Recent Advances in
MEDICINE

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A. M. DAWSON
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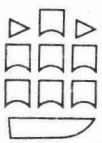

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Preface

Once again the Editors have assembled a distinguished group of contributors to Recent Advances in Medicine. The policy is similar to previous editions in that the contributors have been asked to discuss their subject in a manner which is comprehensible to a reader who is not a specialist in their field. Thus, the book is designed for postgraduate students, generalists and also specialists who wish to understand advances in fields other than their own. We hope that the reader will be as fascinated as we have been by the variety of advances that are reported.

We would like to thank Churchill Livingstone for their help and co-operation.

London, 1984

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1. The natural history of hepatitis B virus infection: impact of molecular biology

Howard C Thomas *Jon Monjardino*

INTRODUCTION

In tropical Africa, Indonesia, China and Japan the majority of people will be infected with the hepatitis B virus at some time during their lives, often at or near birth. Perinatal infection is usually asymptomatic but leads to chronic infection in 50% of children (Beasley & Stevens, 1978). Infection later in life is also often asymptomatic with the development of life long immunity. Approximately half the adults develop symptomatic acute hepatitis followed by recovery, 1% develop a fulminant hepatitis which is fatal in 80% of cases, and 10% develop chronic infection. The latter, both children and adults, exhibit a slowly progressive form of chronic hepatitis which ultimately may result, after 20–30 years, in development of cirrhosis and finally primary hepatocellular carcinoma. The chronic infection afflicts between 200 and 250 million of the world's population and is therefore one of the most important aetiological agents of cirrhosis and of cancer.

STRUCTURE OF THE VIRUS

The antigenic structure of the virus is now established (Fig. 1.1). The hepatitis B surface (HBs) antigen is a lipoprotein which coats the virus and also exists in particulate form (22 nm spheres and tubules) not associated with infectious virus. The

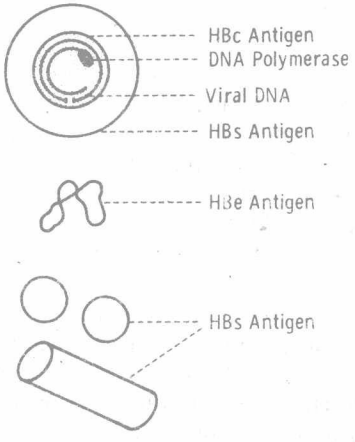

Fig. 1.1. Antigenic structure of the hepatitis B virus. HBe antigen is a soluble protein which is part of the nucleocapsid of the virus and is also present as a free protein in the serum of patients who are exhibiting active viral replication.

nucleocapsid of the virus contains the HBc antigen and a cryptic polypeptide, HBe antigen. The latter also exists, as a soluble protein of molecular weight 35 000 daltons, in the serum of the majority of patients who are supporting active viral replication. The virus contains albumin in its lipid coat and also bears a receptor for polymerised albumin on its surface. It has been suggested that the albumin acts as a ligand binding the virus to the hepatocyte membrane and aiding entry into the cell.

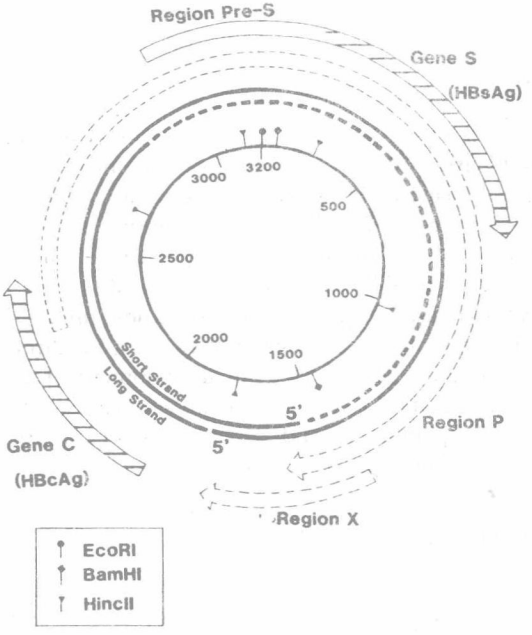

Fig. 1.2 Genome of hepatitis B virus (subtype adw). The long and short strands of DNA are shown. The genes of the virus are present on the long strand. Region P and X are open reading frames (i.e. possible genes) for the DNA polymerase and protein kinase of the virus. The nucleotide sequence and sites at which various enzymes cut the DNA are shown on the central 'clock': the genome contains 3200 bases.

The nucleic acid consists of a partially double stranded circular DNA, 3200 nucleotides long. Of the two strands, one is complete with one scission and the other is shorter with a large gap extending to up to 50% of its length. The 5′ terminus of the short strand has a fixed location and is sited 250 nucleotides to the 5′ side of the scission in the complete strand thereby ensuring a circular configuration. A small protein is covalently attached to the 5′ end of the complete strand (Gerslich et al, 1980).

Further insight into the structural organisation of the virus followed the purification of the viral genome and its production in large quantities by genetic engineering techniques (Valenzuela et al, 1979; Burrell et al, 1979; Shinsky et al, 1979). The nucleotide sequence of the HBV-DNA has been determined, and both surface and core genes have been mapped on the viral genome (Fig. 1.2). Two more open reading frames have also been identified and are presumed to correspond to the DNA polymerase and protein kinase genes.

REPLICATION OF HBV

The availability of cloned HBV-DNA also allowed investigators to make radiolabelled double and single stranded viral DNA probes which were used to detect homologous complementary viral DNA in the serum and liver tissue of infected subjects (Fig 1.3). This technique of molecular hybridisation has allowed the study of the mechanism of replication of the virus.

The virus particles in the serum contain a complete strand of DNA associated with a variable length complementary strand. After infection and uncoating of the virus

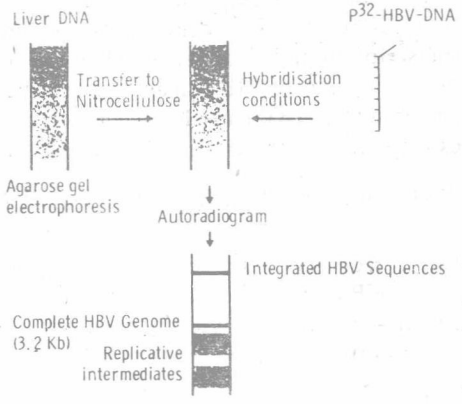

Fig. 1.3 Detection of HBV-DNA in liver DNA by southern blot and DNA hybridisation: P^{32} labelled HBV-DNA is used to detect complementary viral sequences in infected liver or serum.

DNA it is thought that the incomplete strand is completed by the endogenous DNA polymerase and the ends ligated prior to transcription of the viral genes (Hirschman and Garfinkel, 1982). The complete double stranded circular DNA molecules are found in the liver (Ruiz-Opazo et al, 1982) but rarely in the serum (Fowler et al, 1983) and are presumably produced after entry of the virus into the hepatocyte. The coding strand of the virus, which bears all of the known structural genes, is thought to be transcribed by the host RNA polymerase within the nucleus of the infected hepatocytes. The strand of full length RNA thus produced serves as a template (pregenome) for the synthesis of one strand of DNA (minus strand) by reverse transcription (Summers et al, 1982; Fowler et al, 1983), and has the same polarity as the messenger RNAs which travel to the cytoplasm and direct the synthesis of the component proteins of the virus (Gough, 1983).

INTEGRATION OF HBV SEQUENCES

It is now clear that HBV-DNA becomes covalently integrated into the hepatocyte genome at some time during the chronic infection (Brechot et al, 1981; Shafritz et al 1981; Fowler et al, 1983) (Table 1.1). Integrated viral sequences have also been

Table 1.1 Incidence of integrated HBV sequences in patients with chronic HBV infection (Fowler et al, 1983).

Patients	Number studied	HBV-DNA	
		Episomal	Integrated
Chronic Hepatitis (HBs + ve):			
HBeAg + ve	15	15	1*
HBeAb + ve	6	0	3

detected as discrete bands in the liver cell DNA of patients with fulminant hepatitis (Brechot et al, 1982). If the site of integration lies within the HBc coding gene, it will only be when two or more copies are inserted in tandem that the reconstitution of the full HBc sequence will be achieved allowing transcription and expression of this gene. If only single copies are integrated, the HBc gene will be split and only the HBs gene will be available for transcription and expression. Thus the hepatocyte containing integrated multiple tandem copies of HBV may express the various virus gene products necessary for assembly of HBV particles, whereas the hepatocyte containing single integrated copies may only synthesise and secrete HBs antigen.

MECHANISMS RESPONSIBLE FOR ELIMINATION OR PERSISTENCE OF HBV

During acute infection, if recovery is to occur, populations of cells containing replicating and integrated HBV must be eliminated. Recent data suggest that hepatocytes containing replicating virus are lysed by cytotoxic T cells (Eddleston et al, 1982; Montano et al, 1983) sensitised to HBc antigen (Eddleston et al, 1982) and cells which are not replicating virus, by a response to HBs antigen (Thomas et al, 1982) (Fig. 1.4). If this dual elimination system exists, it is easy to imagine that failure of the

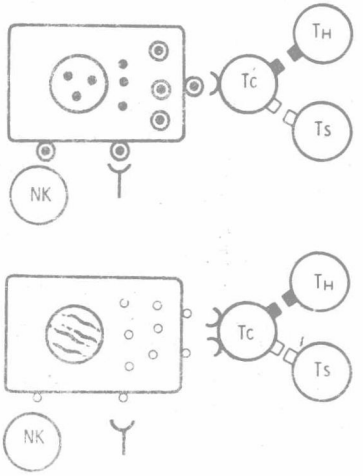

Fig. 1.4 Immune elimination of hepatocytes containing replicating and integrated HBV. A dual elimination system, with different target antigens, may be involved in clearing both types of infected cells. Evidence for the presence of hepatocytes containing integrated sequences in acute infection is controversial. Cytotoxic T cells (Tc) are most probably involved in the lysis of the infected hepatocytes. These cells are controlled by helper (Th) and suppressor (Ts) cells. NK cells may also be involved. Antibody (Y) probably plays a role in virus neutralization.

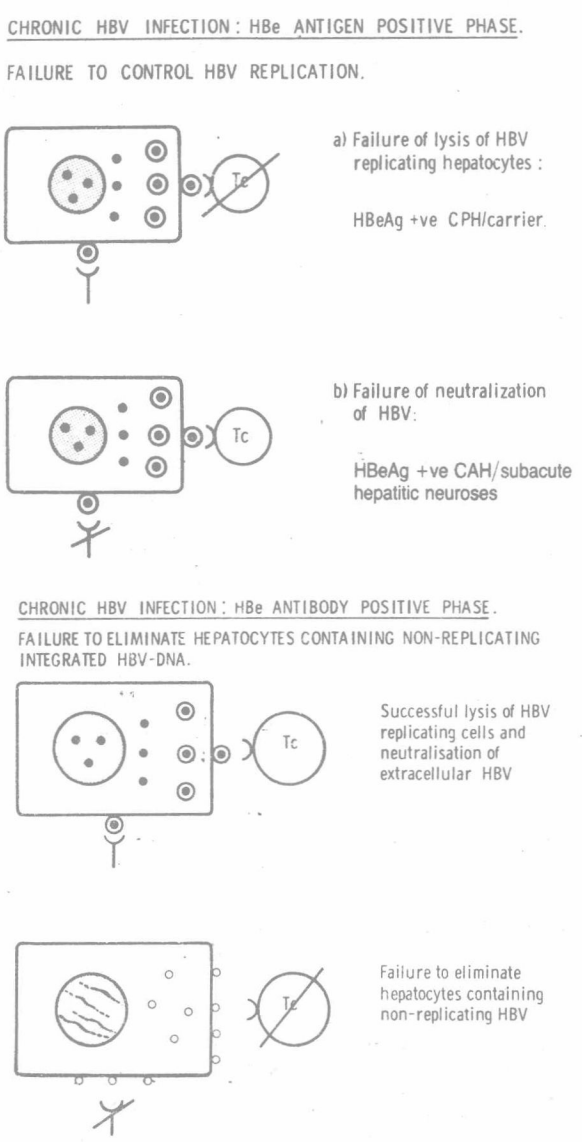

Fig. 1.5 Two phases of chronic HBV infection exist. During the HBe antigen positive phase there is failure of elimination of hepatocytes supporting active viral replication. In the second phase (HBe antibody positive) these cells are cleared and only those containing integrated HBV remain.

former results in chronic viral replication (HBe antigen positive chronic HBs antigenaemia) and selective failure of the second, in chronic HBs antigenaemia in the absence of chronic HBV replication (HBe antibody positive chronic HBs antigenaemia) (Fig. 1.5). In both cases integrated HBV-DNA sequences may be present (Brechot et al, 1981; Shafritz et al, 1981; Fowler et al, 1983), but are generally more readily detected in HBe antibody positive patients (Fowler et al, 1983).

VARIATION IN HOST RESPONSE CAUSES DIFFERING SEVERITY OF HEPATITIS IN ACUTE AND CHRONIC INFECTION

Both acute and chronic infection may result in variable degrees of hepatic necrosis (Fig. 1.6). Since the virus is not cytopathic, this variation is presumed to be related to differences in the hosts immune response to the virus.

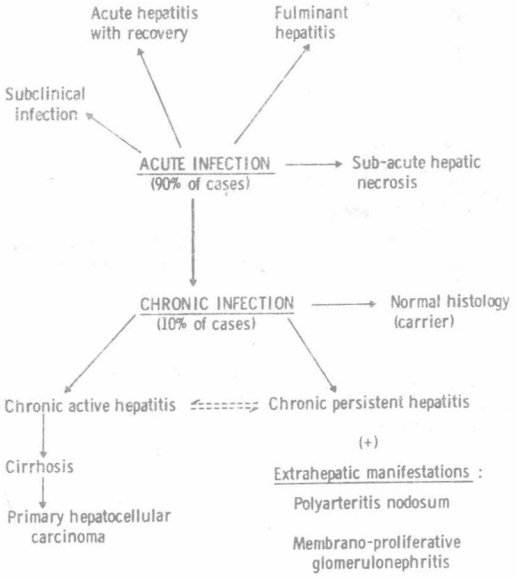

Fig. 1.6 Syndromes associated with acute and chronic HBV infection.

Syndromes occurring during acute infection

Fulminant hepatitis, which is a rare occurrence, has been suggested to be the result of an exaggerated humoral response to the virus which causes a necrotising vasculopathy (Arthus reaction) within the hepatic sinusoids (Woolf et al, 1976). A recent review of patients with presumed acute fulminant B hepatitis revealed that half were cases of delta infection occurring in chronic carriers of HBV (Smedile et al, 1982). Delta agent in an incomplete RNA virus which only replicates in HBV infected people and causes an exacerbation of the hepatitis (Rizzetto et al, 1980). Thus there are at least two mechanisms which may result in fulminant hepatitis.

Acute asymptomatic and symptomatic hepatitis are presumably examples of a more controlled response which results in the gradual lysis of infected cells and neutralisation of infectious virus particles.

Subacute hepatic necrosis which is seen in characteristic form in agammaglobulinaemic subjects, is probably the result of T cell lysis of infected hepatocytes without neutralisation of the virus. Thus, reinfection of regenerating hepatocytes may occur in these patients and is presumably followed by a further cycle of lytic activity. Whether the same lesion occurring in apparently 'immunologically normal' individuals is also

related to a relative failure of humoral immunity in the presence of a normal cellular response, remains to be determined.

Syndromes occurring during chronic infection

Patients with chronic infection manifest lesions varying from active cirrhosis, chronic active and persistent hepatitis to minimal hepatitis. These patients manifest two distinct inflammatory lesions, focal and piecemeal necrosis.

The focal lesions are seen throughout the hepatic lobule and are maximal during the clearance of hepatocytes containing replicating virus during acute hepatitis and in HBe antigen positive patients with chronic HBV infection who are undergoing seroconversion (Liaw et al, 1983) (Fig. 1.7). One factor determining the prognosis of

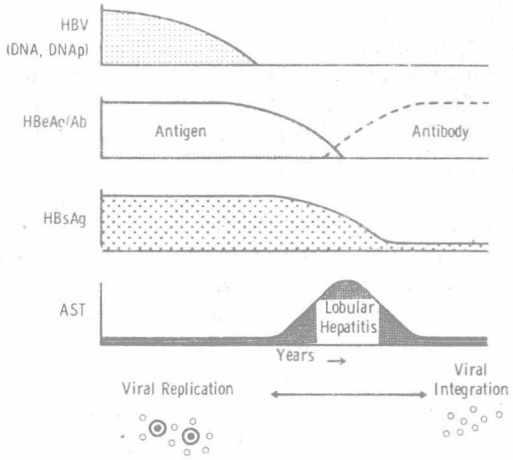

Fig. 1.7 Natural history of chronic HBV infection. The liver damage is maximal during the clearance of hepatocytes containing replicating HBV.

chronic HBV infection may be the duration of this conversion phase (HBe antigen to antibody). During this phase the patients exhibit a chronic lobular hepatitis and on cessation of replication (established HBe antibody phase) revert to chronic persistent or minimal hepatitis (Hoofnagle et al, 1981).

Some patients exhibit periportal piecemeal necrosis which is usually mild in severity in comparison to that seen in autoimmune lupoid chronic active hepatitis. This lesion is rare and when present is associated with the presence of liver membrane auto-antibodies (Wiedmann et al, 1983). It seems probable that these antibodies are formed because of a defect in the regulatory component of the immune system (Eddleston and Williams 1974; Thomas et al, 1982).

Another factor determining the level of inflammatory necrosis of hepatocytes in patients with chronic infection is co-infection with the incomplete RNA virus, delta. Infected patients do develop cirrhosis more rapidly than the HBV carrier without this infection (Weller et al, 1983).

Treatment of chronic HBV infection

The approach to treatment during the phase of HBe antigenaemia and viral replication is different from that needed during the HBe antibody phase, when replication has ceased and continued HBs antigen production is related to the presence of clones of cells containing integrated HBV-DNA. Although the inflammatory lesions are related to the elimination of hepatocytes containing replicating HBV and inhibition of this process results in an improvement in the rate of progression of the inflammatory liver disease, the risk of developing primary liver cell cancer is related to the presence of clones of cells containing integrated viral sequences and this problem has also to be considered during the treatment of the chronic infection. Since the risk of developing primary liver cell cancer is greatest in patients with established cirrhosis and minimal in those with normal liver histology, elimination of clones of hepatocytes containing integrated DNA is most important in the HBe antibody positive cirrhotic patient.

Attempts to inhibit replication of the virus have been partially successful. Adenine arabinoside and its monophosphate derivative have been shown in uncontrolled (Pollard et al, 1978; Chadwick et al, 1978; Weller et al, 1982) and randomised controlled studies (Bassendine et al, 1980; Weller et al, 1983) to produce significant inhibition of viral replication which in 30% of cases is long lasting. Attempts to increase the response rate by longer periods of treatment have been unsuccessful because of the development of a sensory polyneuropathy. For this reason, workers have turned towards the use of the interferons to try and obtain greater response rates. Initially, leucocyte interferons were used (Greenberg et al, 1976) and after one month of therapy, approximately one third of the patients exhibit permanent inhibition of viral replication. Lymphoblastoid interferon is also active in this condition (Weller et al, 1982) and can be given thrice weekly by intramuscular injection (Lok et al, 1983) (Fig. 1.8). Using this regime, treatment can be continued for periods of several months, and randomised controlled trials are now in progress to determine the incidence of long term inhibition of viral replication following this type of regimen. These approaches promise a significant reduction in infectivity and in rate of progression of the inflammatory liver disease to the majority of chronic carriers with active viral replication.

Once viral replication has ceased, the hepatitis usually subsides. The majority of anti-HBe positive carriers have either chronic persistent or minimal hepatitis (Hoofnagle et al, 1981) and run very little chance of developing primary liver cell cancer. The patient who has already developed cirrhosis has a high incidence of developing primary liver cell cancer (Beasley et al, 1982), and it is this patient who needs routine surveillance. It seems probable that the cell which becomes malignant in the HBe antibody positive carrier with cirrhosis is the hepatocyte containing integrated HBV-DNA (Shafritz et al, 1982). Procedures directed to elimination of these cells either by stimulation of the endogenous immune response (Zosko et al, 1978; Bassendine et al, 1980) or by injection of toxin labelled antibodies (Thomas et al, 1982), are under evaluation. In addition, these patients who are at high risk of developing primary liver cell cancer should be screened at regular intervals for a rising serum alphafetoprotein (Thomas et al, 1976). It is now well established that a persistently elevated or rising alphafetoprotein usually proceeds the development of primary liver cell cancer. Tumours detected at an early stage may be amenable to surgical resection or more successful chemotherapy.

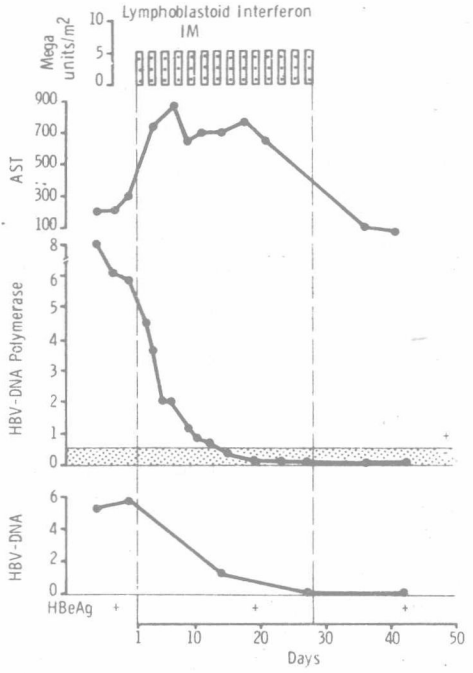

Fig. 1.8 Lymphoblastoid interferon given thrice weekly in low dosage for three months will result in long term inhibition of HBV replication in many patients (Lok et al, 1983).

Although the problems of the existing carrier must be investigated and dealt with, the overall problem of chronic liver disease, and primary liver cell cancer stemming from chronic HBV infection, is best dealt with by a programme of active immunisation. To this end, several vaccines have been developed. The current vaccines are produced from the viral coat protein (HBs antigen) purified from the serum of chronic carriers. This is a safe and highly effective vaccine (Szmuness et al, 1982) but rather expensive. Since the majority of chronic carriers live in the underdeveloped countries, attempts to produce cheaper vaccines are essential. The techniques of genetic engineering and production of synthetic vaccines are now being actively applied to this problem (Zuckerman et al, 1983).

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