *British medical bulletin: viral hepatitis.* London: Churchill Livingstone, 1990: 399–407.
195aCole SM, Gowans EJ, Macnaughton TB, Hau PDLM, Burrell CJ. Direct evidence for cytotoxicity associated with expression of hepatitis delta virus antigen. *Hepatology* 1991; **13:** 845–51.
196 Negro F, Baldi M, Bonino F, *et al.* Chronic HDV (hepatitis delta virus) hepatitis: intrahepatic expression of delta antigen, histologic activity and outcome of liver disease. *J Hepatol* 1988; **6:** 8–14.
197 Chu CM, Liaw YF. Studies on the composition of the mononuclear cell infiltrates in liver from patients with chronic active delta hepatitis. *Hepatology* 1989; **10:** 911–5.
198 Lau JYN, Hansen LJ, Bain VG, Chaggar K, Smith HM, Vergiani D, *et al.* Expression of intrahepatic hepatitis D virus (HDV) in chronic HDV infection: relation to the pathogenesis of chronic liver disease. *J Clin Pathol* 1991 (in press).
199 Lenzi M, Fusconi M, Cassani F, *et al.* Characterisation of LKM1 and LKM2 antigens. *Prog Clin Biol Res* 1987; **234:** 343–5.
200 Smedile A, Farci P, Verme G, *et al.* Influence of delta infection on severity of hepatitis B. *Lancet* 1982; ii: 945–7.
201 Krogsgaard K, Kryger P, Aldershvile J, *et al.* δ-infection and suppression of hepatitis B virus replication in chronic HBsAg carriers. *Hepatology* 1987; **7:** 42–5.
202 Smedile A, Lavarini C, Crivelli O, Raimondo G, Fassone M, Rizzetto M. Radioimmunoassay detection of IgM antibodies to HBV associated delta antigen: clinical significance in delta infection. *J Med Virol* 1982; **9:** 131–8.
203 Smedile A, Rizzetto M, Denniston K, *et al.* Type D hepatitis: The clinical significance of hepatitis D virus RNA in serum as detected by a hybridisation-based assay. *Hepatology* 1986; **6:** 1297–302.
204 Govindarajan S, Gupta S, Valinluck B, Redeker AG. Correlation of IgM anti-hepatitis D virus (HDV) to HDV RNA in sera of chronic HDV. *Hepatology* 1989; **10:** 34–35.
205 Lau JYN, Smith HM, Chagger K, Hansen LY, Portmann BC, Alexander GJM, *et al.* Significance of IgM-anti-HDV in chronic hepatitis D infection. *J Med Virol* 1991; **33:** 273–6.
206 Chaggar K, McFarlane IG, Smith HM, Alexander GJM, Williams R. An enzyme immunoassay for detection of IgA class antibodies against the hepatitis D virus. *J Virol Meth* 1991 (in press).
207 McFarlane IG, Chaggar K, Davies SE, Smith HM, Alexander GJM, Williams R. IgA class antibodies against the hepatitis delta virus antigen in acute and chronic hepatitis delta virus infection. *Hepatology* (in press).
208 Negro F, Bonino F, Di Bisceglia A, Hoofnagle JH, Gerlin JL. Intrahepatic markers of hepatitis delta virus infection: A study by in-situ hybridisation. *Hepatology* 1989; **10:** 916–20.
209 Smedile A, Bergmann F, Baroudy BM, *et al.* Riboprobe assay for HDV RNA: a sensitive method for the detection of the HDV genome in clinical serum samples. *J Med Virol* 1990; **30:** 20–4.
210 Hoofnagle JH, Mullen K, Peters M, *et al.* Treatment of chronic delta hepatitis with recombinant alpha interferon. In: Rizzetto M, Gerin JL, Purcell RH, eds. *The hepatitis delta virus and its infection.* New York: Alan R Liss, 1987: 291–8.
211 Rizzetto M, Rosina F, Saracco G, *et al.* Treatment of chronic delta hepatitis with alpha-2 recombinant interferon. *J Hepatol* 1986; **3:** S229–33.
212 Thomas HC, Farci P, Shein R, *et al.* Inhibition of hepatitis delta virus (HDV) replication with lymphoblastoid human alpha interferon. In: Rizzetto M, Gerin JL, Purcell RH, eds. *The hepatitis delta virus and its infections.* New York: Alan R Liss, 1987: 277–90.
213 Hedin G, Weiland O, Ljunggren K, *et al.* Treatment of fulminant hepatitis B and fulminant hepatitis B and D coinfection with foscarnat. In: Rizzetto M, Gerin JL, Purcell RH, eds. *The hepatitis delta virus and its infections.* New York: Alan R Liss, 1987: 309–20.
214 Viewanath R. Epidemiology: infectious hepatitis in Delhi 1955–56. *Indian J Med Res* 1957; **45** (suppl 1): 1–29.
215 Naidu SS, Viewanath R. Infectious hepatitis in Delhi (1955–1956): a critical study; observations in pregnant women. *Indian J Med Res* 1957; **45** (suppl 1): 71–6.
216 Gupta DN, Smetana HF. The histopathology of viral hepatitis as seen in the Delhi epidemic. *Indian J Med Res* 1957; **45:** (suppl 1): 101–3.
217 Wong DC, Purcell RH, Sreenivasan MA, *et al.* Epidemic and endemic hepatitis in India: evidence for a non-A, non-B hepatitis virus aetiology. *Lancet* 1980; ii: 876–9.
218 Ramalingaswami V, Purcell RH. Waterborne non-A, non-B hepatitis. *Lancet* 1988; i: 571–3.
219 Bradley DW. Enterically-transmitted non-A, non-B hepatitis. In: Zuckerman AJ, ed. *British medical bulletin: Viral hepatitis.* London: Churchill Livingstone, 1990: 442–61.
220 De Cock KM, Bradley DW, Sanford NL, Cogindarajan S, Maynard JE, Refeker AG. Epidemic non-A, non-B hepatitis in patients from Pakistan. *Ann Intern Med* 1987; **106:** 227–30.
221 Balayan MS, Andzhaparidize AG, Savinskaya SS, *et al.* Evidence for a virus in non-A, non-B hepatitis transmitted via the fecal oral route. *Intervirology* 1983; **20:** 23.
222 Arankalle VA, Ticehurst J, Sreenivasan MA, *et al.* Aetiological association of a virus-like particle with enterically transmitted non-a, non-B hepatitis. *Lancet* 1988; i: 550–4.
223 Abe K, Shikata T, Fujisawa K, *et al.* Transmission of non-A, non-B hepatitis agent to chimpanzees from patients of epidemic hepatitis. *Jpn J Med Sci Biol* 1986; **39:** 119–27.
224 Bradley DW, Krawczynski K, Cook EH, *et al.* Enterically transmitted non-A, non-B hepatitis: serial passage of disease in cynomolgus macaques and tarmarins and recovery of disease-associated 27 to 34 nm virus like particles. *Proc Natl Acad Sci USA* 1987; **84;** 6277–81.
225 Krawczynski K, Bradley DW. Enterally transmitted non-A, non-B hepatitis: identification of virus associated antigen in experimentally infectd cynomolgus macaques. *J Infect Dis* 1989; **159:** 1042.
226 Kane MA, Bradley DW, Shrestha SM, *et al.* Epidemic non-A, non-B hepatitis in Nepal. Recovery of a possible etiological agent and transmission studies in possible etiologic agent and transmission studies in marmosets. *JAMA* 1984; **252:** 3140–5.
227 Panda SK, Datta R, Kaur J, Zuckerman AJ, Nayak NC. Enterically transmitted non-A, non-B hepatitis: Recovery of virus like particles from an epidemic in South Delhi and transmission studies in rhesus monkeys. *Hepatology* 1989; **10:** 466–72.
228 Reyes GR, Purdy MA, Kim JP, *et al.* Isolation of a cDNA from the virus responsible for enterically transmitted non-A, non-B hepatitis. *Science* 1990; **247:** 1335–9.
229 Khuroo MS, Teli MR, Skidmore S, Soti MA, Khuroo MI. Incidence and severity of viral hepatitis in pregnancy. *Am J Med* 1981; **70:** 252–5.
230 Gupta DN, Smetana HF. The histopathology of viral hepatitis as seen in the Delhi epidemic. *Indian J Med Res* 1957; **45:** 101–13.
231 Humphrey CD, Cook Jr EH, Bradley DW. Identification of enterically transmitted hepatitis virus particles by solid phase immune electron microscopy. *J Virol Meth* 1990; **29:** 177–88.
232 Fagan EA, Ellis D, Tovey G, Portamnn B, Williams R, Zuckerman AJ. Virus-like particles in liver in sporadic non-A, non-B fulminant hepatitis. *J Med Virol* 1989; **27:** 76–80.
233 Fagan EA, Ellis DS, Tovey G, Lloyd G, Portmann B, Williams R, *et al.* A toga-like virus as cause of fulminant hepatitis attributed to sporadic non-A, non-B. *J Med Virol* 1989; **28:** 150–5.
234 Phillips MJ, Blendis LM, Poucell S, *et al.* Syncytial giant-cell hepatitis—sporadic hepatitis with distinctive pathological features, a severe clinical course, and paramyxoviral features. *N Engl J Med* 1991; **324:** 455–60.
235 Editorial. Hepatitis G. *Lancet* 1991; **337:** 1070.