HEPATITIS B VIRUS INFECTION:

FACTORS INFLUENCING THE OUTCOME

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HEPATITIS B VIRUS INFECTION: FACTORS INFLUENCING THE OUTCOME

Hepatitis B:
factoren die het beloop beinvloeden
(met een samenvatting in het Nederlands)

PROEFSCHRIFT

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aan mijn ouders
aan Rienke

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List of abbreviations

AC	asymptomatic HBsAg carrier
AHB-recov	recovered from acute hepatitis B
AIDS	acquired immune deficiency syndrome
ALT	alanine aminotransferase
anti-d	antibody to HBsAg determinant d
anti-HBc	antibody to hepatitis B core antigen
anti-HBe	antibody to HBeAg
anti-HBs	antibody to HBsAg
anti-y	antibody to HBsAg subdeterminant y
BSA	bovine serum albumin
CAH anti-s	chronic active hepatitis, HBsAg negative, anti-HBs and/or anti HBc positive
CAHB	chronic active hepatitis B
cpm	counts per minute
CPHB	chronic persistent hepatitis B
FACS	fluorescence activated cell sorter
FCS	fetal calf serum
FITC	fluorescein isothyocyanate
GAHu/Ig	goat anti-human immunoglobulin
HBV	hepatitis B virus
HBcAg	hepatitis B core antigen
HBeAg	hepatitis B e antigen
HBsAg	hepatitis B surface antigen
HBsAg/ad	HBsAg subtype ad
HBsAg/ay	HBsAg subtype ay
HDV	hepatitis delta virus
HLA	human leucocyte antigen
IgG	immunoglobulin G
IgM	immunoglobulin M
LSP	liver-specific lipoprotein
PBS	phosphate buffered saline
PI	propidium iodide
vs	versus

CHAPTER I

MOTIVATION, AIMS AND DESIGN OF THE STUDY

Introduction

The course of disease after infection with the hepatitis B virus is very diverse. The early manifestations of the disease may be mild and anicteric, more severe with jaundice or even fulminant with acute liver failure. Most patients recover completely. However, in some individuals the virus is not cleared and they become chronic hepatitis B virus carriers. It is not well known whether the early course of disease in patients who clear the virus is similar to the early course in those who eventually become chronic carriers. Some clinical, serological and histological differences between the various initial disease periods have been described. For example, a mild or asymptomatic early course of disease has been reported to be more frequently followed by persistence of the virus than a jaundiced episode.

Chronic carriers are also not a homogeneous group. A large proportion are asymptomatic. They have no biochemical or histological signs of hepatitis. The rest of the hepatitis B virus carriers have some degree of chronic hepatitis. This varies from mild with little tendency to liver damage to active liver disease with progression to liver cirrhosis. Primary hepatocellular carcinoma is relatively frequently found in patients with hepatitis B positive liver cirrhosis. Chronic liver failure and hepatocellular carcinoma both may lead to death.

This clinical spectrum, ranging from complete recovery to death, is the result of infection with just one type of virus. Although many subtypes of the hepatitis B virus are known, no differences of virulence have become apparent, which suggests that differences in clinical course are due to differences in host response. Subjects with impaired cellular or humoral immunity, in comparison to apparently healthy subjects, frequently fail to eliminate the virus after infection but usually develop milder forms of chronic hepatitis. On examination of liver biopsies T-lymphocytes appear to be the effector cells in the hepatocytolysis which accompanies viral hepatitis. Therefore, the various courses of disease have been ascribed to differences of the host's immune response to the viral infection. The immune response may have at least two effects: 1) (partial) removal of viral products and 2) damage of infected liver cells. An adequate immune response results in clearance of the virus. In some cases this is accompanied by appreciable liver cell damage and jaundice, in others the clearance of the virus occurs without clinical symptoms. In the case of an inadequate immune response, with failure to clear the virus, the liver cell damage may also vary from severe to completely absent. Elimination of the virus and damage to liver cells therefore seem to be mediated by different immunological mechanisms.

It is possible that the course of disease is determined somewhere between the infection and the early symptoms. The key factor may be the efficacy of early viral elimination. Theoretically, viral elimination may be the result of 1) destruction and elimination of all infected liver cells and/or 2) selective intracellular suppression of viral replication. The former is a general mechanism of viral elimination. With respect to hepatitis B virus infection there is some doubt as to whether viral elimination is the exclusive result of destruction of infected liver cells, as viral elimination and liver cell damage do not appear to be congruent in the various clinical forms of

hepatitis B. Although there is no clear evidence for specific suppression of hepatitis B virus replication, selective suppression of viral replication by antiviral antibodies has been described for other viruses. Thus, besides liver cell destruction, selective suppression of viral replication could conceivably play a role in the elimination of the hepatitis B virus. It is not known at what time during the early disease the seemingly different immunological mechanisms mediating viral clearance and liver cell damage come into play. Similarly, the factors that influence these immunological processes are not well known.

Hepatitis B virus infection causes considerable morbidity and mortality throughout the world. Recently, safe and reliable prophylaxis has become available in the form of vaccination. However, despite many efforts, no therapy has been shown to be conclusively effective in patients who have developed chronic hepatitis B

Motivation

Apart from prophylaxis, effective treatment of chronic hepatitis B is an important goal. Rational therapy can only be based on an extensive understanding of the disease processes. The mechanism that triggers the development of chronic hepatitis B is unknown. Even the factors that determine the efficacy of viral elimination and the extent of liver cell damage have been only partially characterized.

Aim of the study

The aim of this study was to find correlations between 1) the various courses of disease after infection with the hepatitis B virus and 2) quantitative parameters of viral replication and liver cell degradation, viral subtypes and the immunological response, in order to obtain more insight into the timing and the mechanisms of the development of chronic hepatitis B.

Design

Patients were grouped according to the several possible courses of disease after infection with the hepatitis B virus:

1) patients with acute hepatitis B;
2) subjects who had recovered from acute icteric hepatitis B;
3) subjects who had recovered from acute non-icteric hepatitis B;
4) chronic hepatitis B virus carriers without hepatitis;
5) chronic hepatitis B virus carriers with chronic persistent hepatitis;
6) chronic hepatitis B virus carriers with chronic active hepatitis and
7) patients who had cleared the hepatitis B virus and had developed chronic "auto-immune" hepatitis.

The study was designed to correlate these various well defined courses of disease after hepatitis B virus infection to:

1) quantitative parameters of viral activity and

2) quantitative parameters of liver cell degradation, separately and compared in time, in order to obtain information about the course of viral activity and liver cell degradation in patients with early hepatitis B who would eventually have different outcomes;

3) the hepatitis B viral subtypes and the subtypes of antiviral antibodies, in order to estimate the importance of viral factors and the subtype-related aspects of humoral immunity;

4) the peripheral T-cell status, in order to determine possible cellular immunoregulatory factors; and

5) the HLA type, in order to establish genetic (immunoregulatory) factors predisposing the host to a particular course of disease.

The following data were necessary:

1) Quantitative parameters of hepatitis B virus replication and viral products in time during the early phase of acute hepatitis B;

2) Quantitative parameters of liver cell damage, simultaneously with the viral parameters;

3) Characterization of subtypes of hepatitis B viral antigens and antibodies in patients with the various courses of acute hepatitis B and chronic hepatitis B carriership;

4) Characterization of immunoregulatory T-lymphocyte subtypes in subjects with the various courses after hepatitis B virus infection;

5) Determination of the (genetic) HLA type in subjects with the various courses after hepatitis B virus infection.

6) Normal control values.

CHAPTER II

HEPATITIS B VIRUS INFECTION

A review of the literature

2.1 The hepatitis B virus

The hepatitis B virus (HBV) is a DNA containing virus that mainly replicates in the liver cells of man and some higher primates, such as the chimpanzee. The viral genome codes for production of infectious virus particles with a diameter of 46 nm and a large amount of non-infectious smaller spherical and tubular particles by the infected liver cells. The latter are 22 nm in diameter and consist of excess viral coat protein, lipids and carbohydrates. The viral coat protein contains the hepatitis B surface antigen (HBsAg). The HBV itself consists of a nucleocapsid core surrounded by an envelope of the HBsAg containing viral coat protein, lipids and carbohydrates. The core includes the DNA genome, a DNA polymerase and a capsid protein, bearing the hepatitis B core antigen (HBcAg) and the hepatitis B e antigen (HBeAg) (fig 2.1).

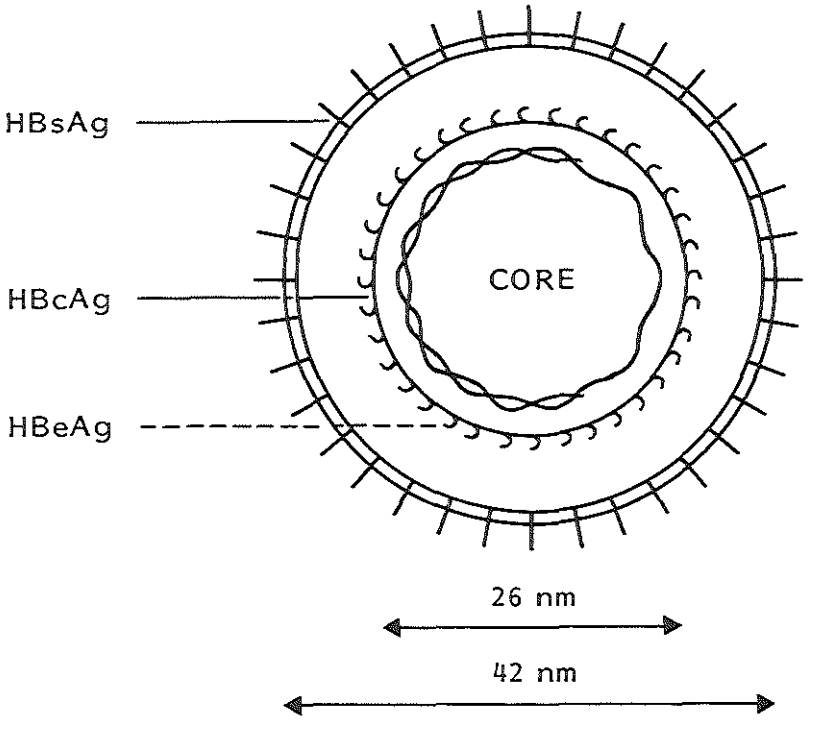

figure 2.1: The structure and the antigens of the hepatitis B virus.

Several subtypes of the hepatitis B virus have been described (1,2), based on differences in the coat protein. There are four major subtypes: adw, ayw, adr and ayr, which have the determinant a in common. The determinants d and y, and w and r are mutually exclusive in one subtype. The antigenic differences between the HBV strains are well established in epidemiological studies (3), inoculation studies in animals (4,5) and DNA sequence studies (6,7).

HBV DNA is a small circular molecule. It was first described by Robinson et al. (8). The DNA molecule is partly double-stranded, partly single-stranded, with a variable ratio. The long strand, or minus strand, has a fixed length of about 3200 nucleotides. The length of the short (plus) strand ranges from 50 to 75 per cent of that of the long (minus)

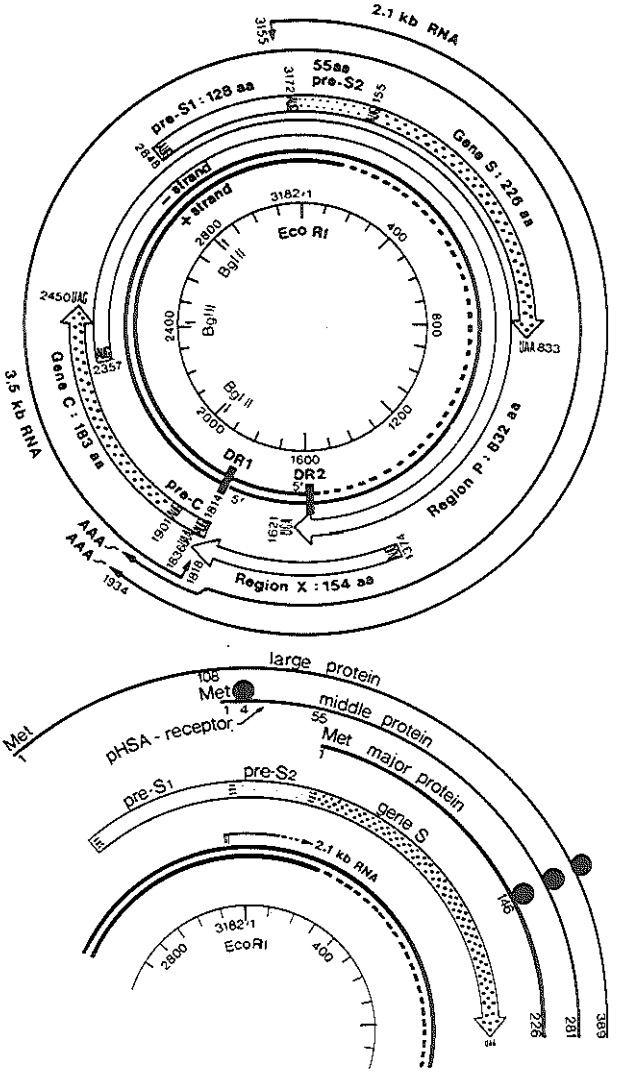

figure 2.2: Genetic organisation of the hepatitis B virus genome. (Reproduced from Tiollais (17), with permission of the publishers.)

strand (9). The plus strand is repaired by a DNA polymerase, using the minus strand as a template (10). The complete nucleotide sequence of HBV DNA is known for several subtypes of the virus (6,7). The minus strand contains four large open reading frames, called the S, P, X and C regions (11) (fig 2.2).

The S region codes for the HBsAg related proteins of the viral envelope. This region is divided into the gene S, the pre-S1 and pre-S2 sub-regions. The gene S region encodes the polypeptides P24 and GP27, both 226 amino acids long, but with different glycosylation. Theoretical analysis of the structure of these polypeptides has permitted a model for the spatial structure of HBsAg, with hydrophylic and hydrophobic regions (12,13). The hydrophylic regions probably bear the antigenic determinants of HBsAg (13). The pre-S2 and pre-S1 regions code for minor additional polypeptides at the N-terminal side of the gene S polypeptides. These larger forms of HBsAg are termed GP31/GP34 and P39/GP42, respectively (14,15), also with different glycosylation. P39 and GP42 have been identified as the translation products of the entire pre S / gene S reading frame (16). Thus, apart from the subtypes, six HBsAg containing polypeptides are known to date. The HBsAg subtypes seem to be correlated to slight differences in the amino acid sequences coded for in the gene S and the pre-S1 regions (13,17). The relative concentration of the pre-S coded polypeptides, in comparison to P24 and GP27, is higher in HBeAg positive than in HBeAg negative serum (18). Since HBeAg is correlated with active HBV replication, this suggests that the pre-S region is activated in that condition. GP31 and GP34 probably bear the receptor for polymerized human serum albumin, which may play a role in the penetration of the HBV into the hepatocyte (19).

The P region is the longest reading frame of the HBV genome, having considerable overlap with all other regions. There is some evidence that it codes for the DNA polymerase molecule (10).

The X region encodes a small polypeptide with slight variations according to the viral subtype (6). The functional meaning of the X region is unknown. Antibodies against peptide products of this region have been detected in human sera (20).

The C region codes for the polypeptides of the capsid, which is related to HBcAg and HBeAg. These polypeptides are hydrophobic and may play a role in the binding of the core to the viral envelope (13). HBeAg occurs as a soluble protein in the serum, either free or in association with immunoglobulin G (21).

The HBV DNA can occur in a freely replicating form, which has been detected in liver cells as well as in HBeAg positive serum of HBV carriers (22). It can also exist in an integrated form in the genome of the hepatocyte. Integrated HBV DNA is mostly found in liver cells of HBeAg negative chronic HBV carriers, without active liver disease and in hepatocellular carcinoma cells (23,24). In such cases the S gene may come to expression of HBsAg, but no products of the P and C regions are observed.

HBV DNA has also been detected in bone marrow and mononuclear blood cells during acute and chronic hepatitis B (25). Other extra-hepatic tissues, such as bile ducts, pancreas, spleen, kidney, skin, endothelial and smooth muscle cells, have also been found to contain HBV DNA (26,27).

The replication cycle of hepatitis B-like viruses has been investigated in other

viruses of the so-called hepadna virus group (28), the duck hepatitis virus, the ground squirrel hepatitis virus and the woodchuck hepatitis virus, which are comparable to the human hepatitis B virus and therefore can be used as a model (29). The replication cycle of hepadna viruses is characterized by the use of an RNA intermediate in the multiplication of the DNA genome (30,31). In these hepatitis B-like viruses multiple plus strand RNA copies are produced by the cellular RNA polymerase from each minus strand DNA molecule. The RNA intermediates either may serve as a template for DNA replication or have messenger function for the viral proteins. The RNA precursors of the DNA genome, termed "pre-genomes", are assembled into the immature nucleocapsid cores and degradated during reverse transcription of the new DNA minus strand. Synthesis of the plus strand DNA starts after completion of the minus strand (17,32).

2.2 Detection of hepatitis B virus products in serum and liver.

Several serological markers can be used to characterize the clinical stage after hepatitis B virus infection. Signs of active viral replication are high titres of HBsAg as well as positive tests for HBeAg, DNA polymerase and HBV DNA in the serum and the presence in liver cells of HBcAg, HBeAg and HBV DNA.

Qualitative detection of the viral antigens HBsAg and HBeAg by sensitive and specific radioimmunoassays and enzyme immunoassays is in wide use (33-35). Quantitative determination of these antigens, however, usually remains restricted to centres with special interest in hepatitis B virus infection (36-39). The absence of HBsAg and/or HBeAg does not prove that the serum is non-infectious, since HBV infections have been described with HBsAg negative, anti-HBc positive sera (40), as well as with HBsAg positive, HBeAg negative anti-HBe positive sera (41).

DNA polymerase activity points to the presence of DNA containing hepatitis B virus particles (42). DNA polymerase activity is usually found in the sera of patients with active viral replication. HBsAg positive sera without DNA polymerase activity may contain hepatitis B virus particles, visible by electron microscopy, but these particles probably do not contain the complete viral DNA within their cores. If that is true, they would be non-infectious (43). However, non-infectivity of HBsAg positive sera is very difficult to prove. DNA polymerase activity is detected by counting the incorporated radiolabel after incubation of serum with radiolabeled thymidine in a suitable medium (44,45).

Hepatitis B core antigen in serum can be detected by radioimmunoassay. A positive test is considered to be a sign of active viral replication (46).

Hepatitis B virus DNA in serum is detected by means of molecular hybridization techniques. Usually HBV DNA, adsorbed from the serum to a nitrocellulose sheet, is hybridized with a HBV DNA probe. Such a probe consists of in vitro produced HBV DNA, labeled with an isotope (47). In a second step the fixed hybridized probe is revealed by autoradiography. Like the tests for DNA polymerase, HBcAg and HBeAg in serum, the HBV DNA hybridization assay is positive in the case of active viral replication.

Hepatitis B virus antigen localizations in liver tissue are demonstrated with immunofluorescence and immunoperoxidase techniques. Specific antibodies labeled with fluorescein or peroxidase are incubated with the liver tissue to bind to

the antigens. After washing the tissue slides are examined microscopically. In this way, the expression of HBsAg and HBcAg can be investigated (48-51). HBeAg is predominantly found in co-occurrence with HBcAg (52,53). The viral antigen expression in liver tissue is believed to be modulated, at least in part, by the host's immune defence and to reflect the grade of insufficiency to eliminate the hepatitis B virus (54).

Detection of hepatitis B virus DNA in liver tissue is performed either with extraction of DNA from the liver tissue and Southern blotting or with in situ hybridization. In the former technique, the extracted DNA is digested with endonucleases, followed by electroforesis and molecular hybridization with isotope-labeled DNA (55). This technique allows the detection of free HBV DNA as well as HBV DNA integrated in the host genome (56-58). The in situ hybridization technique can be applied to formalin-fixed liver biopsy specimens. A DNA probe, labeled with an isotope or a biotin-avidin-peroxidase complex, is incubated with the liver tissue and in a second step the coupe is autoradiographed or examined by microscope, depending on the labeling method (26,59).

2.3 The clinical course after hepatitis B virus infection.

Various courses of disease may develop after infection with the hepatitis B virus (fig 2.3). The HBV can cause acute icteric hepatitis, non-icteric hepatitis or no hepatitis at all. In any of these cases the virus can be cleared, leading to recovery, or persist, leading to chronic HBV carriership. Chronic HBV carriers may be asymptomatic or develop chronic persistent or chronic active hepatitis, in a mild, moderate or severe form. So, in acute disease as well as in chronic carriership various degrees of hepatitis are found, irrespective clearance or persistence of the virus. The discongruency between liver cell degeneration and virus elimination suggests that these two processes are mediated by different mechanisms.

Some factors, such as age, sex and immunological status, may predispose to persistence of the virus. The age at the time of infection is important, as almost all neonates develop chronic HBV carriership after infection with the HBV, whereas children and adults do so much less frequently (60-63). Sex is also a predisposing factor in adults, but not in children (60,61). Chronic HBV carriership is about two times more frequent in men than in women (63,64). The importance of the immunological status is reflected by the high prevalence of chronic HBV carriers in subjects with impaired cellular immunity, such as renal dialysis patients, subjects with Down's syndrome and patients with lymphoma or leukemia (63,64). It has been observed that a mild early course of disease, mostly without jaundice, is relatively frequently followed by persistence of the virus and the development of chronic HBV carriership (62,63,65,66). Exogenous factors which may influence the clinical course after HBV infection include concomitant infection with other viruses, of which the Delta agent is possibly the most important (67), and exposure to hepatotoxins such as ethanol (68).

It is estimated that there are about 200 million chronic HBV carriers worldwide (64). The frequency of HBV carriers ranges from 15-20% in some countries in Africa and Southeast Asia to less than 1% in North America and Western Europe. High risk

groups for acquiring a HBV infection are discernable in the populations of the geographical regions with low overall prevalence, for instance medical personnel, intravenous drug abusers and male homosexuals with frequently changing sexual contacts. These groups have in common that they are involved in the transmission routes of the HBV.

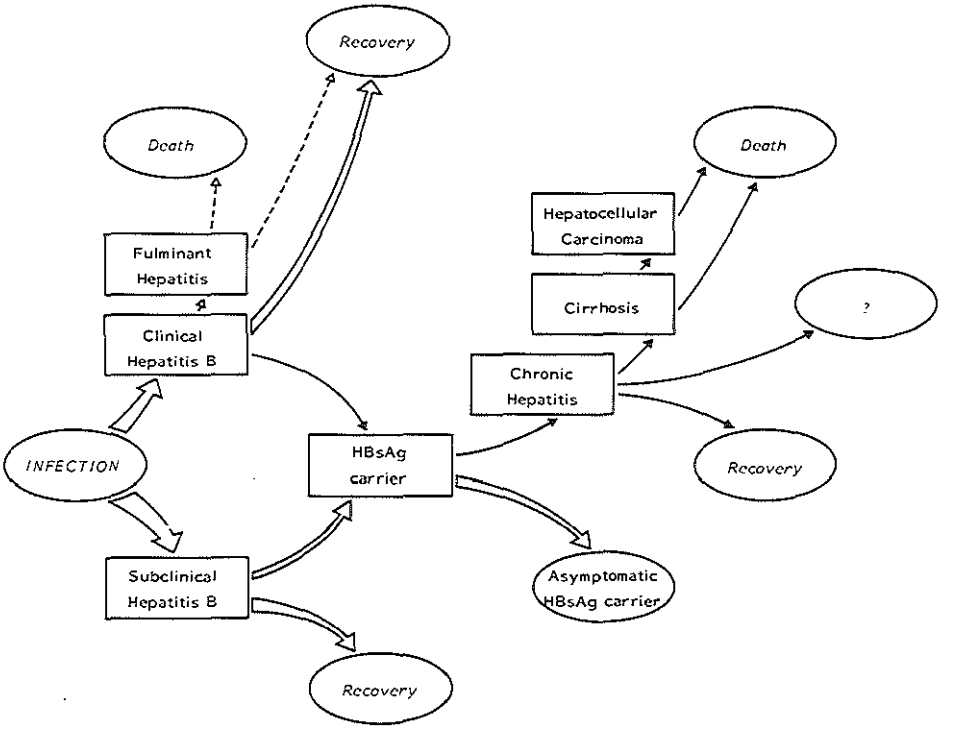

figure 2.3: Courses of disease after hepatitis B virus infection. The relative number of patients following a certain course is roughly indicated by the width of the arrow.

HBV is usually transmitted by means of blood or blood containing products from infectious individuals (69). Transmission may take place through skin lesions or by injections, for instance by a contaminated needle, or through mucous membranes, by sexual contact. This person-to-person spread of the virus is termed "horizontal transmission". Another important mode of HBV transmission is "vertical" spread from an infectious mother to her child during pregnancy or at the time of birth (70).

The natural history of chronic hepatitis B includes changes in the clinical course, accompanied by changes in biochemical and serologic parameters. Three courses of disease can be distinguished:

1) Persistence of chronic active hepatitis and serological markers of viral replication, HBeAg, DNA polymerase activity and HBV DNA.

2) Disappearance of these markers from the serum and seroconversion from HBeAg to antibodies against HBeAg (anti-HBe) (71-74). The seroconversion may be preceded by a period of obvious reactivation of the hepatitis activity (75). Following HBeAg seroconversion, the liver disease becomes asymptomatic. HBsAg remains present or may be serologically cleared as well. As discussed in section 2.1, HBV DNA is suspected to be integrated in the liver cell genome in this type of patients (23,24).

3) Initial disappearance of the serological markers of viral replication and seroconversion to anti-HBe, but subsequently reactivation of chronic active hepatitis with reappearance of markers of viral replication (76,77). In a group of patients with this course of disease the mean age was older compared to patients with the first or second type of disease, and the liver disease was of longer duration with a higher prevalence of cirrhosis (77). The cause of reactivation is unknown, but it has incidentally been observed after immunosuppressive chemotherapy (76), antiviral chemotherapy, major systemic bacterial infections and surgery (78).

The three courses of disease have been described to occur in 59, 29 and 12 % of chronic hepatitis B cases, respectively (78).

Simultaneous presence of HBsAg and antibodies to HBsAg (anti-HBs) has been described in single case reports of chronic hepatitis B. In our patients this phenomenon was found in 32 out of 89 patients (36%), as dicussed in chapter IV (79). In each instance anti-HBs seemed to be directed to another subtype of HBsAg than that present in the same serum. These findings have been confirmed by others (78,80, 81).

Approaches to treatment of chronic hepatitis B mainly comprise immune modulating and antiviral therapy.

Immune modulating therapy is based on the assumption that humoral and cellular mechanisms may play an essential role in the clearance of the virus and in the pathogenesis of liver cell degeneration. From this point of view, immunosuppressive therapy might be effective in suppressing liver cell damage, as has been described for chronic non-viral hepatitis (82). However, immunosuppression has been shown to be ineffective in chronic hepatitis B (83,84), probably because of continuing synthesis of viral products. Immunostimulation theoretically might improve the clearance of the virus, but in practice this has not been shown; the course of disease even worsened in some patients because of increased hepatitis activity (85).

Antiviral therapy includes interferons, adenine arabinoside and acyclovir. In some patients a temporary suppression of viral replication can be achieved. Monotherapy with these agents has not shown to be efficacious over a longer period of time (74,86,87). The effect of combination of antiviral drugs is being studied (88).

2.4 Mechanisms of liver cell degeneration and viral elimination.

The occurrence of viral replication and continuing liver cell damage show a close temporal relationship in one clinical condition, the period of reactivation of liver disease preceding HBeAg seroconversion in chronic active hepatitis, as described in section 2.3 (71-77). This relationship suggests a causal role of active viral replication in the pathogenesis of liver cell degeneration in this condition. However, in various other clinical courses of disease there is a discrepancy between viral replication and liver cell degradation, indicating that the amount of HBV in liver and serum does not correlate with the severity of the hepatitis (77,89). Therefore, the HBV itself does not seem to be directly cytopathic (89,90) and another mechanism is probably involved in the pathogenesis of HBV induced liver cell damage.

Several observations point to the cellular immune system as a mediator of liver cell damage in hepatitis B (91): on microscopic examination of liver biopsies the necrotic liver cells appear to be surrounded by T lymphocytes, which are assumed to cause the liver cell necrosis (92); patients with defective cellular immunity more frequently become chronic HBV carriers after HBV infection (93), but they usually have only minor or no activity of chronic hepatitis (63); reactivation of hepatitis activity has been described to occur during immunosuppressive chemotherapy as well as after discontinuation of such therapy (76,90); stimulation of cellular immunity in chronic HBV carriers is reported to increase the liver cell necrosis (94,95).

Humoral immunity does not seem to be closely related with liver cell necrosis, since patients with agammaglobulinemia may develop severe acute and chronic hepatitis (96) and infusion of large doses of anti-HBs does not result in increased liver cell necrosis (97,98).

The mechanism by which the cellular immune system causes chronic liver cell necrosis has been studied from several hypothetical starting-points. The "classical" theory assumes that T-cell cytotoxicity is evoked by the expression of HBV-associated antigens on the liver cell surface, a mechanism that requires dual recognition of viral and self (HLA-) antigens on the target cells (99). In this theory, chronic hepatitis B is the result of insufficient viral clearance and continuing expression of viral antigens. HBcAg-like expression on the liver cell membrane is especially found in the case of chronic active, HBeAg positive, hepatitis (100, 101). Cytotoxic T lymphocytes with HBcAg specificity have been described in patients with this kind of disease (102). These cells may therefore play a role in hepatocytolysis. Antibodies to HBcAg (anti-HBc), bound to the HBcAg-like determinant on the cell surface (101), may perhaps modulate the cytotoxic attack by covering the infected cells (102).

Other theories assume that the primary defect in chronic hepatitis is related to the immunoregulation of lymphocytotoxicity, either by stimulation of antibody-dependent cellular cytotoxicity by antibodies attached to liver-specific lipoprotein antigens (LSP) on the liver cell surface (103), or by immunoregulatory molecules, originating from necrotic liver cells or lymphocytes (104). In these theories, chronic hepatitis B is not necessarily the result of persistence of viral replication, but rather the effect of changes in liver cell membranes or immunoregulatory lymphocytes.

In attempts to answer these questions, many investigations have been performed on general cellular immune function, the cytotoxic lymphocyte function and the suppressor cell function in hepatitis B (91). A major problem of these tests is the lack

of an experimental in vivo or in vitro model that has sufficient congruence with the different courses of hepatitis B. The studies on general immune function in hepatitis B have produced divergent results with respect by lymphocyte sensitization and transformation by HBV antigens, other antigens and mitogens (105). Alterations found in cytotoxic lymphocyte function have been interpreted as the result of increased T lymphocyte cytotoxic function, antibody-dependent cellular cytotoxicity or natural killer (NK) cell activity. Defective suppressor cell function is presumed to be a regulatory factor in enhanced cytotoxic activity (106). Many types of suppressor cell activity assays have been used to study this issue. Although the results of these non antigen-specific assays are heterogeneous, there is a tendency to decreased suppressor cell activity in patients with chronic active hepatitis B, but a significant correlation with the disease activity has not been found (91).

The interpretation of these immunologic function tests remains difficult, because the methods are indirect and artefacts cannot be excluded. A main artefact in the in vitro tests seems to be the NK cell activity. NK cells in low concentrations already have a profound effector function and overshadow the T lymphocyte cytotoxic function and the antibody-dependent cellular cytotoxicity for which the tests were designed (91). In one study (107) lymphocytes of chronic HBV carriers showed enhanced aspecific cytotoxicity against a series of epithelial, fibroblastoid, lymphoblastoid and myeloid cell lines. The effector cells had properties of NK cells. However, the degree of NK cytotoxicity did not correlate with the degree of hepatitis. Thus, the importance of NK cell cytotoxicity in the pathogenesis of liver cell damage in vivo remains uncertain and the marked in vitro effects of NK cells should probably be regar ded as artefacts.

Direct characterization of the phenotypes of immunoregulatory peripheral T lymphocyte subpopulations by means of monoclonal antibodies has revealed a slight tendency to a decreased number of suppressor cells in various forms of chronic hepatitis B as well as in early acute hepatitis B (91). In two studies (108,109) lowest numbers of T suppressor cells occurred in HBeAg negative chronic active hepatitis B patients. This finding may indicate that ongoing liver disease activity after (partial) clearance of the virus is caused by cytotoxic activity against liver cell constituents rather than HBV antigens (109), as discussed in chapter V of this study. These investigations on T-cell phenotype have some limitations. The peripheral circulating T-cell subsets can only reflect major alterations in the immunoregulatory status of the patients, and may be different from the local situation in the liver. The phenotypic expression of the T-cells has no clearly defined correlation with T-cell function (91). The subsets are detected by monoclonal antibodies which do not distinguish between suppressor and cytotoxic T-cells. Similarly, the functional display in the subpopulation with helper/inducer phenotypic expression is not homogeneous.

The Major Histocompatibility Complex, the Human Leucocyte Antigen system in man, is involved in the regulation of the immune response. The class I HLA antigens are mainly recognized by T-cells that have the ability to develop into cytotoxic cells. Class II antigens play a role in the activation of regulatory T lymphocytes and cooperation between those and other cells of the immune system (110). Therefore, several investigators have looked for a possible relationship between HLA and the course of disease after HBV infection (111). These studies have not provided conclusive evidence for an association between HLA and HBV infection, chronic

HBsAg carriership or HBsAg positive chronic active hepatitis, probably in part because of the divergent patient groups studied and the numbers of HLA antigens tested. In chapter VI a study is presented which examines the relationship between the different courses of disease and both HLA class I and class II antigens.

Viral elimination may be an important factor determining the course of disease. Although HBV is not thought to be directly cytopathic, a close relationship has been established between viral replication and continuing liver cell degeneration, as discussed in sections 2.3 and 2.4. Moreover, a role for HBV in the continuation of liver disease is suggested by the observation that immunosuppressive therapy is not effective in chronic hepatitis B, in contrast to other forms of chronic hepatitis (83,84). Therefore, in addition to a study of the immunologic mechanisms of liver cell degeneration, it is important to obtain insight into the mechanism of viral elimination in acute and chronic hepatitis B.

Antibodies against HBV antigens may play an important role in viral clearance. Freely circulating virus particles are neutralized and prevented from infecting liver cells by anti-HBs, as shown by the efficacy of anti-HBs after vaccination with HBsAg in preventing HBV infection. A similar effect has been ascribed to anti-HBV antibodies which are distinct from anti-HBs (anti-Dane antibodies) (112,113). Re-infection of liver cells by HBV released from infected cells is also possibly prevented in this way. Anti-Dane antibodies can almost never be detected in the serum of patients with chronic active hepatitis B (113). The absence of these antibodies would allow chronic re-infection of hepatocytes. The possible modulating effect of anti-HBc on the cytotoxic attack to infected liver cells has been mentioned above (101,102). Finally, it is possible that antiviral antibodies may suppress the intracellular HBV production, as dicussed below, but this mechanism remains hypothetical.

Two main hypotheses have been formulated regarding HBV elimination from infected liver cells. In the first hypothesis, the virus is eliminated by destruction of all infected liver cells by means of a cytotoxic mechanism (99). The second hypothesis assumes that antiviral antibodies may suppress the synthesis of viral proteins, without necessarily destroying all infected liver cells (104). The first mechanism is a general mechanism of viral elimination. With hepatitis B this mechanism would imply that the degree of liver injury is dependent on the number of infected liver cells. However, observations in chimpanzees (114-116) and man (117) have revealed that the majority of liver cells are already infected with HBV in the early phase of acute hepatitis B. All observed cases recovered normally without massive liver cell necrosis. It is difficult to believe in these instances that viral elimination has occurred only by destruction of all infected liver cells. In the second hypothesis different immunologic mechanisms are proposed for viral elimination and liver cell necrosis. Although such mechanisms remain hypothetical for hepatitis B, suppression of viral replication by antiviral antibodies on the cell surface has been described for measles (118-120), Epstein-Barr virus (121) and herpes simplex virus (122). The hypotheses concerning viral elimination are discussed in chapter III, in connection with a study on viral elimination in acute hepatitis B.

Despite all attempts to synthesize the vast amount of information on hepatitis B virus elimination and liver cell destruction (91,123-126), firm conclusions cannot be drawn. Further studies are needed to supply missing data and resolve apparently conflicting observations.

2.5 References

1. Le Bouvier GL. The heterogeneity of Australia antigen. J Infect Dis 1971; 123: 671-675.
2. Bancroft WH, Mundon FK, Russell PhK. Detection of additional antigenic determinants of hepatitis B antigen. J Immunol 1972; 109: 842-848.
3. Courouce-Pauty A-M, Soulier JP. Further data on HBs antigen subtypes - Geographical distribution. Vox Sang 1974; 27: 533-549.
4. Murphy BL, Maynard JE, Le Bouvier GL. Viral subtypes and cross-protection in hepatitis B virus infections of chimpanzees. Intervirology 1974: 3: 378-381.
5. Tabor E, Gerety RJ, Smallwood LA, Barker LF. Induction of antibody to the "y" determinant of HBsAg in a chimpanzee carrier of HBsAg subtype "adw". J Immunol 1976; 117: 2038-2040.
6. Galibert F, Mandart E, Fitoussi F, Tiollais P, Charnay P. Nucleotide sequence of the hepatitis B virus genome (subtype ayw) cloned in E. Coli. Nature 1979; 281: 646-650.
7. Ono Y, Onda H, Sasada R. Igarashi K, Sugino Y, Nishioka K. The complete nucleotide sequences of the cloned hepatitis B virus DNA; subtype adr and adw. Nucl Acids Res 1983; 11: 1747-1757.
8. Robinson WS, Clayton DA, Greenman RL. DNA of a human hepatitis B virus candidate. J Virol 1974; 14: 384-391.
9. Delius H, Gough NM, Cameron CH, Murray K. Stucture of the hepatitis B virus genome. J Virol 1983; 47; 337-343.
10. Toh H, Hayashida H, Miyata T. Sequence homology between retroviral reverse transcriptase and putative polymerases of hepatitis B virus and cauliflower mosaic virus. Nature 1983; 305: 827-829.
11. Tiollais P, Charnay P, Vyas GN. Biology of hepatitis B virus. Science 1981; 213: 406-411.
12. Misharo S, Imai M, Takahashi K, et al. A 49,000-dalton polypeptide bearing all the antigenic determinants and full immunogenecity of 22nm hepatitis B surface antigen particles. J Immunol 1980; 124: 1589-1593.
13. Tiollais P, Wain-Hobson S. Molecular genetics of the hepatitis B virus. In: Chisari FV, ed. Advances in hepatitis research. New York: Masson Publishing USA 1984: 9-20.
14. Stibbe W, Gerlich WH. Structural relationships between minor and major proteins of hepatitis B surface antigen. J Virol 1983; 46: 626-628.
15. Heermann KH, Goldmann U, Schwartz W, Seyffarth T, Baumgarten H, Gerlich WH. Large surface proteins of hepatitis B virus containing the pre-s sequence. J Virol 1984; 52: 396-402.
16. Pfaff E, Klinkert M-Q, Theilmann L, Schaller H. Characterization of large surface proteins of hepatitis B virus by antibodies to preS-S encoded amino acids. Virology 1986; 148: 15-22.
17. Tiollais P, Pourcel C, Dejean A. The hepatitis B virus. Nature 1985; 317: 489-495.
18. Stibbe W, Gerlich WH. Variable protein composition of hepatitis B surface antigen from different donors. Virology 1982; 123: 436-442.
19. Machida A, Kishimoto S, Ohnuma H, et al. A hepatitis B surface antigen polypeptide (p31) with the receptor for polymerized human as well as chimpanzee albumins. Gastroenterology 1983; 85: 268-274.
20. Moriarty AM, Alexander H, Lerner RA. Antibodies to peptides detect new hepatitis B antigen: serological correlation with hepatocellular carcinoma. Science 1985; 227: 429-433.
21. Takahashi K, Imai M, Miyakawa Y, Iwakiri S, Mayumi M. Duality of hepatitis Be antigen in serum of persons infected with hepatitis B virus: evidence for the nonidentity of e antigen with immunoglobulins. Proc Natl Acad Sci USA 1978; 75: 1952-1956.
22. Brechot C, Hadchouel M, Scotto J, et al. Detection of hepatitis B virus DNA in liver and serum: a direct appraisal of the chronic carrier state. Lancet 1981; 2: 765-768.
23. Brechot C, Hadchouel M, Scotto J, et al. State of hepatitis B virus DNA in hepatocytes of patients with hepatitis B antigens-positive and -negative liver diseases. Proc Natl Acad Sci USA 1981; 78: 3906-3910.
24. Shafritz DA, Shouval D, Sherman HI, Hadziyannis SJ, Kew MC. Integration of hepatitis B virus DNA into the genome of liver cells in chronic liver disease and hepatocellular carcinoma. N Engl J Med 1981; 305: 1067-1073.

25. Pontisso P, Poon MC, Tiollais P, Brechot C. Detection of hepatitis B virus DNA in mononuclear blood cells. Br Med J 1984; 288: 1563-1566.

26. Blum HE, Stowring L, Figus A, Montgomery CK, Haase AT, Vyas GN. Detection of hepatitis B virus DNA in hepatocytes, bile duct epithelium, and vascular elements by in situ hybridization. Proc Natl Acad Sci USA 1983; 80: 6685-6688.

27. Dejean A, Lugassy C, Zafrani S, Tiollais P, Brechot C. Detection of hepatitis B virus DNA in pancreas, kidney and skin of two human carriers of the virus. J Gen Virol 1984; 65: 651-655.

28. Robinson WS. Genetic variation among hepatitis B and related viruses. Ann NY Acad Sci 1980; 354: 371-378.

29. Summers J. Three recently described animal virus models for human hepatitis B virus. Hepatology 1981; 1: 179-183.

30. Summers J, Mason WS. Replication of the genome of a hepatitis B-like virus by reverse transcription of an RNA intermediate. Cell 1982; 29: 403-415.

31. Cattaneo R, Will H, Hernandez N, Schaller H. Signals regulating hepatitis B surface antigen transcription. Nature 1983; 305: 336-338.

32. Molnar-Kimber KL, Summers JW, Mason WS. Mapping of the cohesive overlap of duck hepatitis B virus DNA and of the site of initiation of reverse transcription. J Virol 1984; 51: 181-191.

33. Ling CM, Overby LR. Prevalence of hepatitis B virus antigen as revealed by direct radioimmune assay with 125I-antibody. J Immunol 1972; 109: 834-841.

34. Frosner GG, Brodersen M, Papaevangelou G, et al. Detection of HBeAg and anti-HBe in acute hepatitis B by a sensitive radioimmunoassay. J Med Virol 1978; 3: 67-76.

35. Wolters G, Kuijpers L, Kacaki J, Schuurs A. Solid-phase enzyme-immunoassay for detection of hepatitis B surface antigen. J Clin Path 1976; 29: 873-879.

36. Barker LF, Peterson MR, Murray R. Application of the microtitre complement fixation technique to studies of hepatitis associated antigen in human hepatitis. Vox Sang 1970; 19: 211-216.

37. Krugman S, Hoofnagle JH, Gerety RJ, Kaplan PM, Gerin JL. Viral hepatitis, type B. DNA-polymerase activity and antibody to hepatitis B core antigen. N Engl J Med 1974; 290: 1331-1335.

38. Frosner GG, Schomerus H, Wiedmann KH, et al. Diagnostic significance of quantitative determination of hepatitis B surface antigen in acute and chronic hepatitis B infection. Eur J Clin Microbiol 1982; 1: 52-58.

39. Joller-Jemelka HI, Pfister HF, Grob PJ. Die prognostische Bedeutung der quantitativen HBsAg-Bestimmung bei akuter Hepatitis B. Schweiz Med Wschr 1985; 37: 1249-1256.

40. Lemon SM, Gates NL, Simms ThE, Bancroft WH. IgM antibody to hepatitis B core antigen as a diagnostic parameter of acute infection with hepatitis B virus. J Infect Dis 1981; 143: 803-809.

41. Heijtink RA, Boender PJ, Schalm SW, Masurel N. Hepatitis B virus DNA in serum of pregnant women with HBsAg and HBeAg or antibodies to HBe. J. Infect Dis 1984; 150: 462.

42. Kaplan PM, Greenman RL, Gerin JL, Purcell RH, Robinson WS. DNA polymerase associated with human hepatitis B antigen. J Virol 1973; 12: 995-1005.

43. Kaplan PM, Ford EC, Purcell RH, Gerin JL. Demonstration of sub-populations of Dane particles. J Virol 1976; 17: 885-893.

44. Kaplan PM, Greenman RL, Gerin JL, Purcell RH, Robinson WS. DNA polymerase associated with human hepatitis B antigen. J Virol 1973; 12: 995-1005.

45. Howard CR. The detection of DNA polymerase activity in the diagnosis of HBV infection. J Med Virol 1978; 3: 81-86. 46. Rizzetto M, Shih JW-K, Verme G, Gerin JL. A radio-immunoassay for HBcAg in the sera of HBsAg carriers: serum HBcAg, serum DNA polymerase activity and liver HBcAg immunofluorescence as markers of chronic liver disease. Gastroenterology 1981; 80: 1420-1427.

47. Rigby PW, Diekmann M, Rhodes C, Berg P. Labeling deoxyribonucleic acid to high specific activity in vitro by nick translation with DNA polymerase I. J Mol Biol 1977; 113: 237-251.

48. Alberti A, Realdi G, Tremolada F, Spina GP. Liver cell surface localization of hepatitis B antigen and of immunoglobulins in acute and chronic hepatitis and in liver cirrhosis. Clin Exp Immunol 1976; 25: 396-401.

49. Yamada G, Feinberg LE, Nakane PK. Hepatitis B. Cytologic localization of virus antigens and the role of the immune response. Hum Pathol 1978; 9: 93-109.

50. Gerber MA, Thung SN. The localization of hepatitis viruses in tissues. Int Rev Exp Pathol 1979; 20: 49-76.

51. Rijntjes PJM, Van Ditzhuijsen ThJM, Van Loon AM, Van Haelst UJGM, Bronkhorst FB, Yap SH. Hepatitis B virus DNA detected in formalin-fixed liver specimens and its relation to serologic markers and histopathologic features in chronic liver disease. Am J Pathol 1985; 120: 411-418.

52. Trepo C, Vitvitski L, Neurath R, et al. Detection of e antigen by immunofluorescence in cytoplasm of hepatocytes of HBsAg carriers. Lancet 1976; 1: 486.

53. Arnold W, Nielsen JO, Hardt F, Meyer zum Bueschenfelde KH. Localisation of e-antigen in nuclei of hepatocytes in HBsAg-positive liver diseases. Gut 1977; 19: 994-996.

54. Gudat F, Bianchi L, Sonnabend W, Thiel G, Aenishaenslin W, Stalder GA. Pattern of core and surface expression in liver tissue reflects state of specific immune response in hepatitis B. Lab Invest 1975; 32: 1-9.

55. Southern EM. Detection of specific sequences among DNA fragments separated by gel electrophoresis. J Mol Biol 1975; 98: 503-519.

56. Marion PL, Salazar FH, Alexander JJ, Robinson WS. State of hepatitis B viral DNA in a human hepatoma cell line. J Virol 1980; 33: 795-806.

57. Brechot C, Hadchouel M, Scotto J, et al. State of hepatitis B virus DNA in hepatocytes of patients with hepatitis B surface antigen-positive and -negative liver diseases. Proc Natl Acad Sci USA 1981; 78: 3906-3910.

58. Shafritz DA, Kew MC. Identification of integrated hepatitis B virus DNA sequences in human hepatocellular carcinomas. Hepatology 1981; 1: 1-8.

59. Burrell CJ, Gowans EJ, Rowland R, Hall P, Jilbert AS, Marmion BP. Correlation between liver histology and markers of hepatitis B virus replication in infected patients: a study by in situ hybridization. Hepatology 1984; 4: 20-24.

60. Beasley RP, Hwang L-Y, Lin C-C, et al. Hepatitis B immune globulin (HBIG) efficacy in the interruption of perinatal transmission of hepatitis B virus carrier state. Lancet 1981; 2: 388-393.

61. Beasley RP, Hwang L-Y, Lin C-C, et al. Incidence of hepatitis B virus infections in preschool children in Taiwan. J Infect Dis 1982; 146: 198-204.

62. Redeker AG. Viral Hepatitis: clinical aspects. Am J Med Sci 1975; 270: 9-16.

63. Nordenfelt E, Lindholm T, Loefgren B, Moestrup T, Reinicke V. Different categories of chronic HBsAg carriers: A long-term follow-up. In: Szmuness W, Alter HJ, Maynard JE, eds. Viral hepatitis. Philadelphia: Franklin Institute Press, 1982: 237-242.

64. Szmuness W, Harley EJ, Ikram H, Stevens CE. Sociodemographic aspects of the epidemiology of hepatitis B. In: Vyas GN, Cohen SN, Schmid R, eds. Viral hepatitis. Philadelphia: Franklin Institute Press, 1978: 297-320.

65. Cooper WC, Gershon RK, Sun S-C, Fresh JW. Anicteric viral hepatitis. A clinicopathological follow-up study in Taiwan. N Engl J Med 1966; 274: 585-595.

66. Dudley FJ, Scheuer PJ, Sherlock S. Natural history of hepatitis-associated antigen-positive chronic liver disease. Lancet 1972; 2: 1388-1393.

67. Rizzetto M, Hoyer BH, Purcell RH, Gerin JL. Hepatitis Delta virus infection. In: Vyas GN, Dienstag JL, Hoofnagle JH, eds. Viral hepatitis and liver disease. Orlando: Grune & Stratton, 1984: 371-379.

68. Villa E, Rubbiani L, Barchi T, et al. Susceptibility of chronic symptomless HBsAg carriers to ethanol-induced hepatic damage. Lancet 1982; 2: 1243-1244.

69. Gust ID. Comparison of the epidemiology of hepatitis A and B. In: Szmuness W, Alter HJ, Maynard JE, eds. Viral hepatitis. Philadelphia: Franklin Institute Press, 1982: 129-143.

70. Alexander GJM, Eddleston ALWF. Does maternal antibody to core antigen prevent recognition of transplacental transmission of hepatitis-B-virus infection ? Lancet 1986; 1: 296-297.

71. 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: 195-199.

72. Norkrans G, Nordenfelt E, Hermodsson S, Iwarson S. Long-term follow-up of chronic hepatitis patients with HBsAg, HBeAg and Dane particle associated DNA polymerase in serum. Scand J Infect Dis 1980; 12: 159-160.

73. Hoofnagle JH, Dusheiko GM, Seeff LB, Jones EA, Waggoner JG, Bales ZB. Seroconversion from hepatitis B e antigen to antibody in chronic type B hepatitis. Ann Intern Med 1981; 94: 744-748.

74. Schalm SW, Heijtink RA. Spontaneous disappearance of viral replication and liver cell inflammation in HBsAg-positive chronic active hepatitis: results of a placebo vs interferon trial. Hepatology 1982; 2: 791-794.

75. Liaw Y-F, Chu C-M, Su I-J, Huang M-J, Lin D-Y, Chang-Chien C-S. Clinical and histological events preceding hepatitis B e antigen seroconversion in chronic type B hepatitis. Gastroenterology 1983; 84: 216-219.

76. Hoofnagle JH, Dusheiko GM, Schafer DF, et al. Reactivation of chronic hepatitis B virus infection by cancer chemotherapy. Ann Intern Med 1982; 96: 447-449.

77. Davis GL, Hoofnagle JH, Waggoner JG. Spontaneous reactivation of chronic hepatitis B virus infection. Gastroenterology 1984; 86: 230-235.

78. Hoofnagle JH, Alter HJ. Chronic viral hepatitis. In: Vyas GN, Dienstag JL, Hoofnagle JH, eds. Viral hepatitis and liver disease. Orlando: Grune & Stratton, 1984: 97-113.

79. Heijtink RA, Van Hattum J, Schalm SW, Masurel N. Co-occurrence of HBsAg and anti-HBs: two consecutive infections or a sign of advanced chronic liver disease? J Med Virol 1982; 10: 83-90.

80. Shiels MT, Tasswell HF, Czaja AJ, Nelson C. Concurrent HBsAg and anti-HBs in acute and chronic hepatitis B. Hepatology 1984; 4: 1035.

81. Tsang T-K, Blei AT, O'Reilly DJ, Decker R. Clinical significance of concurrent hepatitis B surface antigen and antibody positivity. Dig Dis Sci 1986; 31: 620-624.

82. Kirk AP, Jain S, Pocock S, Thomas HC, Sherlock S. Late results of the Royal Free Hospital prospective controlled trial of prednisolone therapy in hepatitis B surface antigen negative chronic active hepatitis. Gut 1980; 21: 78-83.

83. Schalm SW, Summerskill WHJ, Gitnick GL, et al. Contrasting features and responses to treatment of severe chronic active liver disease with and without hepatitis Bs antigen. Gut 1976; 17: 781-786.

84. Lam KC, Lai CL, Ng RP, Trepo C, Wu PC. Deleterious effect of prednisolone in HBsAg-positive chronic active hepatitis. N Engl J Med 1981; 304: 380-386.

85. Nilius R, Schentke U, Otto L, et al. Levamisole therapy in chronic hepatitis - Results of a multicentric double blind trial. Hepato-Gastroenterol 1983; 30: 90-92.

86. Hoofnagle JH, Hanson RG, Minuk GY, et al. Randomized controlled trial of adenine arabinoside monophosphate for chronic type B hepatitis. Gastroenterology 1984; 86: 150-157.

87. Weller IVD, Carreno V, Fowler MJF, et al. Acyclovir in hepatitis B antigen-positive chronic liver disease: inhibition of viral replication and transient renal impairment with iv bolus administration. J Antimicrob Chemother 1983; 11: 223-231.

88. Schalm SW, Heijtink RA, Van Buuren HR, De Man RA. Acyclovir enhances the antiviral effect of interferon in chronic hepatitis B. Lancet 1985; 2: 358-360.

89. Alberti A, Tremolada F, Fattovich G, Bortolotti F, Realdi G. Virus replication and liver disease in chronic hepatitis B virus infection. Digest Dis Sci 1983; 28: 962-966.

90. Galbraith RM, Eddleston ALWF, Williams R, et al. Fulminant hepatic failure in leukemia and choriocarcinoma related to withdrawal of cytotoxic drug therapy. Lancet 1975; 2: 528-530.

91. Dienstag JL. Immunologic mechanisms in chronic viral hepatitis. In: Vyas GN, Dienstag JL, Hoofnagle JH, eds. Viral hepatitis and liver disease. Orlando: Grune & Stratton, 1984: 135-166.

92. Eggink HF, Houthoff HJ, Huitema S, Gips CH, Poppema S. Cellular and humoral immune reactions in chronic active liver disease. I. Lymphocyte subsets in liver biopsies of patients with untreated idiopathic autoimmune hepatitis, chronic active hepatitis B and primary biliary cirrhosis. Clin Exp Immunol 1982; 50: 17-24.

93. Blumberg BS, Sutnick AI, London WT. Australia antigen as a hepatitis virus: variation in host response. Am J Med 1970; 48: 1-8.

94. Jain S, Thomas HC, Sherlock S. Transfer factor in the attempted treatment of patients with HBsAg-positive chronic liver disease. Clin Exp Immunol 1977; 30: 10-15.

95. Chadwick RG, Jain S, Cohen BJ, Scott GM, Thomas HC, Sherlock S. Levamisole therapy for HBsAg-positive chronic liver disease. Scand J Gastroent 1980; 15: 973-978.

96. Good RA, Page AR. Fatal complications of virus hepatitis in two patients with agammaglobulinemia. Am J Med 1960; 29: 804-810.

97. Kohler PF, Trembath J, Merrill DA, et al. Immunotherapy with antibody, lymphocytes and transfer factor in chronic hepatitis B. Clin Immunol Immunopathol 1974; 2: 465-471.

98. Reed WD, Eddleston ALWF, Cullens H, et al. Infusion of hepatitis B antibody in antigen-positive active chronic hepatitis. Lancet 1975; 2: 1347-1351.

99. Dudley FJ, Fox RA, Sherlock S. Cellular immunity and hepatitis-associated Australia antigen liver disease. Lancet 1972; 1: 723-726.

100. Trevisan A, Realdi G, Alberti A, Noventa F. Relationship between membrane bound immunoglobulin and viral antigens in liver cells from patients with hepatitis B virus infection. Gastroenterology 1979; 77: 209-214.

101. Trevisan A, Realdi G, Alberti A, Ongaro G, Pomaro E, Meliconi R. Core antigen specific immunoglobulin G bound to the liver cell membrane in chronic hepatitis B. Gastroenterology 1982; 82: 218-222.

102. Mondelli M, Mieli Vergani G, Alberti A, 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: 2773-2778.

103. Cochrane AMG, Moussourous A, Thomson AD, et al. Antibody-dependent cell-mediated (K-cell) cytotoxicity against isolated hepatocytes in chronic active hepatitis. Lancet 1976; 1: 441-444.

104. Chisari FV, Routenberg JA, Anderson DS, Edgington ThS. Cellular immune reactivity in HBV-induced liver disease. In: Vyas GN, Cohen SN, Schmid R, eds. Viral hepatitis. Philadelphia: Franklin Institute Press, 1978: 245-266.

105. Dienstag JL, Bhan AK, Klingenstein RJ, Savarese AM. Immunopathogenesis of liver disease associated with hepatitis B. In: Szmuness W, Alter HJ, Maynard JE, eds. Viral hepatitis. Philadelphia: Franklin Institute Press, 1982: 221-236.

106. Eddleston ALWF, Williams R. Inadequate antibody response to HBAg or suppressor T-cell defect in development of active chronic hepatitis. Lancet 1974; 2: 1543-1545.

107. Dienstag JL, Savarese AM, Bhan AK. Increased natural killer cell activity in chronic hepatitis B virus infection. Hepatology 1982; 2: 107S-115S.

108. Thomas HC, Brown D, Routhier G, et al. Inducer and suppressor T-cells in hepatitis B virus-induced liver disease. Hepatology 1982; 2: 202-204.

109. Van Hattum J, Van Oudenaren A, Schalm SW, Visser JWM, Benner R. T-lymphocyte subpopulations in patients with various courses after hepatitis B virus infection. Scand J Gastroent 1984; 19: 497-501.

110. Thorsby E, Berle E, Nousiainen H. HLA-D region molecules restrict proliferative T-cell responses to antigen. Immunol Rev 1983; 66: 39-56.

111. Kaslow RA, Shaw S. The role of histocompatibility antigens (HLA) in infection. Epidemiol Rev 1981; 3: 90-114.

112. Neurath AR, Trepo C, Chen M, Prince AM. Identification of additional antigenic sites on Dane particles and tubular forms of hepatitis B surface antigen. J Gen Virol 1977; 30: 277-285.

113. 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. Brit Med J 1978; 2: 1056-1058.

114. Barker LF, Chisari FV, McGrath PhP, et al. Transmission of type B viral hepatitis to chimpanzees. J Infect Dis 1973; 127: 648-662.

115. Berquist KR, Peterson JM, Murphy BL, Ebert JW, Maynard JE, Purcell RH. Hepatitis B antigens in serum and liver of chimpanzees acutely infected with hepatitis B virus. Infect Immun 1975; 12: 602-605.

116. Hoofnagle JH, Michalak T, Nowoslawski A, Gerety RJ, Barker LF. Immunofluorescence microscopy in experimentally induced, type B hepatitis in the chimpanzee. Gastroenterology 1978; 74: 182-187.

117. Edgington ThS, Chisari FV. Immunological aspects of hepatitis B infection. Am J Med Sci 1975; 270: 213-227.

118. Joseph BS, Oldstone MBA. Immunologic injury in measles virus infection. II: Suppression of injury through antigenic modulation. J Exp Med 1975; 142: 864-876.

119. Fujinami RS, Oldstone MBA. Antiviral antibody reacting on the plasma membrane alters measles virus expression inside the cell. Nature 1979; 279: 529-530.

120. Fujinami RS, Oldstone MBA. Alteration in expression of measles virus polypeptides by antibody: molecular events in antibody-induced antigenic modulation. J Immunol 1980; 125: 78-85.

121. Aoki T, Geering G, Beth E, Old LJ. Suppression of antigen in Burkitts lymphoma and human melanoma cells grown in selected human sera. In: Nakahara W, Nishoka K, Hirayoma T, Ito Y, eds. Recent advances in human tumour virology and immunology. Tokyo: University of Tokyo Press, 1971: 425-429.

122. Stevens JG, Cook ML. Maintenance of latent herpetic infection: an apparent role for antiviral IgG. J Immunol 1974; 113: 1685-1693.

123. Alberti A, Trevisan A, Fattovich G, Realdi G. The role of hepatitis B virus replication and hepatocyte membrane expression in the pathogenesis of HBV-related hepatic damage. In: Chisari FV, ed. Advances in hepatitis research. New York: Masson Publishing USA 1984: 134-143.

124. Chisari FV. Hepatic immunoregulatory molecules and the pathogenesis of hepatocellular injury in viral hepatitis. In: Chisari FV, ed. Advances in hepatitis research. New York: Masson Publishing USA 1984: 168-178.

125. Mondelli M, Naumov N, Eddleston ALWF. The immunopathogenesis of liver cell damage in chronic hepatitis B virus infection. In: Chisari FV, ed. Advances in hepatitis research. New York: Masson Publishing USA 1984: 144-151.

126. Thomas HC, Pignatelli M, Goodall A, Waters J, Karayiannis P, Brown D. Immunologic mechanisms of cell lysis in hepatitis B virus infection. In: Vyas GN, Dienstag JL, Hoofnagle JH, eds. Viral hepatitis and liver disease. Orlando: Grune & Stratton, 1984: 167-177.

CHAPTER III

VIRUS ELIMINATION IN ACUTE HEPATITIS B; RECOVERY VERSUS CHRONIC HEPATITIS

The contents of this chapter have been submitted for publication under the same title, with the following authors: J. van Hattum, S.W. Schalm, F.J.W. ten Kate and R.A. Heijtink

Summary

The disappearance of viral antigens in relation to liver cell destruction was studied in 63 patients with acute hepatitis B. Hepatitis B virus DNA, DNA polymerase activity, HBeAg and HBsAg were measured quantitatively in serum, and viral antigens were determined in liver biopsies by immunofluorescence. All data were related in time to the alanine aminotransferase (ALT) peak as a marker of maximum liver cell destruction. Six months after initial symptoms 53 patients had recovered and 10 had become chronic HBsAg carriers. At the onset of disease no differences in viral antigens were observed between the two groups with different outcome. In patients who recovered, the HBsAg and the HBeAg levels decreased significantly from two weeks prior to the ALT peak onwards. The disappearance of HBeAg and HBsAg occurred independently of the extent of the liver disease. HBV DNA, DNA polymerase and viral antigens in liver biopsies were mainly detectable before and around the ALT peak. In patients who developed chronic hepatitis, viral antigens in the serum and liver tissue remained consistently high.

These findings indicate that the final course of the acute hepatitis B is set at least two weeks before the maximum hepatitis activity and that quantitative assays of HBsAg or HBeAg appear to be early indicators of prognosis. The disappearance of viral markers, prior to and independently of the extent of liver cell necrosis, suggests that suppression of the viral protein synthesis is an additional mechanism of virus elimination, apart from destruction of infected liver cells.

Introduction

Disappearance of virus multiplication is the hallmark of recovery from a hepatitis B virus (HBV) infection. Chronic hepatitis B is characterized by continuous synthesis of viral proteins, with or without liver cell damage. Better understanding of the mechanism which underlies the suppression or elimination of virus replication may provide a rational basis for the treatment of chronic viral hepatitis.

The two main hypotheses concerning the mechanism of virus elimination in acute hepatitis B differ with respect to the role of liver cell destruction. According to the first hypothesis the virus is eliminated by destruction of all infected liver cells (1). The second hypothesis suggests that antiviral antibodies may suppress the synthesis of viral proteins without necessarily destroying all infected liver cells (2) (see chapter II).

The aim of this investigation was to study viral markers and liver cell destruction quantitatively in time during the early phase of acute hepatitis B in order to find differences in the courses of viral markers between patients who would recover and those who would become chronic hepatitis B virus carriers, and to obtain additional information about the mechanism of hepatitis B virus elimination.

Patients and methods

Patients

Sixty-three consecutive patients, admitted because of acute hepatitis B, were eligible

for this study. They fulfilled the following criteria: no history, symptoms or signs of liver disease prior to presentation; alanine aminotransferase (ALT) at least 2 x upper limit of normal for 5 days or more; hepatitis B surface antigen (HBsAg) and immune globulin M antibody to core antigen (IgM-antiHBc) positive; liver biopsy confirming the presence of acute viral hepatitis without signs of chronic hepatitis; neither immunosuppressive drugs nor general anaesthesia during the four weeks prior to the initial symptoms of hepatitis. Fifty-three patients recovered (group I) with loss of HBsAg and normalization of ALT. Chronic hepatitis developed in 10 patients (group II), in whom HBsAg and elevation of the ALT level persisted for more than six months. A second liver biopsy confirmed chronic hepatitis B.

The majority of patients who had been referred to our ward by the family doctor (31/33) was classified in group I, whereas six of the 10 patients who had been referred after routine testing for HBsAg by the bloodbank, ended up in group II. This pattern of referral may explain the relatively high number of patients that developed chronic hepatitis.

Twenty-seven patients of group I and 6 patients of group II were admitted in an early phase of the disease, with ALT and bilirubin values still increasing (group Ia and IIa, respectively). In such cases the exact date of maximum ALT elevation (ALT peak) could be determined. Twenty-nine patients of group I and four patients of group II were admitted at or after their ALT peak. We observed no differences between the patients who were admitted before ALT peak and those who were admitted at or after ALT peak, regarding age, sex and time intervals from the onset of symptoms to some characteristic points in the courses of ALT, bilirubin, HBeAg and HBsAg (table 3.1). Evidently, all patients were admitted before, at or only a few days after their real ALT peak. The time intervals are not applicable to group II, because most patients of this group had a low-grade hepatitis without jaundice.

Methods

Blood samples were drawn weekly during the period of acute hepatitis and for six weeks after the ALT peak had occurred and then monthly for six months. Serum ALT and bilirubin levels were determined by standard methods; sera were stored at -20°C until determination of serologic HBV markers. Radioimmunoassays (Abbott Laboratories, Chicago, Ill., USA) were used for the detection of HBsAg (AUSRIA II) and HBeAg. HBeAg was quantified by the ratio of the radioactivity of a test sample to that of a negative control (P/N ratio) for sera diluted 1:10. Sera with a P/N ratio below 2.1 were retested undiluted. Diluted sera were considered negative when their P/N ratio was less than 1.7, corresponding to 2.1 for undiluted sera. HBsAg titres were measured by reverse passive haemagglutination tests (AUSCELL, Abbott Laboratories, Chicago, Ill., USA) in two-fold dilution series. DNA polymerase activity was determined according to the method of Howard (3). Hepatitis B virus DNA (HBV DNA) was detected by dot hybridization using the HBV DNA probe pCP 10 after labeling by nick translation (See also chapter II). The HBV DNA probe was kindly provided by Dr. C. Brechot, Institut Pasteur, Paris. IgM anti-HBc was determined by an ELISA-technique, after preincubation of serum samples on anti-IgM coated microtiter plates (Organon, Oss, The Netherlands).

Liver biopsies were reviewed by two independent observers. Direct immunofluorescence microscopy was performed using commercial HBsAg-antiserum (Central Laboratory of the Netherlands Red Cross Blood Transfusion Service, The Netherlands) and an HBcAg-antiserum obtained from a patient recovering from acute hepatitis B, lacking other hepatitis B markers. The number of positive fluorescent hepatocytes was expressed as a percentage of all liver cells.

All data concerning the HBV markers were related in time to the date of the ALT peak.

Statistical analysis was performed with rank sum (Wilcoxon) tests and Spearman's correlation tests.

Results

Viral markers and the course of acute hepatitis B.

In the early phase of acute hepatitis B the courses of viral proteins were measured in patients who recovered (group I) and in those who became chronic HBV carriers (group II). The courses of serum HBeAg, HBsAg and ALT are presented in figure 3.1. Two weeks prior to the ALT peak, the HBsAg and HBeAg titres of patients in group I were in the same range with those of group II. However, at the time of the ALT peak the HBsAg titre had exponentially dropped in group I patients, thus becoming significantly different from the HBsAg level found in patients of group II ($p < 0.05$). The same course was observed for the HBeAg level; it also dropped exponentially in patients of group I, becoming significantly lower ($p < 0.05$) than the HBeAg level in group II at the time of the ALT peak. In patients who developed chronic hepatitis the HBsAg titre and the HBeAg P/N ratio remained consistently high during the observation period. At the time of the ALT peak, 9 out of 53 patients from group I already had a negative HBeAg assay but none of the patients had a negative HBsAg assay. In patients who recovered, HBeAg disappearance occurred within 9.5 weeks after the ALT peak (95% confidence upper limit).

HBV DNA assays were done with the remaining sera from a limited number of patients. In group I 36 sera from 16 patients were tested. Early sera were obtained prior to or in the week of maximal ALT. Mostly an early and a later serum sample were tested per patient. In the early period 10 out of 16 serum samples (six out of 12 patients) were positive for HBV DNA. In the later period only two out of 20 serum samples (two out of 13 patients) were positive (figure 3.2) In the HBV DNA positive samples HBeAg P/N ratio ranged from 3.4 to 29.8 (mean 16.3) and HBsAg titre ranged from 800 to 6400 (mean 3200). In 13 out of the 24 sera negative for HBV DNA the HBeAg test was still positive with a P/N ratio from 1.7 to 22.2 (mean 5.4). In group II all four patients tested for HBV DNA were positive at one or two occasions during the observation period.

DNA polymerase activity was detectable (with a low P/N ratio of 1.4 to 1.6) in only four out of 12 patients of group I at the time of the ALT peak.

The mean maximum ALT level for group I (1766 U/l) was much higher than that of group II (365 U/l) ($p < 0.01$) (table 3.1, figure 3.1). In all patients who developed chronic hepatitis (group II), serum bilirubin levels were always lower than twice the upper limit of normal and no jaundice was observed.

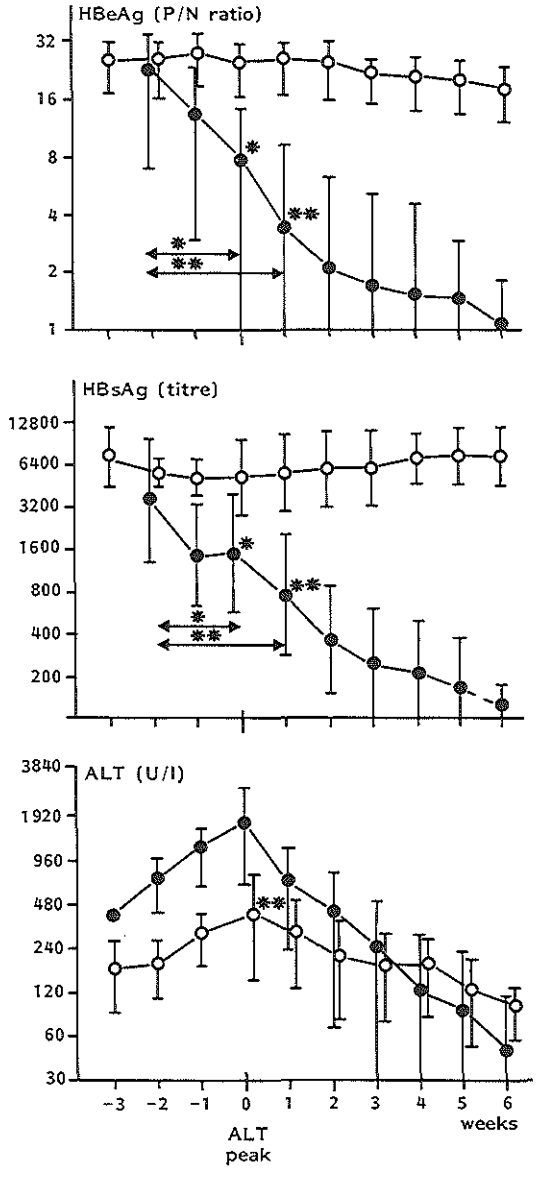

Figure 3.1 The courses of HBeAg P/N ratio, HBsAg titre and ALT in acute hepatitis B for patients who recovered (•) and those who developed chronic hepatitis (o). In patients who recovered the HBeAg P/N ratio and the HBsAg titre dropped significantly two weeks before the ALT peak. At the time of the ALT peak there was a significant difference in HBeAg level, HBsAg titre and ALT level between the two groups (: $p<0.05$; **: $p<0.01$) HBeAg-sera were diluted 1:10; P/N ratios below 1.7 were considered to be negative.*

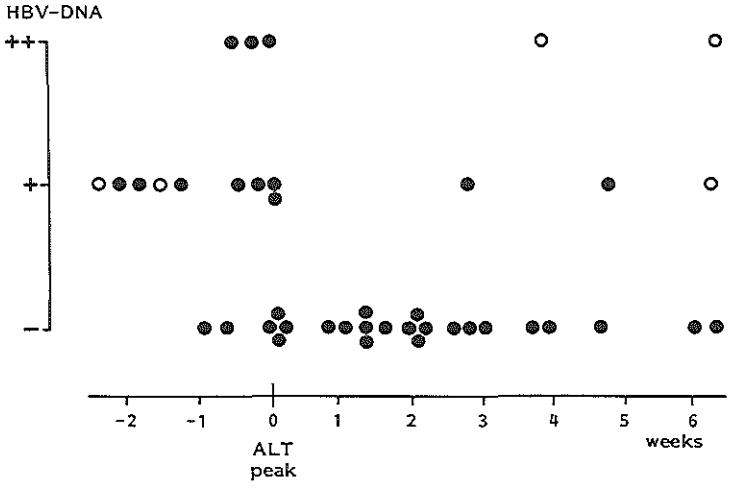

Figure 3.2 HBV DNA in acute hepatitis B for 16 patients who recovered (•) and 4 patients who developed chronic hepatitis (o). In patients who recovered, 10 out of 16 early samples, obtained at or before week zero, were positive for HBV DNA, but only two out of 20 later samples were HBV DNA positive.

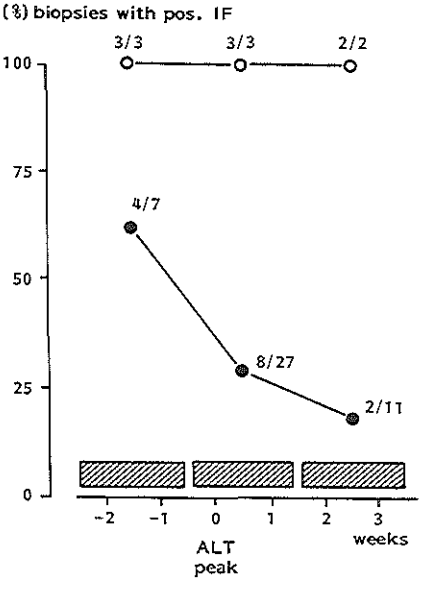

Figure 3.3: The presence of HBV antigens in liver tissue during acute hepatitis B in patients who recovered (•) and those who developed chronic hepatitis (o). The relative number of liver biopsies that showed positive anti-HBs and/or anti-HBc immunofluorescence is shown for three biweekly periods.

TABLE 3.1 Characterization of patients with acute hepatitis who recovered (group I) and those who developed chronic hepatitis (group II).*

group	recovery			chronicity		
	total	admission prior to ALT peak	admission after ALT peak	total	admission prior to ALT peak	admission after ALT peak
number	53	27	26	10	6	4
General features:						
Age (yrs)	30.2±10.6	30.7±11.8	29.7±9.1	27.8±5.8	30.5±5.1	23.8±4.3
Sex (M/F)	40/13	20/7	20/6	8/2	5/1	3/1
Severity of disease:						
Peak ALT (U/l)	1766±1166	1928±969	1598±1322	365±198	349±161	389±241
Bilirubin peak (μmol/l)	227±142	225±129	229±154	16±6	15±5	17±7
HBeAg (P/N ratio)**	8.1±7.4	8.9±7.8	7.6±7.5	24.8±6.5	26.8±9.3	23.5±3.4
HBsAg (titre)**	4.0±1.4	3.9±1.3	4.2±1.7	5.7±0.8	5.8±0.5	5.7±0.9
Time intervals (weeks):						
onset of symptoms to peak ALT	3.3±2.4	3.4±2.2	3.1±2.3	n.a.	n.a.	n.a.
onset of jaundice to peak ALT	1.8±1.5	1.7±1.0	1.8±1.8	n.a.	n.a.	n.a.
peak ALT to ALT 1xN+	6.9±2.8	6.7±3.0	7.4±2.6	n.a.	n.a.	n.a.
peak ALT to HBeAg § negative	2.3±4.3	1.8±3.6	2.8±5.0	n.a.	n.a.	n.a.
peak ALT to HBsAg § negative	7.0±3.9	8.0±4.1	5.5±3.6	n.a.	n.a.	n.a.

* : all data mean ± 1 SD.
** : HBeAg ratio and HBsAg titre at the time of the ALT peak; HBsAg titre given as ^{2}log (titre) x 100.
\+ : N: upper limit of normal.
§ : RIA negative for undiluted sera.
n.a. : not applicable.

The results of immunofluorescence studies using hepatitis B antisera on liver biopsies are shown in figure 3.3. The number of biopsies with more than 5% hepatocytes that exhibited specific anti-HBs and/or anti-HBc fluorescence was highest among the biopsies taken early in the course of acute hepatitis B. In group I positive anti-HBs immunofluorescence was always seen at the liver cell membrane and never in the cytoplasm. In three patients more than 50% of the liver cells exhibited membranous immunofluorescence. In one case nuclear anti-HBc staining was seen in 65% of the liver cells. The biopsies of group II always exhibited strongly positive membranous immunofluorescence, and in 6 cases there was also some cytoplasmatic reaction with anti-HBs.

Viral markers and liver cell degeneration.

In patients who recovered (group I) the above mentioned data were also used to study the course of liver cell damage in relation to the quantitative course of viral proteins. ALT and bilirubin were tested as indices of liver damage. The bilirubin peak corresponded well in time with the ALT peak (mean bilirubin peak one day after ALT peak, SD: 5 days). ALT was used as marker of liver damage in this presentation.

The markers of viral protein synthesis were measured quantitatively or semi-quantitatively and their courses were compared to the course of ALT. HBV DNA and DNA polymerase activity appeared to become negative at the time of the ALT peak, followed by HBeAg at 3 weeks (SD: 3 weeks) and HBsAg at 7 weeks (SD: 4 weeks). The courses of HBeAg and HBsAg could be measured quantitatively reasonably well. Both the HBeAg and the HBsAg levels decreased already rapidly in the two weeks prior to the ALT peak (fig. 3.1).

In order to study a possible relationship between the extent of liver cell degeneration and the amount of viral proteins we compared the following parameters to each other. The ALT peak, the time interval from ALT peak to normal ALT and the product of the ALT peak and the time interval from ALT peak to normal ALT (area under ALT curve) were each compared to the HBeAg P/N ratio at the time of the ALT peak, the time interval from ALT peak to negative HBeAg test and the area under HBeAg curve. The same was done for ALT and the HBsAg curve. The individual data were used in paired tests. No statistically significant correlation was found.

Discussion

In this study quantitative measurements of viral activity and liver cell degeneration were performed simultaneously. It therefore was possible to study the courses of these parameters during acute hepatitis B and to compare them in relation to a time scale. We found that in the early phase of acute disease HBeAg and HBsAg significantly decreased in those patients who eventually recovered. The viral parameters DNA polymerase, HBV DNA, HBeAg and HBsAg each seemed to have their own elimination pattern. The sequence of disappearance, as observed in our series was 1) DNA polymerase/HBV DNA, 2) HBeAg, 3) HBsAg. The disappearance of HBeAg and HBsAg occurred independently of the extent of liver desease.

Other studies on the quantitative course of viral parameters of acute hepatitis B in man usually have been restricted to one viral antigen at a time. Barker et al.(4) demonstrated a decreasing HBsAg titre before clinical hepatitis in 69 patients. Krugman et al.(5) confirmed this observation in 4 patients. Aldershvile et al.(6), who followed 45 patients with HBeAg-positive acute hepatitis type B, found clearance of HBeAg from the peripheral blood within 10 weeks after initial symptoms of hepatitis in recovering patients. Four patients with persistence of HBeAg for more than 10 weeks all became HBsAg carriers. Our results show that HBeAg disappeared within 9.5 weeks from the ALT peak (95% confidence limit), which is about 13 weeks after the onset of symptoms. In our observations, quantitative measurement of HBeAg (which has not been described in acute hepatitis) shows that HBeAg, like HBsAg, starts decreasing prior to clinical symptoms. Alberti et al. (7) found very low and decreasing DNA polymerase activity prior to the ALT peak for 8 patients with acute hepatitis B who recovered. Our findings are in accordance with these results. The higher sensitivity of the HBV DNA determination compared to DNA polymerase activity elongated the time of observation of Dane par ticle markers with 1-2 weeks. A recent study on fulminant hepatitis B showed that serum HBV DNA disappeared prior to HBeAg (8). The same order was observed in our patients, who had a normal course of disease with recovery.

In our study, patients who recovered exhibited a significant decrease of the

HBeAg P/N ratio and the HBsAg titre in the two weeks before as well as after the ALT peak. Furthermore, there was a statistically significant difference in HBeAg and HBsAg levels at the time of the ALT peak between patients who eventually recovered and those who developed chronic hepatitis B. These patients all had high levels of HBeAg and HBsAg that did not decline during the observation period. Quantitative measurement of HBsAg and HBeAg levels therefore seems to be sensitive and practical for the prognosis of acute hepatitis B. The final course of acute hepatitis B appears to be set at least two weeks before the ALT peak, and with the aid of HBsAg titres and/or HBeAg quantification the prognosis can be determined early in the course of disease. Such an early indicator of prognosis might be useful if therapeutic intervention with interferon or other drugs is considered for those patients who fail to clear HBV antigens in the first weeks of an acute hepatitis B.

In view of the hypothesis that viral elimination is caused by destruction of virus containing liver cells (1), we compared the disappearance curves for several viral parameters with the biochemical curve of liver cell degeneration, using ALT as the most specific marker. First we observed that the viral parameters HBeAg and HBsAg were disappearing exponentially while liver cell degeneration increased slowly, reaching its maximum about two weeks later (figure 3.1). Secondly, we found no correlation between the decline in the serum markers of viral protein production and the serum markers of liver cell necrosis (table 3.1). These findings do not fit easily with the hypothesis that viral elimination in acute hepatitis B results predominantly from elimination of infected liver cells. In addition, we as others (9,10,11,12) observed patients with a normal benign course and recovery who exhibited positive HBV immunofluorescence in upto 100% of their liver cells. In these cases recovery with complete virus elimination occurred. This could not have been achieved solely by destruction of all infected liver cells, because there were no signs of massive liver cell necrosis.

Our findings are thus better compatible with the hypothesis of Edgington and Chisari (12), who suggest that viral elimination early in the course of acute hepatitis B can also be the result of suppression of the viral genome in the infected liver cell. Such a mechanism remains hypothetical as far as hepatitis B is concerned, but suppression of viral replication by antiviral antibodies has been described for measles (13,14,15), Epstein-Barr virus (16) and herpes simplex virus (17). The sequential disappearance of viral antigens can now be explained, especially since there is no evidence that this feature is due to different half-life times of HBeAg and HBsAg. The available data suggest that the half-life of HBsAg is about 2.5 days (18).

On the basis of the above-mentioned arguments the decreasing serum levels of viral markers are unlikely to result solely from liver cell destruction. Suppression of viral protein synthesis may be an additional mechanism of viral elimination.

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References

1. Dudley FJ, Fox RA, Sherlock S. Cellular immunity and hepatitis-associated, Australia antigen liver disease. Lancet 1972; 1: 723-726.
2. Chisari FV, Routenberg JA, Anderson DS, Edgington ThS. Cellular immune reactivity in HBV-induced liver disease. In: Vyas GN, Cohen SN, Schmid R, eds. Viral hepatitis. Philadelphia, Pennsylvania: The Franklin Institute Press, 1978; 245-266.
3. Howard CR. The detection of DNA-polymerase activity in the diagnosis of HBV-infection. J Med virol 1978; 3: 81-86.
4. Barker LF, Peterson MR, Murray R. Application of the microtitre complement fixation technique to studies of hepatitis associated antigen in human hepatitis. Vox Sang 1970; 19: 211-216.
5. Krugman S, Hoofnagle JH, Gerety RJ, Kaplan PM, Gerin JL. Viral hepatitis, type B. DNA-polymerase activity and antibody to hepatitis B core antigen. N Eng J Med 1974; 290: 1331-1335.
6. Aldershvile J, Froesner GG, Nielsen JO, Hardt F, Deinhardt F, Skinhoj P. Hepatitis B e antigen and antibody measured by radioimmunoassay in acute hepatitis B surface antigen-positive hepatitis. J Infect Dis 1980; 141: 293-298.
7. Alberti A, Diana S, Eddleston ALWF, Williams R. Changes in hepatitis B virus DNA-polymerase in relation to the outcome of acute hepatitis type B. Gut 1979; 20: 190-195.
8. Brechot C, Bernuau J, Thiers V, et al. Multiplication of hepatitis B virus in fulminant hepatitis B. Brit Med J 1984; 288: 270-271.
9. Barker LF, Chisari FV, McGrath PhP, et al. Transmission of type B viral hepatitis to chimpanzees. J Infect Dis 1973; 127: 648-662.
10. Berquist KR, Peterson JM, Murphy BL, Ebert JW, Maynard JE, Purcell RH. Hepatitis B antigens in serum and liver of chimpanzees acutely infected with hepatitis B virus. Infect Immun 1975; 12: 602-605.
11. Hoofnagle JH, Michalak T, Nowoslawski A, Gerety RJ, Barker LF. Immunofluorescence microscopy in experimentally induced, type B hepatitis in the chimpanzee. Gastroenterology 1978; 74: 182-187.
12. Edgington ThS, Chisari FV. Immunological aspects of hepatitis B virus infection. Am J Med Sci 1975; 270: 213-227.
13. Joseph BS, Oldstone MBA. Immunologic injury in measles virus infection. II: Suppression of injury through antigenic modulation. J Exp Med 1975; 142: 864-876.
14. Fujinami RS, Oldstone MBA. Antiviral antibody reacting on the plasma membrane alters measles virus expression inside the cell. Nature 1979; 279: 529-530.
15. Fujinami RS, Oldstone MBA. Alterations in expression of measles virus polypeptides by antibody: molecular events in antibody-induced antigenic modulation. J Immunol 1980; 125: 78-85.
16. Aoki T, Geering G, Beth E, Old LJ. Suppression of antigen in Burkitts lymphoma and human melanoma cells grown in selected human sera. In: Nakahara W, Nishoka K, Hirayoma T, Ito Y, eds. Recent advances in human tumour virology and immunology. Tokyo: University of Tokyo Press, 1971: 425-429.
17. Stevens JG, Cook ML. Maintenance of latent herpetic infection: an apparent role for antiviral IgG. J Immunol 1974; 113: 1685-1693.
18. Drouet J, Courouce-Pauty AM, Soulier JP, Chanard J, Vallee G, Funck-Brentano JL. Kinetics of HBs antigen in man. Biomedicine 1975; 22: 158-166.

CHAPTER IV

CO-OCCURRENCE OF HBsAg AND ANTI-HBs; TWO CONSECUTIVE INFECTIONS OR A SIGN OF ADVANCED CHRONIC LIVER DISEASE?

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Summary

Simultaneous presence of HBsAg and anti-HBs was detected in 32 out of 89 Dutch chronic hepatitis B virus carriers of Caucasian race. The subtype frequencies and the distribution of risk factors for acquiring a hepatitis B virus infection were compared in the chronic HBV carriers and 38 patients with acute hepatitus B who recovered. In the chronic HBV carriers, HBsAg was subtyped ad in 28 and ay in four cases. Anti-HBs could be subtyped in 25 cases using reference antigens discriminating between d, y, and w1-w4 determinants. In 20 out of these 25 patients HBsAg subtype ad (HBsAg/ad) was accompanied by antibody to determinant y (anti-y), whereas HBsAg/ay and anti-d were simultaneously detected in the serum of one patient. The antibody pattern in sera from the remaining patients was complex. In the acute hepatitis B group, HBsAg was subtyped ay in 25 and ad in 13 cases. Thus, in our patients a predominance of ay was found in acute hepatitis with recovery and ad in chronic HBV carriers. This difference was partly caused by the prevalence of ay in drug-users and ad in homosexuals. Non-drug users appeared to have an equal chance to acquire HBsAg/ad or HBsAg/ay acute hepatitis.

Eighteen anti-HBs positive chronic HBV carriers were matched for age, histology, and HBeAg status with 18 anti-HBs negative chronic HBV carriers. No differences in risk factors for acquiring a hepatitis B infection were found. These results do not support the hypothesis that co-occurrence of HBsAg and anti-HBs is due to two consecutive infections with hepatitis B virus. The frequency of the co-occurrence of HBsAg and anti-HBs was found to be related to the degree of progressive liver disease, since anti-HBs was found in three out of 23 asymptomatic HBV carriers, in four out of 20 chronic persistent hepatitis B patients, in 20 out of 41 chronic active hepatitis B patients, and in all five patients with chronic active hepatitis B and cirrhosis. The high frequency of anti-HBs in patients with advanced liver disease may be the result of a disturbed immunologic response mechanism.

Introduction

Simultaneous presence of hepatitis B surface antigen (HBsAg) and antibodies against antigenic determinants of HBsAg (anti-HBs) has recently been described in single cases of HBsAg-positive chronic liver disease by various authors (1-8).

Since several subtypes of the hepatitis B virus are known (9,10), co-occurrence of HBsAg and anti-HBs may be explained by two subsequent infections with different subtypes, one of which gives rise to hetero-typic antibodies ("two infection hypothesis") (3). Alternative mechanisms, also presented by Le Bouvier et al. (3), are: 1) the presence of "hidden" determinants on HBsAg which cause antibody formation without subsequent interaction with HBsAg-bearing particles; 2) some form of persistent double infection with HBV of two different genotypes, while one or the other antigenic product is "outweighed" by its monospecific subtypic antibody at the time of blood sampling; and 3) quasi-monospecific antibody, evoked by an antigenic stimulus unrelated to HBV infection. Of our group of 89 HBsAg-positive chronic hepatitis patients, 32 (36%) showed HBsAg as well as anti-HBs in their sera. This offered the opportunity for a more detailed investigation of the relation between HBV antibodies and HBV antigens, and to establish the validity of the widely accepted

"two infection hypothesis". For the latter reason we also compared the subtype frequencies and the distribution of risk factors for acquiring a HBV infection in patients with acute and chronic hepatitis.

Patients and methods

Patients

The study group consisted of 89 chronic HBV carriers and 38 patients with acute symptomatic hepatitis B and complete recovery. Eight patients of the chronic group had been observed with acute symptomatic hepatitis B and subsequently had developed chronic hepatitis, as described in chapter III. All patients were born of Dutch parents of Caucasian race.

The patient group with biopsy-confirmed chronic HBV carriership was divided into 4 subgroups: I) asymptomatic HBV carriers (AC) (n=23); II) chronic persistent hepatitis B (CPH) (n=20); III) chronic active hepatitis B (CAH) (n=41); IV) CAH with cirrhosis (CIRR) (n=5). Patients were grouped according to standard histologic criteria (11) and the following supplementary criteria: AC: HBsAg positive > 6 months, no symptoms of liver disease, serum alanine amino transferase (ALT) continuously normal > 6 months; CPH: HBsAg positive > 6 months, ALT < 2 x upper limit of normal; CAH: HBsAg positive > 6 months, ALT > 2 x upper limit of normal.

Acute hepatitis B was diagnosed if a patient was HBsAg positive and showed ALT > 5 x upper limit of normal for a period of at least 5 days and a liver biopsy in conformity with acute viral hepatitis.

From the initial group of 89 chronic patients, 18 anti-HBs negative patients could be matched with patients in the anti-HBs positive group for histology, HBeAg/anti-HBe status, and, in the majority (13 cases), for year of birth (divided in 5-year periods). The number of patients that could be matched was limited, due to the limited number of HBeAg positive/anti-HBs negative patients with chronic active hepatitis (group III), and the lack of anti-HBe positive/anti-HBs negative patients with cirrhosis (group IV).

Methods

The sera were stored at -20 °C after collection. Radioimmunoassays (Abbott Laboratories, Chicago, Il 1., USA) were used for the detection of HBsAg (AUSRIA II), anti-HBs (AUSAB), HBeAg, and anti-HBe (ABBOTT-HBe).

Subtyping of HBsAg and anti-HBs was performed according to the method of Hoofnagle et al. (12). This method is based upon the neutralization of anti-HBs by HBsAg in solution prior to the performance of the AUSAB test. The residual amount of anti-HBs is then compared with the original amount detected using HBsAg and anti-HBs negative normal human serum as additive. Before incubation of anti-HBs positive sera with reference antigens of known sub type or of anti-HBs reference sera with HBsAg positive sera, pilot experiments were conducted to assure an optimal ratio of antibody in the neutralization reaction. In all cases of subtyping of HBsAg in anti-HBs positive sera, HBsAg was present in a relatively large concentration, so that the serum could be diluted until the contribution of the patient's anti-HBs was negligible.

For the subtyping of HBsAg we used guinea pig antisera containing anti-ad and anti-ay, which were incubated with human HBsAg/ay and HBsAg/ad, respectively, to obtain antisera specific for d and y (anti-d, anti-y). The reference antigen- and antibody containing sera were kindly supplied by the National Institutes of Allergy and Infectious Diseases, Bethesda, Md., USA. After subtyping anti-HBs in some HBsAg positive sera, monospecific anti-d and anti-y serum became available. Subtyping of HBsAg with these reagents was in accordance with the results obtained by monospecific antibodies prepared in vitro. Subtyping of anti-HBs was made possible by reference sera kindly supplied by Dr. A.M. Courouce-Pauty (Centre National de Transfusion Sanguine, Paris, France). The antigen subtypes used in the neutralization reaction were: ayw1, ayw2, ayw3, ayw4, adw2, and adw4.

The results were expressed as the percentage of reduction of counts per minute (cpm) measured in the AUSAB test after preincubation with antigen compared to preincubation with normal human serum. A percentage of more than 50 was considered positive. The results were reproducible within 10%. In the case of subtyping HBsAg, the reduction was always less than 10% or more than 60%, dependent on the subtype of the monospecific antiserum used.

Results

Subtype of HBsAg

In the group of 32 patients with anti-HBs positive sera, HBsAg was subtyped ad in 28 and ay in four cases. The number of d versus(vs) y determinant positive sera (15 vs 3) in the matched anti-HBs positive group was not significantly different from that in the matched anti-HBs negative group (17 vs 1) (chi-square with Yates' correction: 0.281, P'0.50).

Subtype of anti-HBs

Antibodies could be subtyped in 25 out of the 32 anti-HBs positive sera. In the remaining seven cases insufficient material was available, or the concentration was too low (cpm < 5x negative control value in AUSAB test). In the latter cases anti-HBs was repeatedly positive in the same serum sample or could be detected in the same concentration in other samples of that particular patient. In Table 4.1 the results of anti-HBs subtyping are grouped according to antibody pattern. In the majority of cases (20 of 25) HBsAg/ad was accompanied by anti-y antibodies as the sole type of antibody. One patient combined HBsAg/ay with anti-d. This patient had probably been infected in Southeast Asia during a 2-year stay.

In two cases the only type of antibody detected was anti-w3, in one patient accompanied by HBsAg/ay and in the other by HBsAg/ad. The two remaining cases exhibited a rather complex pattern: HBsAg/ad : anti-y and anti-w4 (adw4) and HBsAg/ad : probably anti-w2 (ayw2), anti-w3 (ayw3) and anti-w4 (ayw4).

The results of the neutralization tests on the serum of patient no.24 illustrate that the affinity of antibodies for the reference antigens is not uniform. The percentage of reduction with subtypes ayw1 (53%) and adw4 (52%) remained unchanged if the concentration of these antigens was changed eightfold.

TABLE 4.1 Neutralization of anti-HBs by reference antigens in 25 HBsAg containing sera

Patient no.	HBsAg subtype	Percentage neutralization by HBsAg subtype					
		ayw1	ayw2	ayw3	ayw4	adw2	adw4
1	ad	97	98	97	94	0	9
2	ad	61	71	72	71	2	0
3	ad	97	98	96	96	0	0
4	ad	95	98	96	90	3	4
5	ad	85	96	90	88	1	8
6	ad	91	97	95	91	4	0
7	ad	61	63	98	80	11	13
8	ad	99	100	100	95	0	0
9	ad	99	98	98	100	0	0
10	ad	94	92	100	89	20	5
11	ad	86	95	94	80	0	0
12	ad	92	97	96	99	6	7
13	ad	85	98	93	96	0	0
14	ad	93	96	93	93	6	6
15	ad	100	100	100	95	7	0
16	ad	90	97	98	100	0	0
17	ad	98	99	99	99	0	13
18	ad	56	75	63	75	6	8
19	ad	99	99	89	95	8	13
20	ad	85	93	95	89	3	0
21	ay	0	0	0	31	58	95
22	ad	11	7	89	29	21	30
23	ay	20	10	66	30	32	17
24	ad	53	94	96	100	15	52
25	ad	0	100	100	95	0	38

Risk factors

The 18 matched anti-HBs positive and anti-HBs negative patients were grouped according to their risk factors for acquiring a hepatitis B infection (Table 4.2). In about half of the cases a risk factor could not be found for both the anti-HBs positive and the anti-HBs negative groups. In the other half, the risk was almost equally distributed among the two groups. For comparison, the risk distribution for the whole group of anti-HBs positive patients is added to Table 4.2.

TABLE 4.2 Risk factor distribution among anti-HBs positive (anti-HBs +) and anti-HBs negative (anti-HBs -) chronic HBV carriers

	matched groups		total group
	anti-HBs +	anti-HBs -	anti-HBs +
Drug abuse	0	1	1
Homosexuality	4	5	6
Multiple transfusions	1	0	3
Medical profession	0	1	1
Relatives with HBsAg	3	3	4
No risk factor	10	8	17
Total	18	18	32

Subtype frequencies and risk factors compared in chronic and acute hepatitis B

Since the number of HBsAg/ad and HBsAg/ay antigens and the risk factors are almost equally distributed among the groups of anti-HBs positive (n = 32) and anti-HBs negative (n = 18) chronic patients, these groups were combined and a comparison was made of subtype distribution and risk factor with the group of 38 acute hepatitis B patients who recovered.

Table 4.3 illustrates that HBsAg/ad predominated in chronic HBV carriers (90%) and HBsAg/ay in acute hepatitis (66%). In one case of the acute hepatitis group with recovery, a combination of HBsAg/ay and anti-d had been detected during the acute hepatitis. The development of chronic liver disease was observed in eight cases with acute hepatitis, as described in chapter III. In all cases except one, the subtype was HBsAg/ad; twice anti-HBs (subtype anti-y) was already present in the first sample with HBsAg obtained during the acute hepatitis.

With regard to risk factors, drug abuse was predominantly found in patients with acute hepatitis and recovery, whereas homosexuality and contact with relatives was mostly observed in patients who had developed chronic hepatitis.

TABLE 4.3 Risk factor distribution in cases of chronic HBV carriers and acute hepatitis B patients subdivided according to subtype of HBsAg

	chronic		acute	
	ad	ay	ad	ay
Drug abuse	1	1	1	11
Homosexuality	11	0	2	2
Multiple transfusions	3	0	1	1
Medical profession	1	1	2	0
Relatives with HBsAg	6	1	0	1
No risk factor	23	2	7	10
Total	45	5	13	25

Anti-HBs in relation to histology and HBe status

The frequency of anti-HBs positive sera in four groups of patients with different stages of liver disease is given in Table 4.4. The percentage of anti-HBs positive sera increased with the severity of the liver disease. Anti-HBs was found most frequently in HBeAg positive sera (20 out of 34, 59%) but this was probably due to the association of HBeAg with chronic active hepatitis.

TABLE 4.4 Frequency of anti-HBs in 89 patients with different stages of chronic HBV carriership and their HBeAg and anti-HBe status (*)

Histology	anti-HBs+	HBeAg+ anti-HBe-	HBeAg- anti-HBe+	HBeAg+ anti-HBe+	HBeAg- anti-HBe-
I AC	3/23 (13%)	0/0	3/23	0/0	0/0
II CPH	4/20 (20%)	1/2	2/17	1/1	0/0
III CAH	20/41 (49%)	19/32	1/7	0/0	0/2
IV CIRR	5/5 (100%)	0/0	5/5	0/0	0/0
Total	32/89 (36%)	20/34	11/52	1/1	0/2

*: frequency given as anti-HBs positive / anti-HBs positive and anti-HBs negative patients.

Discussion

Our attention was drawn to the phenomenon of anti-HBs in HBsAg positive chronic hepatitis after observing a rise in anti-HBs titer in a patient participating in a double-blind trial with human leucocyte interferon in CAH patients (13,14). In this patient, who received a placebo, HBsAg titer, DNA polymerase activity and HBeAg titer decreased concomitantly with the rise of anti-HBs. Approximately one year later, three other participants (one treated patient and two control patients) showed anti-HBs transiently, while maintaining HBsAg.

In agreement with other studies, it seemed possible that our HBsAg positive/anti-HBs positive patients had been infected on two consecutive occasions by different subtypes of HBV. In order to find additional evidence for the "two infection hypothesis" (3), we determined the HBsAg subtypes in a population of chronic and acute hepatitis patients. Predominantly HBsAg/ad was found in chronic HBV carriers, in accordance with the observations in neighbouring countries (15-18). In contrast, HBsAg/ay prevailed in acute hepatitis. This discrepancy, which was also found by others (17,19,20) is generally attributed to the predominance of HBsAg/ad in earlier days and the association of HBsAg/ay with intravenous drug abuse at present. However, seven out of eight acute hepatitis cases that subsequently became chronic showed the subtype HBsAg/ad in our observation during the last years. Moreover, in the acute hepatitis group that recovered the non-drug users appeared to have had an equal chance to acquire HBsAg/ad or HBsAg/ay acute hepatitis, but the prevalence of HBsAg/ay in drug-associated hepatitis was confirmed in our study. This limited number of observations suggest that the course after an HBsAg/ad or HBsAg/ay infection may be different in our country and that the predominance of HBsAg/ad in chronic hepatitis is not associated with the prevalence of HBsAg/ad in earlier days.

It is still conceivable that the presence of HBsAg/ad and HBsAg/ay viruses in the Dutch population makes it possible to acquire two consecutive infections with different subtypes in the absence of immunity after the first infection. However, the infection rate of hepatitis B is very low in the Netherlands (3.5 per 100,000). For a patient who once has contracted a hepatitis B infection, the chance to acquire a second infection seems to be extremely small. It might be argued that the chance for a chronic carrier to be exposed for the second time cannot be compared with that of a primary infection in the population as a whole, since in about half of the chronic cases risk factors for acquiring hepatitis B may be present. We therefore expected a higher frequency of risk factors among chronic HBsAg carriers with anti-HBs. However, the prevalence of risk factors in HBsAg positive/ anti-HBs negative chronic hepatitis was similar. This finding and the observation of increasing frequency of anti-HBs with progressive liver disease seem hardly compatible with the "two infection hypothesis".

We also considered the possibility that both determinants d and y are present on complete HBsAg-bearing particles. One of the determinants would then be "hidden". The immune system would only produce both subtypes of antibodies if incomplete HBsAg particles, possibly with both determinants exposed, are released into the circulation, for example when liver cells are destroyed by active inflammation.

However, this hypothesis is not supported by chemical and enzymatic treatment of HBsAg (21). Furthermore, the genetic base for antigenic differences between HBV strains is well established in epidemiological studies (16), inoculation studies in animals (22,23), and DNA sequence studies (24-26).

Another explanation for the simultaneous presence of HBsAg and anti-HBs may be a host-dependent abnormality in the immune response. The development of chronic hepatitis is ascribed to a (combination of) inadequate antibody response and a defect in T-cell function (27,28). However, the detection of HBsAg-specific immune complexes (29,30) in chronic hepatitis B patients suggests the existence of an antibody response to HBsAg in such cases. Since the frequency of anti-HBs with progressive liver disease in our study parallels that of HBsAg-specific circulating immune complexes (29,30), it may be suggested that the presence of "heterotypic" soluble anti-HBs, which at least in vitro can be neutralized by heterotypic HBsAg, represents a "nonspecific" part of the immune response. Some support for this hypothesis is found in an earlier study (31), where 16 of our HBsAg positive chronic active hepatitis patients were vaccinated with an equine influenza A virus. In 13 (80%) patients a significant rise was observed against a number of nonrelated influenza A strains, while in large vaccination trials with influenza virus nonspecific (heterotypic) antibody formation is observed normally only in about 5% (32).

Since in this study the formation of heterotypic antibodies was observed for HBV as well as influenza A virus in a relative large number of patients with chronic hepatitis B, and anti-HBs was also found frequently by others in such patients (33) and in HBsAg positive ful minant hepatitis (34), we suggest an underlying principle of qualitative deficiency in immune response of the host with degrees in severity leading to different forms of hepatitis.

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References

1. Biswas R, Gerlich W, Schober A, Thomssen R. Bestimmung der Antikoerper gegen Subtypen des Hepatitis-B- Oberflaechenantigens (HBsAg). Zentralblatt fur Bakteriologie und Hygiene I. Abteiling Orig. A 1976; 235: 310-315.
2. Koziol DE, Alter HJ, Kirchner JP, Holland PV. The development of HBsAg-positive hepatitis despite the previous existence of antibody to HBsAg. J Immunol 1976; 117: 2260-2262.
3. Le Bouvier GL, Capper RA, Williams AE, Pelletier M, Katz AJ. Concurrently circulating hepatitis B surface antigen and heterotypic anti-HBs antibody. J Immunol 1976; 117: 2262-2264.
4. Sasaki T, Ohkubo Y, Imai M, Miyakawa Y, Mayumi M. Co-occurrence of hepatitis B surface antigen of a particular subtype and antibody to a heterologous subtypic specificity in the same serum. J Immunol 1976; 117: 2258-2259.
5. Hess G, Arnold W, Koesters W, Biswas R, Huetteroth TH, Meyer zum Bueschenfelde KH. Simultaneous presence of HBsAg and anti-HBs in the serum of different subtypes (Serological and immunofluorescent studies). Z Immun-Forsch 1977; 153: 143-151.
6. Tabor E, Gerety RJ, Smallwood LA, Barker LF. Coincident hepatitis B surface antigen and antibodies of different subtypes in human serum. J Immunol 1977; 118: 369-370.
7. Courouce-Pauty A-M, Drouet J, Kleinknecht D. Simultaneous occurrence in the same serum of hepatitis B surface antigen and antibody to hepatitis B surface antigen of different subtypes. J Infect Dis 1979; 140: 975-978.
8. Brandt KH, Katchaki JN, Bronkhorst FB, Meinders AE. Co-occurrence of hepatitis Bs-antigen and heterotypic anti-HBs in the same serum. Neth J Med 1980; 23: 233-236.
9. Bancroft WH, Mundon FK, Russell PK. Detection of additional antigenic determinants of hepatitis B antigen. J Immunol 1972; 109: 842-848.
10. Le Bouvier GL. The heterogeneity of Australia antigen. J Infect Dis 1971; 123: 671-675.
11. Leevy CM, Popper H, Sherlock S. Diseases of the Liver and Biliary Tract. Standardization of Nomenclature, Diagnostic Criteria, and Diagnostic Methodology. Fogarty International Center Proceedings no.22, DHEW Publication no. (NIH) 76-725, 1976; 1-31.
12. Hoofnagle JH, Gerety RJ, Smallwood LA, Barker LF. Subtyping of hepatitis B surface antigen and antibody by radioimmunoassay. Gastroenterology 1977; 72: 290-296.
13. Weimar W, Heijtink RA, Ten Kate FJW, et al. Double-blind study of leucocyte interferon administration in chronic HBsAg positive hepatitis. Lancet 1980; 1:336-338.
14. Schalm SW, Heijtink RA. Temporary disappearance of viral replication in hepatitis B surface antigen-positive severe chronic active hepatitis. J Infect Dis 1981; 144: 282.
15. Skinhoy P. Hepatitis-associated antigen, subtypes d and y. An epidemiological and clinical evaluation. Scand J Infect Dis 1973; 5: 85-89.
16. Courouce-Pauty A-M, Soulier JP. Further data on HBs antigen subtypes - Geographical distribution. Vox Sang 1974; 27: 533-549.
17. Froesner GG, Berg PA. Wechsel des vorherschenden Subtyps des Hepatitis B Antigens bei akuten Hepatitis B Infektionen. Z Immun-Forsch 1975; 150: 259-266.
18. Donea-Debroise B, Brocteur J, Andre A, Remacle MB. The use of radio-immuno-inhibition assay for the study of y, d and w determinants of hepatitis B surface antigen. Biomed 1979; 30: 260-264.
19. Nielsen JO, Le Bouvier GL, The Copenhagen Hepatitis Acute Program. Subtypes of Australia antigen among patients and healthy carriers in Copenhagen. N Engl J Med 1973; 288: 1257-1261.
20. Gust ID, Dimitrakakis M, Lucas CR. Changing patterns in the distribution of hepatitis B subtypes. Vox Sang 1980; 38: 81-86.
21. Neurath AR, Strick N, Huang CY. Properties of delipidated hepatitis B surface antigen (HBsAg) and preparation of its proteolytic cleavage fragments carrying HBsAg-specific antigenic determinants. Intervirology 1978; 10: 265-275.
22. Murphy BL, Maynard JE, Le Bouvier GL. Viral subtypes and cross-protection in hepatitis B virus infections of chimpanzees. Intervirology 1974; 3: 378-381.

23. Tabor E, Gerety RJ, Smallwood LA, Barker LF. Induction of antibody to the "y" determinant of HBsAg in a chimpanzee carrier of HBsAg subtype "adw". J Immunol 1976; 117: 2038-2040.

24. Galibert F, Mandart E, Fitoussi F, Tiollais P, Charnay P. Nucleotide sequence of the hepatitis B virus genome (subtype ayw) cloned in E. coli. Nature 1979; 281: 646-650.

25. Valenzuela P, Gray P, Quiroga M, Zaldivar J, Goodman HM, Rutter WJ. Nucleotide sequence of the gene coding for the major protein of hepatitis B surface antigen. Nature 1979; 280: 815-819.

26. Ono Y, Onda H, Sasada R, Igarashi K, Sugino Y, Nishioka K. The complete nucleotide sequences of the cloned hepatitis B virus DNA; subtype adr and adw. Nucl Acids Res 1983; 11: 1747-1757.

27. Dudley FJ, Fox RA, Sherlock S. Cellular immunity and hepatitis-associated. Australia antigen liver disease. Lancet 1972; 1: 723-726.

28. Eddleston ALWF, Williams R. Inadequate antibody response to HBsAg or suppressor T-cell defect in development of active chronic hepatitis. Lancet 1974; 2: 1543-1545.

29. Carella G, Digeon M, Feldmann G, Jungers P, Drouet J, Bach JF. Detection of hepatitis B antigen circulating immune complexes in acute and chronic hepatitis. Scand J Immunol 1977; 6: 1297-1304.

30. Lambert PH, Tribollet E, Celada A, Madalinski K, Frei PC, Miescher PA. Quantitation of immunoglobulin-associated HBs antigen in patients with acute and chronic hepatitis, in healthy carriers and in polyarteritis nodosa. J Clin Lab Immunol 1980; 3: 1-8.

31. Heijtink RA, Masurel N, Weimar W, Schalm SW. Influenza vaccination in HBsAg positive chronic active hepatitis patients treated with interferon. Med Microbiol Immunol 1980; 169: 31-38.

32. Mulder J, Masurel N. Pre-epidemic antibody against 1957 strain of Asiatic influenza in serum of older people living in The Netherlands. Lancet 1958; 1: 810-814.

33. Schlicht I, Gadow J, Ortmans H, et al. Deficiency of antibody formation to HBsAg in the pathogenesis of chronic hepatitis and cirrhosis? Acta Hepato-Gastroenterol 1979; 26: 450-456.

34. Trepo CG, Robert D, Motin J, Trepo D, Sepetjian M, Prince AM. Hepatitis B antigen (HBsAg) and/or antibodies (anti-HBs and anti-HBc) in fulminant hepatitis: Pathogenic and prognostic significance. Gut 1976; 17: 10-13.

CHAPTER V

T-LYMPHOCYTE SUBPOPULATIONS IN PATIENTS WITH VARIOUS COURSES AFTER HEPATITIS B VIRUS INFECTION

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Summary

The course of disease after hepatitis B virus infection is probably determined by the cellular immune response of the host, which is partly regulated by the T helper and T suppressor cells. Immunoregulatory T-cell subsets were counted in the peripheral blood of 97 patients with various courses after hepatitis B virus infection: 23 of these patients were asymptomatic HBsAg carriers without detectable liver disease, 13 had chronic persistent hepatitis B, 19 had chronic active hepatitis B (11 HBeAg, 8 anti-HBe), 7 had chronic active hepatitis with anti-HBs, and 35 were healthy controls with anti-HBs after recovery from acute hepatitis B. Peripheral blood mononuclear cells were specifically labeled with monoclonal Leu-1 (T-cells), Leu-2a (T suppressor/cytotoxic cells), and Leu-3a (T helper cells) antibodies and analyzed by flow cytometry.

Leu-3a/Leu-2a ratios for patients with persistence of the virus did not differ from those found for patients who cleared the hepatitis B virus. These findings suggest that elimination of hepatitis B virus as such is unlikely to be related to the relative number of peripheral T-cell subsets. However, evidence was found that the number of T suppressor cells had decreased in the subgroup of patients with ongoing chronic active hepatitis and anti-HBe. This subgroup of patients who develop chronic active hepatitis after partial clearance of the virus probably have an enhanced immunoreactivity compared with those running the commoner courses of this disease.

Introduction

The extremely variable course of disease after infection with hepatitis B virus (HBV) is usually explained by differences in the immunological host response to the hepatocytes carrying viral antigens (1,2). These immunological reactions may be regulated by T lymphocyte subpopulations, especially T helper and T suppressor cells (3), as discussed in chapter II, section 2.4. Several authors have observed differences in peripheral T-cell subset activity among patients with various forms of chronic hepatitis (4-7). The results of these T-cell activity assays are not homogeneous, but a tendency is perceivable to decreased suppressor cell activity in patients with chronic active hepatitis B. Few data are available on T lymphocyte subpopulations in patients with various forms of hepatitis B (8). We therefore studied T lymphocyte subsets in the peripheral blood of patients with and without virus elimination and with and without chronic hepatitis after hepatitis B virus infection.

Patients and methods

Patients.

One hundred and twenty-four patients with a hepatitis B infection, all of Dutch extraction and Caucasian race, were eligible for the study. Reliable T-cell subset counts could be obtained in 97 cases. The patients were grouped in accordance with standard criteria (9): 23 asymptomatic HBsAg carriers without biochemical or histologic abnormalities (AC-B), 13 patients with chronic persistent hepatitis B (CPH-B), 19 with chronic active hepatitis B (CAH-B), 7 with chronic active hepatitis

after elimination of hepatitis B antigens (CAH-anti-HB), and 35 healthy controls without clinical or biochemical abnormalities and with demonstrable anti-HBs after recovery from acute hepatitis B (C-anti-HB).

Methods.

HBsAg, anti-HBs, HBeAg, anti-HBe, and anti-HBc were determined by radioimmunoassays (Abbott Laboratories, Chicago, Ill., USA). Mononuclear cells were isolated by Ficoll-Isopaque gradient centrifugation by the method of Böyum (10), washed twice, suspended in a medium consisting of 25% fetal calf serum, 65% RPMI, and 10% dimethylsulfoxide, frozen to -80 °C in a polystyrene box, and stored in liquid nitrogen. Subsequently, vials with frozen mononuclear cells were thawed to 37 °C and, after being washed, were resuspended in 5% bovine serum albumin in phosphate-buffered saline. Cell counting was accomplished with a Coulter counter. The yield of mononuclear cells was 75%, according to cell counts obtained before isolation and after thawing. The cell suspension was diluted to a concentration of 10 million cells/ml. One hundred microliters of the suspension were incubated with 1 microgram of fluorescein isothiocyanate-labeled (FITC) anti-Leu antibodies (Becton Dickinson Monoclonal Antibody Center, Sunnyvale, Va., USA) or 5 microlitre of a FITC-labeled goat anti-human Ig antiserum diluted 1:15 (GAH/Ig/FITC, Nordic Immunological Laboratories, Tilburg, The Netherlands). A direct immunofluorescence technique was thus applied. The cells were analyzed for the presence of Leu-1, Leu-2a, Leu-3a and Ig determinants on the cell membrane. Anti-Leu-1 is directed against a non-subtype specific human T-cell antigen, anti-Leu-2a against a human T cytotoxic / suppressor cell antigen, and anti-Leu-3a against a human T helper / inducer cell antigen. GAH/Ig/FITC detects human B lymphocytes and monocytes, the latter via the Fc receptor. After incubation for 30 min and washing at 4 °C, the cells were analyzed by flow cytometry.

Flow cytometry analysis was performed with a fluorescence-activated cell sorter (FACS II, Becton Dickinson FACS Systems, Sunnyvale, Calif., USA). For each sample at least 10,000 cells were analyzed. Percentages of positive cells were calculated by plotting the histogram profiles for both unlabeled and FITC-anti-Leu-antibody labeled cells. Viable and nonviable cells could be distinquished on the basis of their low-angle scatter characteristics (11). In this manner three groups of mononuclear cells could be distinguished (Fig.1): a group with a low scatter signal that appeared to consist of nonviable cells (according to a propidium iodide stain) and two other groups consisting of viable lymphocytes and monocytes.
The fluorescence histogram was measured twice, first for all cells and subsequently for the viable lymphocytes (Fig.1). When samples contained more than 75% dead cells, the lymphocyte window could not be localized exactly, resulting in a less precise counting of the lymphocyte subpopulation; this was encountered in 27 cases. The data for these 27 out of 124 patients were not included in the analysis. These patients were equally distributed among the various patient groups.

Statistical analysis was performed with the Wilcoxon rank sum test (12) and the rank correlation test (13); results were considered significant when the p value was less than 0.05.

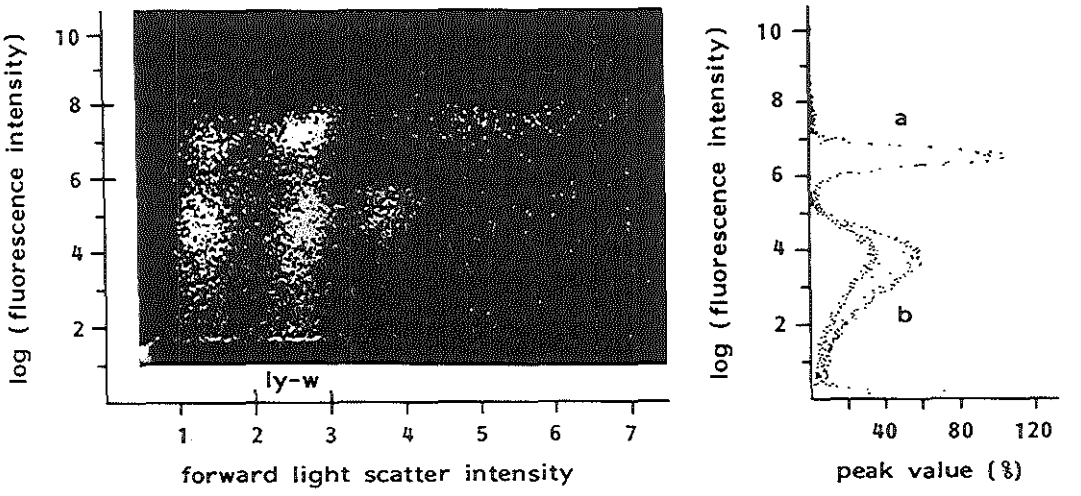

Figure 5.1. Typical example of flow cytometry analysis of mononuclear cells by fluorescence-activated cell sorter. With forward light scatter three groups of mononuclear cells can be distinguished in arbitrary units: nonviable cells with a low scatter signal (1-2), viable lymphocytes (2-3) and viable monocytes with a higher scatter signal (3-4). By measuring the fluorescence, specifically labeled cells can be distinguished at a higher level (6-7) than the unlabeled cells. The lymphocyte window (ly-w) separates viable lymphocytes from other mononuclear cells. The histogram (right) shows the number of cells with a certain fluorescent intensity. By comparing the histogram of a cell population before (b) and after (a) specific labeling, the percentage of labeled cells can be calculated.

Results

In all patient groups we found a statistically high correlation ($p < 0.001$) between the distribution of the lymphocyte subpopulations obtained by counting the viable lymphocytes only and that found by counting all cells. In these patients approximately 45% of the cells were viable and 55% were nonviable.

The distribution of the T-cell subset population, as presented in Table 1, is based on the counting of viable lymphocytes. We found average values of 56% Leu-1 positive cells, 19% Leu-2a positive cells, and 40% Leu-3a positive cells, there was close correspondence between the sum of the T-cell subsets and the total T-cell percentage.

Regarding viral elimination, the T-cell subset counts of the patients who had cleared the virus (C-antiHB and CAH-antiHB) were compared to those of patients with persistence of the virus (AC-B, CPH-B and CAH-B together). No statistical differences were found between these two categories of patients.

TABLE 5.1 Distribution of monuclear cells in patients with various courses of disease after hepatitis B virus infection

Patient group	n=	all* leucocytes	%** monocytes	% B-cells	% Leu-1	% Leu-2a	% Leu-3a	3a/2a
Healthy HBsAg carriers	23	5.6±1.3	4.7±2.6	28±13	54±15	18±10	41±12	2.8±1.5
Chronic persistent hepatitis B	13	5.7±1.0	8.1±6.0	23±10	52±10	20± 7	37± 9	2.1±1.1
Chronic active hepatitis B	19	6.2±1.1	5.3±3.9	26±19	55±18	17±8***	41±14	2.9±1.4
Chronic active hepatitis non-B +anti-HBs	7	4.9±1.1	3.9±2.3	31±16	54±14	17± 8	39±12	3.1±2.0
Healthy controls recovered from hepatitis B	35	7.4±2.3	6.0±4.8	26±14	60±12	21± 7	42±12	2.4±1.4

* mean absolute number ± 1 SD ($x10^{-3}$) per mm^3 blood
** mean ± 1 SD
*** $P < 0.05$ in comparison to healthy controls

With respect to liver cell damage, the patient groups were compared separately. Although there was a considerable range in the percentages of cells, the Leu-2a positive (suppressor/cytotoxic) cells were significantly reduced ($p < 0.05$) in patients with chronic active hepatitis B (CAH-B) compared with healthy controls who had recovered from hepatitis B (C-antiHB). Patients of the CAH-B subgroup with anti-HBe exhibited a more pronounced reduction in Leu-2a positive cells ($p < 0.02$), whereas the concentration of these cells was normal in the subgroup with HBeAg (Table 2). The patients in the CPH-B and AC-B groups, all with anti-HBe, had normal T-cell subset concentrations. The number of Leu-2a positive cells in the seven patients with chronic hepatitis after virus elimination (CAH-antiHB) was as low as that found for the CAH-B group, but in this small patient group a 5% significance was not reached ($0.05 < p < 0.10$ in comparison with healthy controls).

Comparison of 24 patients with a high Leu-3a/Leu-2a ratio (75-100 percentile) with 24 patients with a low ratio (0-25 percentile) yielded no differences in age, sex, blood group, HLA, or bilirubin and ALT levels.

TABLE 5.2 T Lymphocyte subpopulations and HBeAg / anti-HBe status in patients with chronic active hepatitis B

	n=	%Leu-2a*	%Leu-3a	3a/2a
HBeAg	11	19±7	39±14	2.5±1.1
anti-HBe	8	14±3**	44±17	3.4±1.7
Controls	35	21±7	42±12	2.4±1.4

* mean ± 1 SD
** $P < 0.02$ in comparison to controls

Discussion

This study was designed to investigate patients with an HBV infection who differed in their subsequent HB elimination and liver cell destruction.

Since elimination of the virus is thought to be mediated by immune responses, as discussed in detail in chapter II, section 2.4, we hypothesized a difference in immunoregulatory T-cell subsets between patients who had cleared the hepatitis B virus and those with persistence viral. Combining the patient groups in this respect, we found no statistical differences between these groups, indicating no general defect in immunological host response as cause of viral persistence. Obviously, with the methods used a HBV related antigen specific immunologic defect cannot be excluded.

Regarding liver cell degeneration by studying the various courses of disease separately, we found a decreased number of T suppressor/cytotoxic cells in the peripheral blood of patients with chronic active hepatitis B. This effect, however, is due to the subgroup which had partly eliminated the HB virus (CAH-B with anti-HBe), confirming the results of Thomas et al.(8). The T-cell subset counts for patients with other forms of chronic HBV carriership (AC-B, CPH-B, and CAH-B with persisting HBeAg) did not show statistically significant differences compared to the controls. Patients with chronic active hepatitis and clearance of the HB virus (CAH-antiHB) tended to have a lower number of T suppressor/cytotoxic cells, but this was not statistically significant for the small number of patients studied. These results suggest that elimination of hepatitis B virus is not causally related to the relative number of T helper and T suppressor cells in the blood. The persistence of active inflammation of the liver after partial or total clearance of the virus, however, might be mediated by a high T helper/T suppressor cell ratio.

In fact, the CAH subgroup with anti-HBe is a group of patients with an unusual course of disease. Most patients with chronic hepatitis B become biochemically and histologically inactive after conversion to anti-HBe (14-16) (see also Chapter II, section 2.3). The continuing chronic active hepatitis in CAH-B patients with anti-HBe might therefore be related to ongoing immunoreactivity against hepatocytes facilitated by a low level of suppressor cells. The same mechanism of liver cell destruction might be operational in patients with chronic active hepatitis after clearance of viral antigens (CAH-antiHB), although a conclusion is prohibited by the small number of patients in this group. Based on these observations, patients with chronic active hepatitis B who continue to have high hepatitis activity after seroconversion from HBeAg to anti-HBe may theoretically be served by immunosuppressive therapy, in contrast to CAH-B patients with a more regular course of disease (17,18).

Alternatively, chronic active hepatitis might affect the number of T suppressor cells. However, the normal number of Leu-2a cells in CAH patients with HBeAg in the blood, also observed by Thomas et al. in studies with OKT monoclonal antibodies (13), does not support this explanation.

The results of counting T-cell subsets by means of monoclonal antibodies should be interpreted cautiously, since the antigenic properties of the T-cell surface do not correspond directly to the functional classification of these cells. One problem of interpretation concerns the fact that Leu-2a positive cells are not a homogeneous group, since Leu-2a specific antibodies react not only to T suppressor cells but also to cytotoxic T lymphocytes. The low number of peripheral Leu-2a positive cells might therefore be due to a decreased number of peripheral cytotoxic T lymphocytes,

caused by an accumulation of cytotoxic T-cells in liver tissue. Immunohistologic analysis of the inflammatory infiltrate in chronic active hepatitis shows predominantly OKT-8 reactive cells (that is, T suppressor cells and/or cytotoxic T lymphocytes) in the liver (19). However, it is unknown whether long-term depression of the suppressor/cytotoxic cells in the blood can be explained by the accumulation of these cells in the liver.

In conclusion, the peripheral blood T-cell subsets investigated in chronic HBV carriers do not differ significantly from those in patients who cleared the virus. Reduced numbers of T suppressor/cytotoxic cells were found in patients with persistence of chronic active hepatitis after cessation of active viral replication. This finding suggests that liver cell destruction in these patients is no longer a direct consequence of the hepatitis B virus infection but is mediated by an imbalance among immunoregulatory T-cells.

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References

1. Dudley FJ, Fox RA, Sherlock S. Cellular immunity and hepatitis-associated, Australia antigen liver disease. Lancet 1972; 1: 723-726.
2. Chisari FV, Routenberg JA, Anderson DS, Edgington TS. Cellular immune reactivity in hepatitis B virus-induced liver disease. In: Vyas GW, Cohen SW, Schmid R, eds. Viral hepatitis. Philadelphia, Pa.: Franklin Press, 1978; 245-266.
3. Thomas HC, Montano L, Goodall A, De Koning R, Oladapo J, Wiedman KH. Immunological mechanisms in chronic hepatitis B virus infection. Hepatology 1982; 2: 116S-121S.
4. Kakumu S, Yata K, Kashio T. Immunoregulatory T cell function in acute and chronic liver disease. Gastroenterology 1980; 79: 613-619.
5 Tremolada F, Fattovich G, Panebianco G, Ongaro G, Realdi G. Suppressor cell activity in viral and non-viral chronic active hepatitis. Clin Exp Immunol 1980; 40: 89-95.
6. Chisari FV, Castle KL, Xavier C, Anderson DS. Functional properties of lymphocyte subpopulations in hepatitis B virus infection, I: Suppressor cell control of T lymphocyte responsiveness. J Immunol 1981; 126: 38-44.
7. Kashio T, Hotta R, Kakumu S. Lymphocyte suppressor cell activity in acute and chronic liver disease. Clin Exp Immunol 1981; 44: 459-466.
8. Thomas HC, Brown D, Routhier G, et al. Inducer and suppressor T cells in hepatitis B virus-induced liver disease. Hepatology 1982; 2: 202-204.
9. Leevy CM, Popper H, Sherlock S. Diseases of the liver and biliary tract. Standardization of nomenclature, dignostic criteria, and diagnostic methodology. Fogarty International Center Proceedings no. 22, DHEW publication No (NIH) 76-725, 1976; 1-31.
10. Böyum A. Separation of leucocytes from blood and bone marrow. Scand J Clin Lab Invest 1968; 21 (suppl 97): 51-89.
11. Loken MR, Herzenberg LA. Analysis of cell populations using a fluorescence activated cell sorter. Ann NY Acad Sci 1975; 254: 163-171.
12. Wilcoxon F. Individual comparisons by ranking methods. Biometrics Bull 1945; 1: 80-83.
13. Kendall MG. Tests of significance. In: Rank correlation methods, 4th ed. London: Griffin, 1970; 49-66.
14. Realdi G, Alberti A, Rugge M, et al. Seroconversion from hepatitis Be antigen to anti-HBe in chronic hepatitis B virus infection. Gastroenterology 1980; 79: 195-199.
15. Hoofnagle JH, Dusheiko GM, Seef LB, Jones EA, Waggoner JC, Bales ZB. Seroconversion from hepatitis Be antigen to antibody in chronic type B hepatitis. Ann Intern Med 1981; 94: 744-748.
16. Schalm SW, Heijtink RA. Spontaneous disappearance of viral replication and liver cell inflammation in HBsAg-positive chronic active hepatitis. Hepatology 1982; 2: 791-794.
17. Schalm SW, Summerskill WHJ, Gitnick GL, et al. Contrasting features and responses to treatment of severe chronic active liver disease with and without hepatitis Bs antigen. Gut 1976; 17: 781-786.
18. Lam KC, Lai CL, Ng RP, Trepo C, Wu PC. Deleterious effect of prednisolone in HBsAg-positive chronic active hepatitis. N Engl J Med 1981; 304: 380-386.
19. Eggink HF, Houthoff HJ, Huitema S, Gips CH, Poppema S. Cellular and humoral immune reactions in chronic active liver disease. I. Lymphocyte subsets in liver biopsies of patients with untreated idiopathic autoimmune hepatitis, chronic active hepatitis B and primary biliary cirrhosis. Clin Exp Immunol 1982; 50: 17-24.

CHAPTER VI

HLA ANTIGENS IN PATIENTS WITH VARIOUS COURSES AFTER HEPATITIS B VIRUS INFECTION

The contents of this chapter have been submitted for publication, under the same title, with the following authors: J. van Hattum, G.M.Th. Schreuder, S.W. Schalm.

Summary

In order to find a possible relationship between the course of disease and the phenotype frequency of HLA determinants, 396 Dutch subjects of Caucasian race were studied. Six groups of individuals with various courses after HBV infection were compared to healthy controls. The hepatitis B patients were grouped according to standard criteria: 1) 47 had recovered from acute symptomatic hepatitis B, 2) 60 had recovered from asymptomatic hepatitis B, 3) 26 were asymptomatic HBV carriers, 4) 16 had chronic persistent hepatitis B, 5) 37 had chronic active hepatitis B and 6) 10 had chronic active hepatitis after elimination of hepatitis B antigens. 29 Class I and 13 Class II HLA-antigens were assayed by standard microlymphocytotoxicity tests.

The phenotype frequency of the Class II antigen DQw1 appeared to be significantly lower in patients with chronic active hepatitis B. Other HLA specificities showing deviations from control values were not statistically significant after correction for the number of antigens tested. In conclusion, no evidence was found that the elimination of hepatitis B virus is related to HLA phenotype. However, HLA DQw1 may affect the morphologic type of chronic hepatitis B, since its presence may protect against chronic active hepatitis.

Introduction

The course of disease after hepatitis B virus (HBV) infection is extremely divergent, varying from recovery to the development of chronic active hepatitis, liver cirrhosis and death. The course of disease appears not to be related to variation in virulence of the HBV itself (1). However, there are many reasons to assume that the course of disease after HBV infection is determined by the host's immune system (2,3), as discussed in chapter II.

The Major Histocompatibility Complex (MHC) is involved in the regulation of the immune response. The class I antigens (HLA-A, -B and -C) are mainly recognized by T-cells that have the ability to develop into cytotoxic cells and the class II molecules (HLA-DR and -DQ) play a role in the activation of regulatory T lymphocytes (4).

Several investigations have been performed to detect a relationship between HLA and the course of hepatitis B. So far, the results are inconclusive and no statistically significant correlation could be found, except in one study (5). The main difficulties in the interpretation of the data are the small numbers of patients investigated, the inhomogeneity for race and the lack of uniform definition of the liver disease. Moreover, no study has been performed which included the presently known HLA loci and all possible courses of disease.

We therefore studied the phenotype frequencies of HLA antigens of class I and class II loci in groups of subjects, homogenous for race and with well-defined courses of disease after HBV infection.

Patients and methods

Patients.

Threehundred ninety six subjects were included in the study. They were unrelated, Caucasian and of Dutch extraction for two generations. The phenotype frequencies of HLA antigens of 196 subjects with HBV infection were compared with those of 200 healthy random controls. The patients were eligible for this study on the basis of serological signs of current or past hepatitis B, and they were grouped according to the courses of their liver disease, defined by standard criteria (6): 1) 47 patients, recovered from well documented acute symptomatic hepatitis B, with disappearance of HBV antigens and development of antibodies to the hepatitis B surface antigen (anti-HBs) and to the hepatitis B core antigen (anti-HBc), 2) 60 subjects, recovered from asymptomatic hepatitis B, anti-HBs and/or anti-HBc positive. These subjects had had no signs of hepatitis ever before, and were found by routine screenings of blood donors; 3) 26 asymptomatic hepatitis B surface antigen (HBsAg) carriers without biochemical or histological abnormalities; 4) 16 patients with chronic persistent hepatitis B, with transaminases lower than 2x upper limit of normal during more than two years and restriction of the inflammatory infiltrate to the portal areas in the liver biopsies; 5) 37 patients with chronic active hepatitis B, with transaminases higher than 2x upper limit of normal during more than six months and the typical periportal piecemeal necrosis in the liver biopsies; 6) 10 patients with continuing chronic active hepatitis after elimination of HBV antigens (anti-HBs, anti-HBc positive). The 200 controls were randomly selected, healthy blood donors, serologically negative for HBsAg and anti-HBc.

All patients, except those of group 2, had a liver biopsy to establish the diagnosis. In patients with chronic hepatitis (groups 4, 5 and 6) at least two biopsies were performed with an interval of at least six months.

Methods.

HBsAg, anti-HBs and anti-HBc were determined by radioimmunoassays (Abbott Laboratories, Chicago, Ill., USA). Mononuclear cells were isolated from 50 ml of heparinized blood by centrifugation on Ficoll-isopaque (Pharmacia, Sweden) (7). The cell suspension was washed twice, resuspended in a medium consisting of 25% fetal calf serum, 65% RPMI and 10% dimethyl sulfoxyde, and frozen to -80 °C in plastic vials. HLA-A, -B and -C typing was performed using the standard NIH lymphocyte microlymphocytotoxicity technique with a set of 120 well-defined sera (8). For HLA-DR and -DQ typing a panel of 80 platelet-absorbed sera was used in the two-colour fluorescence test (9,10,11).

The phenotype frequencies of 42 HLA determinants of each group were statistically compared to those of the control group (12): for each group the Hardy-Weinberg test was applied to exclude heterogeneity per locus, and the phenotype frequencies, relative risks and their significances were calculated according to Woolf's method as modified by Haldane. P-values were corrected for the 42 antigens tested in each set of comparisons between one patient group and the control group.

TABLE 6.1 HLA - antigen phenotype frequencies in 196 patients with various courses after hepatitis B virus (HBV) infection, compared to 200 healthy controls.

Group HLA	1 (47)	2 (60)	3 (26)	4 (16)	5 (37)	6 (10)	controls (200)
A1	0.298	0.186*	0.423	0.125	0.351	0.500	0.340
A2	0.681	0.644	0.538	0.625	0.486	0.400	0.525
A3	0.234	0.254	0.346	0.250	0.189	0.200	0.255
A9	0.170	0.169	0.192	0.188	0.135	0.200	0.195
A10	0.043	0.119*	0.077	0.063	0.081	0.000	0.035
A11	0.085	0.119	0.038	0.125	0.054	0.400*	0.125
A28	0.106	0.085	0.038	0.125	0.216*	0.000	0.085
Aw19	0.213	0.271	0.269	0.250	0.324	0.200	0.230
B5	0.043	0.034	0.192	0.188	0.108	0.100	0.120
B7	0.213	0.254	0.269	0.375	0.189	0.100	0.255
B8	0.234	0.119	0.269	0.188	0.270	0.300	0.225
B12	0.213	0.424	0.346	0.250	0.405	0.400	0.300
B13	0.043	0.017	0.038	0.125*	0.027	0.000	0.030
B14	0.043	0.017	0.038	0.000	0.027	0.000	0.040
B15	0.234	0.186	0.154	0.125	0.243	0.300	0.155
B16	0.021	0.068	0.077	0.063	0.162*	0.000	0.070
B17	0.128	0.085	0.077	0.063	0.054	0.000	0.100
B18	0.087	0.085	0.000	0.063	0.027	0.000	0.075
Bw22	0.064	0.119*	0.038	0.063	0.054	0.100	0.045
B27	0.106	0.068	0.038	0.063	0.027	0.100	0.075
B35	0.319*	0.186	0.154	0.125	0.054	0.100	0.175
B37	0.043	0.000	0.038	0.063	0.027	0.200*	0.036
B40	0.085	0.186	0.077	0.063	0.081	0.100	0.125
Cw1	0.085	0.017	0.115*	0.125*	0.027	0.000	0.035
Cw2	0.043	0.068	0.038	0.067	0.027	0.111	0.085
Cw3	0.319	0.458*	0.154	0.188	0.351	0.556	0.312
Cw4	0.298	0.220	0.231	0.188	0.108	0.300	0.197
Cw5	0.085	0.224	0.115	0.063	0.108	0.222	0.154
Cw6	0.237	0.130	0.211	0.333	0.118	0.250	0.144
DR1	0.170	0.217	0.154	0.313	0.135	0.100	0.205
DR2	0.213	0.217	0.308	0.313	0.189	0.200	0.270
DR3	0.234	0.167	0.346	0.125	0.243	0.400	0.286
DR4	0.319	0.400*	0.423*	0.125	0.351	0.200	0.245
DR5	0.319	0.200	0.154	0.500*	0.270	0.100	0.231
DRw6	0.234	0.350	0.231	0.125	0.162	0.400	0.290
DR7	0.277	0.133	0.192	0.188	0.324	0.200	0.250
DRw8	0.106*	0.067	0.000	0.000	0.108*	0.000	0.025
DRw9	0.021	0.017	0.077	0.063	0.028	0.000	0.030
DRw10	0.021	0.000	0.000	0.063	0.000	0.200*	0.050
DQw1	0.574	0.683	0.654	0.750	0.378**	0.800	0.678
DQw2	0.383	0.276*	0.538	0.250	0.417	0.500	0.402
DQw3	0.660*	0.552	0.654	0.688	0.629	0.400	0.447

* $p < 0.05$
** $p = 0.001$; p corrected for 42 tests = 0.043
1: recovered from acute symptomatic hepatitis B;
2: recovered from asymptomatic hepatitis B;
3: asymptomatic HBV carriers;
4: chronic persistent hepatitis B;
5: chronic active hepatitis B;
6: chronic active hepatitis and HBV elemination.

Results

The groups investigated were homogeneous, since no statistical significant heterogeneity per locus was observed by the Hardy-Weinberg test in the six groups.

The phenotype frequencies of the HLA determinants tested in the groups of subjects are listed in table 6.1. Comparison of each group independently with the controls showed several statistical deviations at the 5% level for the antigens of the A, B, C and D loci. Nearly all of these deviations were not significant after correction. However, we found that in patients with chronic active hepatitis B (group 5), the class II antigen DQw1 had a significantly lower phenotype frequency than the controls (table 6.2).

In the second instance, we combined groups that shared certain characteristics with respect to the two possible effects of the immunological response to HBV infection. We joined the groups 1, 2 and 6 (viral elimination), 3, 4 and 5 (no viral elimination), 2 and 3 (no hepatitis), and 4, 5 and 6 (chronic hepatitis). These larger groups were compared to the controls and, for viral elimination and hepatitis, to each other. Significant deviations at the 5% level were found for the following antigens (table 6.3): B5 (low phenotype frequency in 'HBV-elimination'), B35 (high frequency in 'HBV elimination') and DRw6 (low frequency in 'no HBV elimination'). However, we found no statistical deviations after correction for the number of comparisons made.

TABLE 6.2 HLA-DQw1 in patients with various courses after hepatitis B virus infection

group	HLA-DQw1 +ve	— ve	relative risk	χ^2	p	pc
1	26	21	0.59	2.70	0.10	ns
2	41	19	1.01	0.00	0.92	ns
3	17	9	0.88	0.10	0.75	ns
4	12	4	1.32	0.26	0.62	ns
5	14	23	0.29	11.45	0.001	0.043
6	8	2	1.62	0.50	0.49	ns
all	118	78	0.72	2.49	0.11	ns
controls	135	64				

pc : p corrected for 42 antigens tested, compared to controls.

TABLE 6.3 HLA antigen phenotype frequencies in patients with and without hepatitis B virus (HBV) elimination.

HLA	HBV elimination (117)	no HBV elimination (79)	controls (200)
B5	0.043*	0.152	0.120
B35	0.233*	0.101	(0.175)
DRw6	0.301	0.177*	0.290

* : $p \leq 0.05$, compared to the other groups, except that within paren theses.

Discussion

The results of HLA studies in hepatitis B have been discongruent and sometimes contradictory. No HLA deviations have been described that were statistically significant after correction for the number of antigens tested, except one (5). A review of a large number of studies was given by Kaslow and Shaw in 1981 (13); they wrote that these studies provided no conclusive evidence for an association between hepatitis B infection, persistent HBsAg carriage or HBsAg positive chronic active hepatitis and HLA, because of the divergent patient selections and the number of HLA loci and antigens tested. No study had been performed wich included all possible courses of disease and both HLA class I and class II antigens. In our study, we tried to avoid the problems mentioned above and composed six independent groups of subjects with characteristic, well defined courses of disease after HBV infection. The subjects were homogeneous for race and they were not related to each other. All known HLA loci were typed, including the class II determinants DR and DQ.

Significant numbers of patients have been included into the patient groups to provide reliable conclusions about the frequency of the HLA antigens examined, with exception of the smaller groups 4 and 6. Comparison of each group with the controls showed several deviations at the uncorrected 5% level (table 6.1).The phenotype frequency of B35 was elevated in patients who recovered (group 1), and was lowest in the group with chronic active hepatitis B (group 5). A tendency to increased frequency of B35 has been described in four studies of HBsAg positive chronic hepatitis and in two studies of HBsAg carriers, whereas in one study of HBsAg carriers the frequency of B35 was lower than normal (13). Our results suggest that B35 could be weakly associated with the ability to eliminate the hepatitis B virus (table 6.3). However, HLA B35 has a low frequency in our general population as well as in the patient groups, limiting its pathogenetic importance.

Elevated frequencies of HLA A1, B8 and DR3 are described in HBsAg negative auto-immune chronic active hepatitis (14). In our patients with chronic active hepatitis after clearance of the HBV (anti-HBs, anti-HBc positive) (group 6) no deviations of HLA A1, B8 and DR3 were found, although this group is too small to make firm conclusions.

The frequency of class II antigens has hardly been investigated in patients with various courses after HBV infection. Lepage et al. (15) studied 25 patients with chronic active hepatitis B and found no deviations in DR specificities. Forzani et al. (5) found a complete absence of DR4 in 44 Italian patients with HBsAg positive chronic active hepatitis without delta infection. That finding was statistically significant, also after correction for the number of tests performed. However, in view of the low frequency of DR4 in the control population (0.185 in the Italian population), the finding of a still lower frequency is of limited pathogenetic importance. The increased frequency of HLA DR3 described by Forzani et al. (5) was not statistically significant after correction for the total number of antigens tested within the patient group.

In this study we describe a low frequency of DQw1 in patients with chronic active hepatitis B (group 5) in comparison with the controls. This deviation was statistically

significant after correction for the number of antigens tested. Further correction for the number of groups tested is in our opinion not applicable, since the six independent patient groups represent divergent courses of disease and have in common only the fact of previous infection with hepatitis B virus (16). The low frequency of DQw1 was characteristic for group 5, and was not observed in the other patient groups (table 6.1). DQw1 is a common HLA determinant in the Dutch population (phenotype frequency 0.678). Therefore, a reduction of its phenotype frequency may be of pathogenetic importance.

DQw1 belongs to the MHC class II determinants, which are involved in the antigen presen tation to T-cells (4). The proliferative T-cell response has been shown to be restricted by products of the HLA DR region. Restriction is the phenomenon that antigen sensitized T-cells only recognize the antigen on presenting cells that are sharing a HLA class II (-DR or -DQ) determinant. Van Eeden et al. (17) tested a large number of distinct monocyte T-cell combinations for their proliferative response to PPD and showed that HLA DQw1 T-cells were significantly associated with low-responsiveness to antigen stimulation.

Chronic active hepatitis B is characterized by an ongoing attack of T lymphocytes on infected liver cells (18). Simultaneous expression of MHC class II determinants and viral antigens on the liver cell surface may play a role in maintaining this attack. In this study, DQw1 frequency appeared to be significantly lower than normal only in the group of chronic active hepatitis B patients. This finding suggests a protective role of DQw1 against chronic active hepatitis type B and is compatible with the in vitro finding of low responsiveness of DQw1 T-cells to antigenic stimulation.

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References

1. World Health Organization Technical Report 1977; series 602: 38.
2. Thomas HC, Montano L, Goodall A, De Koning R, Oladapo J, Wiedman KH. Immunological mechanisms in chronic hepatitis B virus infection. Hepatology 1982; 2: 1165-1215.
3. Chisari FV, Routenberg JA, Anderson DS, Edgington ThS. Cellular immune reactivity in HBV-induced liver disease. In: Vyas GN, Cohen SN, Schmid R, eds. Viral hepatitis. Philadelphia, Pennsylvania: The Franklin Institute Press, 1978; 245-266.
4. Thorsby E, Berle E, Nousiainen H. HLA-D region molecules restrict proliferative T-cell responses to antigen. Immunol Rev 1983; 66: 39-56.
5. Forzani B, Actis GC, Verme G, et al. HLA-DR antigens in HBsAg-positive chronic active liver disease with and without associated delta infection. Hepatology 1984; 4: 1107-1110.
6. Leevy CM, Popper H, Sherlock S, eds. Diseases of the liver and biliary tract. Standardization of nomenclature, diagnostic criteria, and diagnostic methodology. Fogarty International Centre proceedings no.22. DHEW publication no. (NIH) 76-725. Washington: U.S.Government Printing Office, 1976: 1-31.
7. Böyum A. Separation of leucocytes from blood and bone marrow. Scand J Clin Lab Invest 1968; 21 (suppl 97): 51-89.
8. Van Rood JJ. Microlymphocytotoxicity method. In: Ray JG, ed. Manual of tissue typing techniques. Bethesda: National Institutes of Health, 1979: 104-105.
9. Van Leeuwen A, Van Rood JJ. Description of B-cell methods. In: Terasaki PI, ed. Histocompatibility testing 1980. Los Angeles: UCLA Press 1980: 278-279.
10. Schreuder GMTh, Van Leeuwen A, Termijtelen A, Parlevliet J, D'Amaro J, Van Rood JJ. Cell membrane polymorphisms coded for in the HLA D/DR region. In: Relation between D and DR. Human Immunol 1982; 4: 301-312.
11. Bodmer WF (rapporteur) et al. Nomenclature for factors of the HLA system 1984. Tissue Antigens 1984; 24: 73-80.
12. Cavalli Sforza LL, Bodmer WF. The genetics of human populations. San Francisco: Freeman, 1971: 53-56.
13. Kaslow RA, Shaw S. The role of histocompatibility antigens (HLA) in infection. Epidemiol Rev 1981; 3: 90-114.
14. Tait BD, Mackay IR, Kastelan A, Dausset J, Mayer S, Okochi K. Chronic liver disease including chronic active hepatitis. In: Terasaki PI, ed. Histocompatibility testing 1980. Los Angeles: UCLA tissue typing laboratory, 1980: 657-661.
15. Lepage V, Degos F, Carella G, De Lima M, Giraud MC, Degos L. HLA-Cw7 and HBsAg negative chronic active hepatitis. Tissue Antigens 1981; 18: 105-107.
16. Svejgaard A, Platz P, Ryder LP. HLA and disease susceptibility: clinical implications. Clin Immunol Allergy 1984; 4: 567-580
17. Van Eeden W, Elferink BG, Hermans J, De Vries RRP, Van Rood JJ. Role of HLA class II products in proliferative T-lymphocyte responses to PPD: evidence for a regulatory influence associated with MBI. Scand J Immunol 1984; 20: 503-510.
18. Eggink HF, Houthoff HJ, Huitema S, Gips CH, Poppema S. Cellular and humoral immune reactions in chronic active liver disease; I: lymphocyte subsets in liver biopsies of patients with untreated idiopathic autoimmune hepatitis, chronic active hepatitis B and primary biliary cirrhosis. Clin Exp Immunol 1982; 50: 17-24.

CHAPTER VII

GENERAL DESCRIPTION AND DISCUSSION

Starting-points

This study was designed to find correlations between the various courses of disease after hepatitis B virus (HBV) infection and factors that could conceivably have influenced the course of disease.

The aim of the study was to find correlations between parameters of viral replication and liver cell damage, factors related to the viral subtypes and the immunological response and the various well defined courses of disease after hepatitis B virus infection, in order to gain insight into the timing and the mechanism of the development of chronic hepatitis B.

The course of disease after hepatitis B virus infection is heterogeneous (see fig. 2.3). Acute hepatitis B may cause hepatitis with jaundice, hepatitis without jaundice or no hepatitis at all. In any of these instances, the HBV may be cleared, leading to recovery, or may persist, resulting in chronic HBV carriership. Chronic HBV carriers may have varying degrees of chronic hepatitis, or no hepatitis at all. Viral clearance seems to run independently from hepatitis activity, in acute as well as in chronic hepatitis B. However, a close relationship between viral replication activity, partial viral clearance and hepatitis activity has been described (1-6) in a group of patients who have developed chronic active hepatitis, suggesting a causal role of active viral replication in the pathogenesis of persisting liver cell degradation. In this patient group, serological tests for hepatitis B e antigen (HBeAg) become negative during the natural history, which is considered to be a sign of partial viral clearance (4). Liver cell damage is often increased during some weeks preceding the seroconversion. After HBeAg seroconversion the virus is usually not completely cleared, but incorporated into the host's liver cell genome (7,8). In the other courses of disease such a correlation between viral clearance and liver cell damage has not become apparent. In particular, it is not clear whether in early acute hepatitis B liver cell damage plays a causal role in viral elimination, or is merely the result of the viral infection of liver cells.

Patients

The patient groups that were formed in this study had to be distinctive with respect to viral elimination and hepatitis activity.

Viral elimination and liver cell damage were studied simultaneously in patients with acute hepatitis B, who had no previous signs of HBV infection, as described in chapter III. After six months these patients either recovered or became chronic HBV carriers.

Factors related to the viral subtypes and factors related to the immunological response, described in the chapters IV, V and VI, were studied in the following groups of subjects, diagnosed as having:

- recovered from acute icteric hepatitis B; these patients had effective viral clearance together with considerable liver cell damage;
- recovered from acute non-icteric hepatitis B, with effective viral clearance, but without marked liver cell damage;

- chronic non-symptomatic HBV carriership, without effective viral clearance and without liver cell damage;
- chronic persistent hepatitis B, without effective viral clearance and with mild non-progressive liver cell damage;
- chronic active hepatitis B, without effective viral clearance and with mild to severe progressive liver cell damage;
- chronic active non-viral auto-immune hepatitis, with effective viral clearance and mild to severe progressive liver cell damage.

The patient groups were defined according to standard criteria (9).

Acute hepatitis B was diagnosed if alanine aminotransferase (ALT) was more than 2x the upper limit of normal for five days or more; hepatitis B surface antigen (HBsAg) and immune globulin M antibody to hepatitis B core antigen (IgM-anti-HBc) were positive; liver biopsy was in conformity with acute viral hepatitis without signs of chronic hepatitis; no history, symptoms or signs of liver disease had been present prior to presentation and no immunosuppressive drugs nor anaesthesia had been administered during four weeks prior to the initial symptoms of hepatitis. This extensive definition was used to prevent inclusion of patients with an exacerbation of pre-existent chronic hepatitis in the acute hepatitis group.

Recovery from acute icteric hepatitis was defined as normalization of ALT and loss of HBsAg in the serum, with development of antibodies to HBsAg (anti-HBs) and/or persistence of IgG-anti-HBc. These patients had previously fulfilled the criteria of acute hepatitis B as decribed above.

Recovery from acute non-icteric hepatitis was defined as normal ALT, negative HBsAg in the serum, positive anti-HBs and/or anti-HBc tests and no history, previous symptoms or signs of liver disease. These subjects were found by routine screening of blood donors.

Chronic non-symptomatic hepatitis B virus carriers were characterized by a normal ALT and positive serum HBsAg during more than six months, no history, symptoms or signs of liver disease and a liver biopsy in conformity with asymptomatic HBsAg carriership. Patients were only eligible for this group if they had at least one liver biopsy and were without signs of hepatitis in all biopsies, to be sure that patients with very mild chronic hepatitis would not be included in this group.

Chronic hepatitis was diagnosed if ALT elevation and positive serum HBsAg persisted for more than six months and a second liver biopsy, taken more than six months apart from the former, confirmed chronic hepatitis, according to the standard criteria (9).

Chronic persistent hepatitis B was defined by the following criteria, in addition to those of chronic hepatitis: ALT never higher than 2x the upper limit of normal; serum HBsAg positive for more than six months; liver biopsies in conformity with viral hepatitis, the mononuclear infiltrate being limited to the portal zones. Some spill-over of lymphocytes between intact hepatocytes immediately around the portal zones was allowed.

Chronic active hepatitis B was defined by the following criteria, additional to those of chronic hepatitis: ALT repeatedly or continuously higher than 2x the upper limit of normal; serum HBsAg positive for more than six months; liver biopsies in conformity with viral hepatitis and chronic active hepatitis, the mononuclear infiltrate being

extended into the liver lobules, with piecemeal necrosis (9).

Chronic active non-viral auto-immune hepatitis was defined by the following criteria, in addition to those of chronic hepatitis: ALT repeatedly or continuously higher than 2x the upper limit of normal; serum anti-HBs, and/or anti-HBc, anti-nucleic factor and liver membrane antibodies positive; liver biopsies in conformity with chronic active hepatitis, the mononuclear infiltrate being extended into the lobules, with piecemeal necrosis (9).

The patient groups as defined above were investigated in various parts of this study. The number of patients included in different parts of the study varies as a result of the availability of the patients to participate to the investigations at different times and because of specific additional requirements for particular parts of the study, for example, the patients had to be of Caucasian race in the chapters IV, V and VI.

All chronic HBV carriers were tested for markers of hepatitis delta virus (HDV) infection (10,11). The patients included in this study did not have positive tests for delta antigen or antibody to delta antigen. No patients have developed signs of acquired immune deficiency syndrome (AIDS) (12) during a follow-up of at least three years after collection of the sera that were investigated in this study. No alcoholics and no subjects with impaired cellular immunity, such as renal dialysis patients, subjects with Down's syndrome or patients with lymphoma or leukemia were included in the study.

Methods

Some methods had to be developed to detect HBV related antigens, where no standard procedures were available. In particular this was the case in the determination of the HBsAg titre and the HBeAg P/N ratio (chapter III) and the subtyping of HBsAg and anti-HBs (chapter IV). The other methods used in this clinical study are standard. They are described in the chapters III, IV, V and VI. Standard statistical methods are used in each investigation, with a preference for rank correlation methods (13,14) to minimize artefacts caused by non-normally distributed data.

The studies performed

Description, results and conclusions

The first aim of the study was to find correlations between parameters of viral replication and liver cell damage after hepatitis B virus infection, in order to gain insight into the timing and the mechanism of the development of chronic hepatitis B.

Parameters of viral replication and liver cell damage were studied in 63 patients with acute hepatitis B (chapter III). The patients were included in an early stage of acute hepatitis B and were followed until recovery or for at least six months. Parameters of viral replication (HBV DNA, DNA polymerase, HBeAg and HBsAg) and liver cell damage (ALT, bilirubin) were measured quantitatively or semi-quantitatively in the serum. Viral antigens (HBsAg and HBcAg) were determined in liver tissue by immunofluorescence.

The data were compared in time, using the point of maximal ALT elevation as the reference point.

After six months 53 patients had recovered and 10 had become chronic HBV carriers. Two weeks before maximal ALT, patients with different outcome had no statistically significant differences in the viral antigens measured. From that time, the HBsAg and HBeAg levels fell significantly in patients who eventually recovered and remained constantly high in patients who would become chronic HBV carriers. The sequence of disappearance from the serum was: HBV DNA and DNA polymerase, HBeAg, HBsAg. Viral antigens in liver biopsies were usually only detectable before the ALT peak and remained high only in those patients who became chronic carriers. The disappearance of HBsAg and HBeAg levels in serum showed no correlation with ALT or bilirubin levels in paired data of individual patients.

This study indicates that the course of disease and especially early clearance of the virus is already determined at least two weeks before the maximal ALT. The disappearance of viral markers, which reflects a decreased viral replication, occurs independently of the timing and the extent of liver cell damage. Decreasing viral replication is therefore probably not mediated by necrosis of infected hepatocytes. A further conclusion is that quantitative assays of HBsAg and HBeAg appear to be early indicators of the prognosis.

The second aim of the study was to find correlations between factors related to the viral subtypes and the various courses of disease after hepatitis B virus infection, in order to gain insight into the mechanism of the development of chronic hepatitis B.

The subtype frequencies of HBsAg and anti-HBs as well as risk factors for acquiring a HBV infection were compared in a study of 127 patients (chapter IV). Eighty-nine patients were chronic HBV carriers with various degrees of hepatitis activity and 38 had acute hepatitis B with recovery. All subjects were of Caucasian extraction.

HBsAg was simultaneously present with anti-HBs in 32 of the 89 chronic HBV carriers. The HBsAg subtype was ad in 28 and ay in four cases. Anti-HBs could be subtyped in 25 cases. Twenty of them had anti-HBs type anti-y in combination with HBsAg type ad . In one patient anti-d was found in combination with HBsAg type ay . The other four patients had a complex antibody subtype pattern. In the acute hepatitis B group, the HBsAg subtype was ay in 25 and ad in 13 cases. Subtype ay was common in intravenous drug users, who had mainly acute hepatitis B with recovery, and HBsAg subtype ad was predominant in homosexuals, who were mainly chronic HBV carriers. Non-drug users had equal chances of acquiring HBsAg subtype ad or ay acute hepatitis. However, 7 out of 8 acute hepatitis B patients who developed chronic hepatitis B had HBsAg subtype ad. Chronic HBV carriers with simultaneous presence of HBsAg and anti-HBs did not have an increased risk of acquiring HBV infection compared to chronic HBV carriers without anti-HBs. The frequency of chronic HBV carriers with simultaneous HBsAg and anti-HBs correlated with the degree of hepatitis activity: three out of 23 (3/23) asymptomatic carriers, 4/20 patients with chronic persistent hepatitis B, 20/41 with chronic active hepatitis B and 5/5 with chronic active hepatitis and liver cirrhosis.

These data show that HBsAg and anti-HBs relatively frequently occur together in chronic HBV carriers and that the frequency of co-occurrence increases with the

severity of HBV related liver disease. The detectable anti-HBs subtypes were always reactive to HBsAg subtypes other than the HBsAg subtypes present in the sera. These findings, as well as reports of a similar pattern of HBsAg-specific circulating immune complexes (15,16) and non-specific antibody formation after influenza vaccination (17) in chronic HBV carriers suggest that the non-specific anti-HBs formation may be part of a qualitative defect in the immune response to HBV antigens in such patients. The hypothesis that co-occurrence of HBsAg and anti-HBs is caused by two consecutive infections with HBV of different subtypes is not supported by the equal distribution of risk factors for HBV infection that was found in chronic HBV carriers groups with and without anti-HBs. There is still some doubt whether HBV subtypes ad and ay have the same pathogenetic properties in view of the higher incidence of subtype ad in chronic hepatitis despite an equal incidence of ad and ay in acute hepatitis in non-drug users.

The third aim of the study was to find correlations between the immune response and the various courses of disease after hepatitis B virus infection. The parts of the study that are related to this question are described in chapters V and VI.

In chapter V the determination of immunoregulatory T-cell subsets in the peripheral blood of 97 patients with various courses after HBV infection is discussed. Twenty three of the patients were asymptomatic HBV carriers, 13 had chronic persistent hepatitis B, 19 had chronic active hepatitis B, 7 had chronic active hepatitis with anti-HBs or anti-HBc, and 35 had recovered from acute hepatitis B with development of anti-HBs. Peripheral blood mononuclear cells were specifically labeled with monoclonal Leu-1 (reactive to T-cells), Leu-2a (reactive to T suppressor and cytotoxic cells), Leu-3a (reactive to T helper cells) antibodies and analyzed by flow cytometry. The T-cell subset counts of the combined groups of patients who had cleared the virus were compared to those of the combined patient groups with persistence of the virus. With respect to liver cell damage, the patient groups were compared separately.

No statistical differences were found between patients with and without clearance of the virus. Comparison of the patient groups separately showed a significant reduction of Leu-2a positive cells ($p < 0.05$) in patients with chronic active hepatitis B compared with healthy subjects who had recovered from hepatitis B. Chronic active hepatitis B patients of the subgroup with anti-HBe exhibited a more pronounced reduction in Leu-2a positive cells ($p < 0.02$), whereas the concentration of these cells was normal in the subgroup with HBeAg. The patients in the other HBV positive groups with anti-HBe had normal T-cell subset concentrations. The number of Leu-2a positive cells in the seven patients with chronic hepatitis after virus elimination was as low as that found for the chronic active hepatitis B group ($0.05 < p < 0.10$ in comparison with healthy controls).

These findings suggest that elimination of HBV is unlikely to be related to the relative number of peripheral T-cell subsets, and that viral persistence is not caused by a general defect in the host's immune response. With the methods used, a HBV related antigen specific immunologic defect cannot be excluded. However, persistence of active inflammation of the liver after partial or total clearance of the virus might be mediated by a high T helper/T suppressor cell ratio, which may be an

expression of enhanced immunoreactivity against hepatocytes rather than against viral antigens in these patients.

Genetic factors possibly influencing the course of disease after HBV infection are discussed in chapter VI. The phenotype frequency of HLA antigens was studied in 396 unrelated subjects of Dutch, Caucasian extraction. Six groups of individuals with various courses after HBV infection were compared to healthy controls. The hepatitis B patients were grouped according to the criteria formulated above: 1) 47 had recovered from acute symptomatic hepatitis B, 2) 60 subjects, recovered from asymptomatic hepatitis B, 3) 26 asymptomatic HBV carriers without biochemical or histological abnormalities 4) 16 patients with chronic persistent hepatitis B, 5) 37 patients with chronic active hepatitis B, 6) 10 patients with continuing chronic active hepatitis after elimination of HBV antigens (anti-HBs, anti-HBc positive). The 200 controls were randomly selected healthy blood donors, serologically negative for HBsAg and anti-HBc. All patients, except those of group 2, had a liver biopsy to establish the diagnosis. 29 class I and 13 class II HLA antigens were assayed by standard microlymphocytotoxicity tests.

Comparison of each group independently with the controls showed several statistical deviations at the 5% level for antigens of all HLA loci. Nearly all deviations were not significant after correction for the number of tests performed. However, we found that in patients with chronic active hepatitis B (group 5), the class II antigen DQw1 had a significantly lower phenotype frequency than in the controls. Combination of patient groups with and without viral elimination as well as groups with and without hepatitis activity yielded no additional HLA deviations that were statistically significant after correction.

In conclusion, evidence was found that the class II HLA antigen DQw1 may protect against chronic active hepatitis. The reduction of HLA DQw1 may be of pathogenetic importance because it is a common HLA determinant in the Dutch population. The MHC class II determinants are involved in the antigen presentation to T-cells (18). HLA DQw1 T-cells have shown to be associated with low-responsiveness to antigen stimulation (19). Simultaneous expression of MHC class II determinants and viral antigens on the liver cell surface may play a role in maintaining the ongoing attack of T lymphocytes on infected liver cells in chronic active hepatitis B. The finding of a low DQw1 frequency only in the group of chronic active hepatitis B patients suggests a protective role of DQw1 against chronic active hepatitis type B and is compatible with the in vitro finding of low responsiveness of DQw1 T-cells to antigenic stimulation. No evidence was found that the elimination of HBV is related to HLA phenotype.

General discussion

The results of this study suggest that different immunological mechanisms are involved in the clearance of the HBV and the development of liver cell damage at different stages in the courses of disease after HBV infection.

In the early acute disease the clearance of the HBV seemed to precede liver cell damage in patients who recovered. The precise cause of the defective early viral

elimination in patients who developed chronic hepatitis remains unclear. Factors related to this problem were investigated in the other parts of this study. A slight preference of HBV with HBsAg subtype ad to coincide with chronic hepatitis B was observed in Dutch patients with chronic hepatitis B and in patients with acute hepatitis B who eventually became chronic HBV carriers. Therefore, some influence of the viral HBsAg subtype on the course of disease cannot be excluded. Simultaneous presence of HBsAg and non-specific anti-HBs was found relatively frequently in chronic HBV carriers with more severe liver damage. It was found only in three patients with acute hepatitis B. Two of them eventually became chronic HBV carriers. Nevertheless the co-occurrence phenomenon, suggesting an immunologic defect resulting in production of less-specific humoral factors, seems to be correlated closer to liver cell damage than to HBV clearance as such. No indication of a general defect in the immune response of the host was found by investigation of immunoregulatory T-cell subsets in patients who did not clear the virus. A genetic basis for persistence or clearance of the HBV was not found by detection of HLA antigens in relatively large groups of subjects.

Insufficient clearance of the virus is a common feature of chronic HBV carriers at first sight. However, the degree of viral clearance may be different in the various courses of chronic HBV carriership and in individual patients during different stages of their course of disease, as discussed in chapter II, section 2.4. For that reason, the chronic HBV carriers included in this study had carefully and clearly defined courses of disease. HBsAg subtypes, non-specific anti-HBs formation, immunoregulatory T-cell subsets and HLA phenotype frequencies, however, did not correlate with the degree of viral clearance in these patients.

Liver cell damage in the early stage of acute hepatitis B did not correlate in time or severity with the decreasing quantitative parameters of viral replication in cases that eventually recovered. This is of importance with respect to the two main mechanisms of viral clearance that have been proposed. The first hypothesis states that elimination of the HBV is achieved by destruction of all infected liver cells (20). The second hypothesis postulates suppression of viral protein synthesis by antiviral antibodies (21). The results of this study support the proposed mechanism of selective suppression of viral protein without destruction of all infected liver cells.

Factors related to liver damage in chronic HBV carriers were investigated in the other parts of this study. Non-specific anti-HBs was observed relatively frequently in chronic HBV carriers with more advanced liver damage. This kind of patients also produced aspecific antibodies after Influenza vaccination. Therefore, these observations are interpreted as being a sign of a qualitative defect of the host's immune response rather than being related merely to the viral subtypes. A low number of T suppressor cells was found in patients with ongoing chronic active hepatitis after partial or complete clearance of the virus. This would be consistent with immunoreactivity against liver cell constituents, without the immunostimulating effects of expression of viral antigens on the liver cell surface in such patients. Genetic factors were found to be possibly involved in the regulation of cytotoxicity

against hepatocytes. The HLA DQw1 phenotype frequency was significantly low in the patient group with chronic active hepatitis, which is in concordance with the finding in vitro that DQw1 lymphocytes are low-reactive to antigenic stimulation (19).

Other factors that cause damage to the liver or interfere with the host's immune system, may influence the course of disease after HBV infection. The cytotoxic hepatitis delta virus, which replicates only in hosts who are HBV positive, decreases the HBV DNA synthesis and may cause variable degrees of liver damage, additive with that caused by HBV (10). The chronic HBV patients included in this study did not have positive tests for delta antigen or antibody to delta antigen. A high prevalence of chronic HBV carriers is found in subjects with impaired cellular immunity, such as renal dialysis patients, subjects with Down's syndrome and patients with lymphoma or leukemia (22,23) and in AIDS patients (12). Such patients were not included in the study.

The results of this study allow some additional remarks about the therapeutic approach of hepatitis B, which is an important end-goal of many investigations in this field.

In acute hepatitis B the decisive course seems to be set at least two weeks before maximal ALT. At this time no differences in quantitative viral parameters were observed between patients who would recover and those who would become chronic HBV carriers. Early antiviral therapy theoretically might be effective in preventing chronic HBV carriership in the latter category of patients. These patients with a potentially chronic course could possibly be selected for therapy at an early stage by repeated quantitative HBsAg or HBeAg assays, which appeared to be early and sensitive parameters of viral clearance in acute hepatitis B.

In chronic hepatitis B, immunosuppressive therapy has been shown to be only partially effective by decreasing the hepatitis activity (24,25), probably because the continuing viral antigen expression seems to play a major role in the cytotoxic activity (26). In fact, this kind of therapy showed detrimental effects on the prognosis of patients treated that way. Therefore, immunosuppressive therapy is no longer given to chronic hepatitis B patients. In this study a subgroup of patients with chronic active hepatitis B who continued to have high hepatitis activity after HBeAg seroconversion was discernable from other chronic HBV carriers because of a low number of T suppressor cells. This finding would be consistent with enhanced immunoreactivity against liver cell membrane antigens rather than viral antigens, corresponding to the supposed cytotoxic mechanism in chronic active non-viral hepatitis (27,28). The latter disease responds well to immunosupressive therapy (28). Therefore, immunosuppressive therapy theoretically might be effective in the HBeAg negative subgroup of patients with chronic active hepatitis B.

References

1. Hoofnagle JH, Dusheiko GM, Seeff LB, et al. Seroconversion from hepatitis B e antigen to antibody in chronic type B hepatitis. Ann Intern Med 1980; 94: 744-748.
2. Norkrans G, Nordenfelt E, Hermodsson S, et al. Long-term follow-up of chronic hepatitis patients with HBsAg, HBeAg and Dane particle associated DNA polymerase in serum. Scand J Infect Dis 1980; 12: 159-160.
3. 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: 195-199.
4. Liaw Y-F, Chu C-M, Su I-J, et al. Clinical and histological events preceding hepatitis B e seroconversion in chronic type B hepatitis. Gastroenterology 1983; 84: 216-219.
5. Hoofnagle JH, Dusheiko GM, Schafer DF, et al. Reactivation of chronic hepatitis B virus infection by cancer chemotherapy. Ann Intern Med 1982; 96: 447-449.
6. Davis GL, Hoofnagle JH, Waggoner JG. Spontaneous reac- tivation of chronic type B hepatitis. Gastroenterology 1984; 86: 230-235.
7. Brechot C, Hadchouel M, Scotto J,et al. State of hepatitis B virus DNA in hepatocytes of patients with hepatitis B antigens-positive and -negative liver diseases. Proc Natl Acad Sci USA 1981; 78: 3906-3910.
8. Shafritz DA, Shouval D, Sherman HI, Hadziyannis SJ, Kew MC. Integration of hepatitis B virus DNA into the genome of liver cells in chronic liver disease and hepato- cellular carcinoma. N Engl J Med 1981; 305: 1067-1073.
9. Leevy CM, Popper H, Sherlock S. Diseases of the liver and biliary tract. Standardization of nomenclature, dignostic criteria, and diagnostic methodology. Fogarty International Center Proceedings no. 22, DHEW publication No (NIH) 76-725, 1976; 1-31.
10. Rizzetto M, Hoyer BH, Purcell RH, Gerin JL. Hepatitis Delta virus infection. In: Vyas GN, Dienstag JL, Hoofnagle JH, eds. Viral hepatitis and liver disease. Orlando: Grune & Stratton, 1984: 371-379.
11. Heijtink RA, Kruining J, Kuijpers L, Jacobs A, Geudens C, Wolters G. An ELISA for Delta markers in hepatitis B infection. In: Verme G, Bonino F, Rizzetto M, eds. Viral hepatitis and Delta infection. New York: Allan R Liss, 1983: 263.
12. Curran JW. AIDS - Two years later. N Engl J Med 1983; 309: 609-610.
13. Wilcoxon F. Individual comparison by ranking methods. Biometrics Bull 1945; 1: 80-83.
14. Kendall MG. Rank correlation methods. London: Griffin, 1970.
15. Carella G, Digeon M, Feldmann G, Jungers P, Drouet J, Bach JF. Detection of hepatitis B antigen circulating immune complexes in acute and chronic hepatitis. Scand J Immunol 1977; 6: 1297-1304.
16. Lambert PH, Tribollet E, Celada A, Madalinski K, Frei PC, Miescher PA. Quantitation of immunoglobulin-associated HBs antigen in patients with acute and chronic hepatitis, in healthy carriers and in polyarteritis nodosa. J Clin Lab Immunol 1980; 3: 1-8.
17. Heijtink RA, Masurel N, Weimar W, Schalm SW. Influenza vaccination in HBsAg positive chronic active hepatitis patients treated with interferon. Med Microbiol Immunol 1980; 169: 31-38.
18. Thorsby E, Berle E, Nousiainen H. HLA-D region molecules restrict proliferative T-cell responses to antigen. Immunol Rev 1983; 66: 39-56.
19. Van Eeden W, Elferink BG, Hermans J, De Vries RRP, Van Rood JJ. Role of HLA class II products in proliferative T-lymphocyte responses to PPD: evidence for a regulatory influence associated with MBI. Scand J Immunol 1984; 20: 503-510.
20. Dudley FJ, Fox RA, Sherlock S. Cellular immunity and hepatitis-associated Australia antigen liver disease. Lancet 1972; 1: 723-726.
21. Chisari FV, Routenberg JA, Anderson DS, Edgington ThS. Cellular immune reactivity in HBV-induced liver disease. In: Vyas GN, Cohen SN, Schmid R, eds. Viral hepatitis. Philadelphia: Franklin Institute Press, 1978: 245-266.
22. Nordenfelt E, Lindholm T, Loefgren B, Moestrup T, Reinicke V. Different categories of chronic HBsAg car- riers: A long-term follow-up. In: Szmuness W, Alter HJ, Maynard JE, eds. Viral hepatitis. Philadelphia: Franklin Institute Press, 1982: 237-242.

23. Szmuness W, Harley EJ, Ikram H, Stevens CE. Sociodemo- graphic aspects of the epidemiology of hepatitis B. In: Vyas GN, Cohen SN, Schmid R, eds. Viral hepatitis. Philadelphia: Franklin Institute Press, 1978: 297-320.

24. Schalm SW, Summerskill WHJ, Gitnick GL, et al. Con- trasting features and responses to treatment of severe chronic active liver disease with and without hepatitis Bs antigen. Gut 1976; 17: 781-786.

25. Lam KC, Lai CL, Ng RP, Trepo C, Wu PC. Deleterious effect of prednisolone in HBsAg positive chronic active hepatitis. N Engl J Med 1981; 304: 380-386.

26. Mondelli M, Mieli Vergani G, Alberti A, et al. Speci- ficity of T lymphocyte cytotoxicity to autologous hepatocytes in chronic hepatitis B virus infection: antigen expressed on hepatocytes. J Immunol 1982; 129: 2773-2778.

27. Cochrane AMG, Moussourous A, Thomson AD, et al. Antibody-dependent cell-mediated (K-cell) cytotoxicity against isolated hepatocytes in chronic active hepatitis. Lancet 1976; 1: 441-444.

28. Kirk AP, Jain S, Pocock S, et al. Late results of the Royal Free Hospital prospective controlled trial of prednisolone therapy in hepatitis B surface antigen negative chronic active hepatitis. Gut 1980; 21: 78-83.

CHAPTER VIII

SUMMARY AND CONCLUSIONS

Aim of the study

The aim of this study is formulated in chapter I: "To find correlations between 1) the various courses of disease after infection with the hepatitis B virus and 2) quantitative parameters of viral replication and liver cell degradation, viral subtypes and the immunological response, in order to obtain more insight into the timing and the mechanisms of the development of chronic hepatitis B".

Motivation

The main motivation for this study was the lack of proven effective therapy for patients who have developed chronic hepatitis B. The theoretical basis for designing a treatment is incomplete. The key factors that determine the development into chronic hepatitis B are unknown, as well as the point of time at wich these factors play their decisive role in the course of disease.

All parts of this study deal with the spectrum of various, well defined, courses of disease wich may develop after infection with the hepatitis B virus (HBV). The acute infection usually results in complete recovery with clearance of the virus. Defective clearance of the virus leads to chronic HBV carriership. Both in acute infection and chronic carriership the degree of hepatitis may vary from severe to completely absent, indicating a discrepancy between viral elimination and liver cell destruction. Therefore, the immunological mechanisms regulating viral elimination and liver cell destruction may be, at least partially, different.

Design

This study was designed to get more information on some aspects that are related to the timing and the mechanism of the development of the wide range of courses of disease after infection with just one type of virus. The following questions were put forward: at what time during the acute desease is the course directed to chronicity?; are early changes in viral replication, viral elimination or liver cell degradation indicative for the change of course to chronicity?; are viral subtypes or antibodies directed against viral subtypes related to the development of a particular clinical course?; are immunoregulatory peripheral T-cell subsets correlated with the various clinical courses after HBV infection?; is there a genetic basis which predisposes to effective or defective viral clearance, tolerance of chronic viral presence without hepatitis, or intolerance with chronic active hepatitis?

Patients

The patient groups consisted of: patients with acute hepatitis B, subjects who had recovered from acute icteric hepatitis B, subjects who had recovered from acute non-icteric hepatitis B, chronic HBV carriers without hepatitis, chronic HBV carriers with chronic persistent hepatitis, chronic HBV carriers with chronic active hepatitis and patients who had cleared the HBV and had developed chronic active auto-immune hepatitis.

Hepatitis B virus infection, a review

A review of the literature concerning HBV infection is given in chapter II. Topics which are related to the aims of this study and the methods that are used are emphasized.

The HBV is a DNA containing virus. It mainly replicates in human liver cells. The coat protein contains the hepatitis B surface antigen (HBsAg). The viral core contains the DNA and the hepatitis B core antigen (HBcAg) and hepatitis B e antigen (HBeAg) (see fig 2.1). The main viral subtypes are expressed in the HBsAg types ad and ay, having determinant a in common. The small DNA molecule is partly double-stranded. The incomplete strand is repaired by DNA polymerase using the complete strand as a template. The complete strand contains large open reading frames, coding for HBsAg, DNA polymerase and polypeptides containing HBcAg and HBeAg, (see fig 2.2). The HBV DNA can occur in the liver cells either in a freely replicating form or integrated in the liver cell genome. Integrated HBV DNA is mostly found in the liver cells of HBeAg negative HBsAg positive chronic HBV carriers and in hepatocellular carcinoma cells.

HBV products detected in serum and liver can be used as markers after the clinical stage after HBV infection. Qualitative HBsAg and HBeAg detection is widely used, in contrast to quantitative determination of these antigens. HBsAg, HBcAg and HBeAg localizations in liver tissue are demonstrated with immunofluorescence and immunoperoxydase techniques. The viral antigen expression in liver tissue is believed to be inversely related to the clearance of the virus. DNA polymerase activity is a marker of the presence of DNA containing HBV particles. HBV DNA in serum and liver is detected by molecular hybridization techniques. DNA polymerase activity and HBV DNA both point to active viral replication.

The spectrum of clinical courses after HBV infection has already been summarized at the beginning of this chapter, stressing the discrepancies between viral clearance and hepatitis activity. However, the natural history of chronic active hepatitis B shows a close inverse relationship between partial viral clearance and hepatitis activity in cases that have reached the stage of HBeAg seroconversion. This observation suggests a causal role of active viral replication in the pathogenesis of persisting liver cell degeneration. Therapeutic intervention of chronic hepatitis B by means of immunosuppressive or immunostimulatory methods has not been proven to be effective. A temporary suppression of viral replication can be achieved by antiviral therapy.

The mechanisms of liver cell degeneration and viral elimination, both seeming to be immunological by nature, are not identical, as mentioned above. Liver cell degeneration is likely to be mediated by the cellular immune system. No close relationship of humoral immunologic factors and liver cell necrosis has become apparent. According to the classical theory, the cell-mediated immunity may account for liver cell necrosis by T-cell cytotoxicity, evoked by the expression of HBV-associated antigens on the liver cell surface. This mechanism requires dual recognition of viral and self (HLA-) antigens on the liver cell membrane. Chronic hepatitis B would then be the result of insufficient viral clearance and continuing expression of viral antigens on the cell surface. Other theories stress the role of changes in liver cell membranes or immunoregulatory substances in the development to

chronicity. Many studies have been performed with respect to these questions, in particular on the function of the general cellular immune system, cytotoxic lymphocytes and suppressor cells, but no homogeneous results have emerged and no significant correlation with hepatitis B disease activity has been found.

Viral elimination may be mediated by the cellular as well as by the humoral immune system. Humoral immunity probably plays an important role in the initial viral clearance in acute hepatitis B. Antiviral antibodies are also known to suppress viral replication in some other viruses than the HBV. One hypothesis assumes that this mechanism is also applicable to HBV infection and that antiviral antibodies may suppress the synthesis of HBV proteins. According to this hypothesis, the infected liver cells do not need to be destroyed to clear the virus. In contrast, another main hypothesis postulates that HBV elimination is the result of destruction of all infected liver cells.

The investigations performed

Chapter III deals with the questions concerning the time at which the course of disease during acute hepatitis B is directed to chronicity and whether early changes in viral replication, viral elimination or liver cell damage are indicative for the change of course to chronicity.

The disappearance of viral antigens in relation to liver cell destruction was studied in 63 patients with acute hepatitis B. Hepatitis B virus DNA, DNA polymerase activity, HBeAg and HBsAg were measured quantitatively in serum, and viral antigens were determined in liver biopsies by immunofluorescence. All data were related in time to the alanine aminotransferase (ALT) peak as a marker of maximum liver cell destruction. Six months after initial symptoms 53 patients had recovered and 10 had become chronic HBsAg carriers. At the onset of disease no differences in viral antigens were observed between the two groups with different outcome. In patients who recovered, the HBsAg and the HBeAg levels decreased significantly from two weeks prior to the ALT peak onwards. The disappearance of HBeAg and HBsAg occurred independently of the extent of the liver disease. HBV DNA, DNA polymerase and viral antigens in liver biopsies were mainly detectable before and around the ALT peak. In patients who developed chronic hepatitis, viral antigens in the serum and liver tissue remained consistently high.

These findings indicate that the final course of the acute hepatitis B is set at least two weeks before the maximum hepatitis activity and that quantitative assays of HBsAg or HBeAg appear to be early indicators of prognosis. The disappearance of viral markers, prior to and independently of the extent of liver cell necrosis, suggests that suppression of the viral protein synthesis is an additional mechanism of virus elimination, apart from destruction of infected liver cells.

In chapter IV the relationship between viral subtypes or antibodies against viral subtypes and the development of a particular clinical course is discussed, as well as some aspects of humoral immunity.

Simultaneous presence of HBsAg and anti-HBs was detected in 32 out of 89 Dutch chronic HBV carriers of Caucasian race. The subtype frequencies and the distribution of risk factors for acquiring a HBV infection were compared in the chronic

HBV carriers and 38 patients with acute hepatitis B who recovered. In the chronic HBV carriers, HBsAg was subtyped ad in 28 and ay in four cases. Anti-HBs could be subtyped in 25 cases using reference antigens discriminating between d, y, and w1-w4 determinants. In 20 out of these 25 patients HBsAg subtype ad (HBsAg/ad) was accompanied by antibody to determinant y (anti-y), whereas HBsAg/ay and anti-d were simultaneously detected in the serum of one patient. The antibody pattern in sera from the remaining patients was complex. In the acute hepatitis B group, HBsAg was subtyped ay in 25 and ad in 13 cases. Thus, in our patients a predominance of ay was found in acute hepatitis with recovery and ad in chronic HBV carriers. This difference is partly caused by the prevalence of ay in drug-users and ad in homosexuals. Non-drug users appeared to have an equal chance to acquire HBsAg/ad or HBsAg/ay acute hepatitis.

Eighteen anti-HBs positive chronic HBV carriers were matched for age, histology, and HBeAg status with 18 anti-HBs negative chronic HBV carriers. No differences in risk factors for acquiring a hepatitis B infection were found. These results do not support the hypothesis that co-occurrence of HBsAg and anti-HBs is due to two consecutive infections with hepatitis B virus. The frequency of the co-occurrence of HBsAg and anti-HBs was found to be related to the degree of progressive liver disease, since anti-HBs was found in three out of 23 asymptomatic HBV carriers, in four out of 20 chronic persistent hepatitis B patients, in 20 out of 41 chronic active hepatitis B patients, and in all five patients with chronic active hepatitis B and cirrhosis. The high frequency of anti-HBs in patients with advanced liver disease may be the result of a disturbed immunologic response mechanism.

In chapter V the investigations on peripheral T-cell subsets in the various clinical courses after HBV infection are discussed.

The course of disease after a HBV infection is probably determined by the cellular immune response after the host, which is partly regulated by the T helper and T suppressor cells. Immunoregulatory T-cell subsets were counted in the peripheral blood of 97 patients with various courses after HBV infection: 23 of these patients were asymptomatic HBsAg carriers without detectable liver disease, 13 had chronic persistent hepatitis B, 19 had chronic active hepatitis B (11 HBeAg, 8 anti-HBe), 7 had chronic active hepatitis with anti-HBs, and 35 were healthy controls with anti-HBs after recovery from acute hepatitis B. Peripheral blood mononuclear cells were specifically labeled with monoclonal Leu-1 (T-cells), Leu-2α (T suppressor/cytotoxic cells), and Leu-3α (T helper cells) antibodies and analyzed by flow cytometry.

Leu-3α/Leu-2α ratios for patients with persistence of the virus infection did not differ from those found for patients who cleared the HBV antigens. These findings suggest that elimination of HBV as such is unlikely to be related to the relative number of peripheral T-cell subsets. However, evidence was found that the number of T suppressor cells had decreased in the subgroup of patients with ongoing chronic active hepatitis and anti-HBe. This subgroup of patients who develop chronic active hepatitis after partial clearance of the virus probably have an enhanced immunoreactivity compared with those running the commoner courses of this disease.

Chapter VI concerns the genetic basis which may predispose to a particular course of disease after HBV infection.

In order to find a possible relationship between the course of disease and the phenotype frequency of HLA determinants, 396 Dutch subjects of Caucasian race were studied. Six groups of individuals with various courses after HBV infection were compared to healthy controls. The hepatitis B patients were grouped according to standard criteria: 1) 47 had recovered from acute symptomatic hepatitis B, 2) 60 had recovered from asymptomatic hepatitis B, 3) 26 were asymptomatic HBV carriers, 4) 16 had chronic persistent hepatitis B, 5) 37 had chronic active hepatitis B and 6) 10 had chronic active hepatitis after elimination of hepatitis B antigens. 29 Class I and 13 Class II HLA-antigens were assayed by standard microlymphocytotoxicity tests.

The phenotype frequency of the Class II antigen DQw1 appeared to be significantly lower in patients with chronic active hepatitis B. Other HLA specificities showing deviations from control values were not statistically significant after correction for the number of antigens tested. In conclusion, no evidence was found that the elimination of HBV is related to HLA phenotype. However, HLA DQw1 may affect the morphologic type of chronic hepatitis B, since its presence may protect against chronic active hepatitis.

A general description and discussion of the study is given in chapter VII. The starting-points, patient selection and methods, as well as the studies performed are discussed in view of the aims of the study.

Conclusions

The main results of the study indicate that different immunologic mechanisms are involved in the clearance of the HBV and the development of liver cell necrosis at different time-points in the various courses after HBV infection. The conclusions can be grouped with respect to HBV elimination and liver cell degeneration in acute and chronic hepatitis B.

The HBV clearance in early acute hepatitis B seems to precede maximal liver cell necrosis by at least two weeks in cases of recovery. No signs of HBV clearance have become apparent during early acute hepatitis B in cases that eventually became chronic HBV carriers, indicating that the course of disease is set before clinical symptoms became apparent. HBsAg subtype ad may be slightly related to deficient HBV clearance in the group of Dutch patients studied.

Liver cell damage in early acute hepatitis B shows no correlation in time or extent with HBV clearance, supporting the hypothesis that viral clearance may (partially) be achieved by suppression of virus production without destroying liver cells. Non-specific anti-HBs was found to occur more frequently in patients with advanced stages of HBV-related liver disease and seems to be closer correlated to liver damage than to HBV clearance. It is interpreted to be a sign of a qualitative defect in the immune response to HBV infection. The decreased number of T suppressor cells found in patients with ongoing chronic active hepatitis B after HBeAg seroconversion may be an indication of enhanced immuno-reactivity in these patients. The low phenotype frequency of HLA DQw1 in the patient group with chronic

active hepatitis B shows that genetic factors are possibly involved in the regulation of hepatitis activity.

The findings reported in this thesis have possible implications for the treatment of hepatitis B. Antiviral drugs may be more effective if given early in the course of disease. Repeated quantitative assays of HBsAg and HBeAg may be useful in the identification of patients with a potentially chronic course. A subset of chronic hepatitis B patients, without HBeAg, have some hallmarks of "autoimmune" hepatitis, and these patients may possibly benefit from immunosuppression.

Hoofdstuk VIII

SAMENVATTING EN CONCLUSIES

Doel van het onderzoek

In hoofdstuk I is het doel van dit onderzoek als volgt geformuleerd: "het vinden van correlaties tussen 1) de verschillende beloopsvormen van hepatitis B en 2) kwantitatieve parameters betreffende virale replicatie en levercelverval, subtypen van het hepatitis B virus en de immunologische respons, ten einde beter inzicht te verkrijgen in het tijdstip waarop en het mechanisme waardoor chronische hepatitis B ontstaat".

Aanleiding

De belangrijkste aanleiding voor dit onderzoek is het ontbreken van een blijvend werkzame behandeling van chronische hepatitis B. De theoretische basis voor een dergelijke therapie is nog incompleet. De reden waarom hepatitis B bij bepaalde patienten chronisch wordt is niet bekend, evenmin als het tijdstip waarop dit gebeurt.

In dit onderzoek komen telkens de verschillende beloopsvormen ter sprake die zich kunnen voordoen na besmetting met het hepatitis B virus (HBV). Acute hepatitis B geneest meestal, en hierbij wordt het virus opgeruimd. Indien het virus niet volledig wordt geelimineerd dan ontstaat chronisch hepatitis B dragerschap. De mate van hepatitis kan zowel bij de acute hepatitis B als bij chronisch dragerschap aanzienlijk varieren, zodat er geen duidelijk verband lijkt te bestaan tussen de mate van levercelbeschadiging en eliminatie van het virus. Mogelijk zijn daarom verschillende (immunologische) mechanismen verantwoordelijk voor levercelbeschadiging en viruseliminatie.

Opzet van het onderzoek

Dit onderzoek werd zo opgezet dat aspecten betreffende het tijdstip waarop en het mechanisme waardoor chronische hepatitis B ontstaat konden worden onderzocht. Hierbij werden de volgende vragen geformuleerd: op welk tijdstip wordt gedurende de acute fase van de ziekte het beloop in chronische richting gestuurd?; geven vroege veranderingen in virale replicatie, virale eliminatie of levercel beschadiging gedurende de acute fase aanwijzingen voor een al dan niet chronisch beloop ?; hebben virale subtypen of antistoffen tegen subtypen een correlatie met de ontwikkeling van een bepaald ziektebeloop ?; is er verband tussen het voorkomen van bepaalde soorten T-cellen in het bloed en de verschillende beloopsvormen van hepatitis B ?; is er een genetische basis die voert tot voldoende of onvoldoende opruiming van het virus, het laatste in combinatie met tolerantie van het virus bij chronisch HBV-dragerschap zonder hepatitis, of met intolerantie van het virus bij chronische hepatitis B.

Patienten

De patienten-groepen werden als volgt samengesteld: patienten met acute hepatitis B, personen die genazen na acute icterische hepatitis B, personen die genazen na acute anicterische hepatitis B, chronische HBV dragers zonder hepatitis,

chonische HBV dragers met chronisch persisterende hepatitis, chronische HBV dragers met chronisch actieve hepatitis en patienten die het HBV hebben opgeruimd maar chronisch actieve auto-immuun hepatitis hebben ontwikkeld.

Hepatitis B, een overzicht

In hoofdstuk II wordt een overzicht gegeven van de literatuur betreffende hepatitis B. De nadruk wordt gelegd op onderwerpen die betrekking hebben op het doel van dit onderzoek en de gebruikte methoden.

Het HBV is een DNA-virus. Het vermenigvuldigt zich voornamelijk in menselijke levercellen. Het manteleiwit bevat het hepatitis B oppervlakte (surface) antigeen (HBsAg). In de viruskern (core) bevindt zich het DNA, het hepatitis B core antigeen (HBcAg) en het hepatitis B e antigeen (HBeAg) (zie fig. 2.1). De belangrijkste HBsAg subtypen zijn ad en ay. Zij hebben determinant a gemeenschappelijk. Het kleine DNA-molecuul is circulair en bestaat gedeeltelijk uit twee strengen. Met behulp van DNA-polymerase wordt de incomplete streng verder opgebouwd. De complete streng bevat de genetische code voor HBsAg, DNA-polymerase en polypeptiden die HBcAg en HBeAg bevatten (zie fig. 2.2). Het HBV-DNA kan in de levercellen zowel in vrije vorm als geintegreerd in het gastheer-genoom voorkomen. In de vrije vorm kan het virus zich repliceren. Geintegreerd HBV-DNA komt voornamelijk voor in levercellen van HBeAg-negatieve HBsAg-positieve chronische HBV-dragers, nadat het virus (-genoom) gedeeltelijk is opgeruimd, en in cellen van hepatocellulair carcinoom.

Het aantonen van bestanddelen van het HBV in serum en lever kan worden gebruikt ter indicatie van de fase waarin het beloop van de hepatitis B zich bevindt. HBsAg en HBeAg in serum worden, gewoonlijk kwalitatief en bij uitzondering kwantitatief, bepaald met radio-immuno-assays of enzyme-immuno-assays. HBsAg, HBcAg en HBeAg worden in leverweefsel aangetoond met immunofluorescentie-en immunoperoxydase-technieken. DNA-polymerase-activiteit wordt gemeten met behulp van incorporatie van radio-actief thymidine. HBV-DNA kan in serum en lever worden aangetoond met behulp van moleculaire hybridisatie. Zowel DNA-polymerase-activiteit als aantoonbaar HBV-DNA zijn een uiting van actieve virale replicatie.

In het natuurlijke beloop van hepatitis B kan de mate van hepatitis zowel bij de acute hepatitis B als bij chronisch dragerschap aanzienlijk varieren, zodat er geen eenduidig verband lijkt te bestaan tussen de mate van levercelbeschadiging en eliminatie van het virus. Het natuurlijke beloop van chronisch actieve hepatitis B na verdwijnen van HBeAg, meestal gepaard gaande met vermindering van de mate van hepatitis, vertoont echter een omgekeerd verband tussen (gedeeltelijke) eliminatie van het virus en de levercelbeschadiging. In dit geval speelt actieve virale replicatie mogelijk een causale rol in de pathogenese van persisterende levercelbeschadiging.

Therapeutische interventie van chronische hepatitis B middels immunosuppressiva of immunostimulantia is niet effectief gebleken. Met antivirale therapie kan tijdelijke suppressie van de virale replicatie worden verkregen.

De mechanismen van levercelbeschadiging en viruseliminatie zijn waarschijnlijk niet identiek. Bij levercelbeschadiging speelt de cellulaire immuniteit de belangrijkste

rol. Humorale factoren zijn hierbij niet van invloed gebleken. Het cellulaire immuunsysteem kan levercelnecrose veroorzaken door middel van T-cel cytotoxiciteit. Hiervoor is gelijktijdige herkenning van virale en HLA-antigenen op het levercelopperylak noodzakelijk. Volgens de klassieke theorie is chronische hepatitis B het gevolg van onvoldoende virale eliminatie en voortgaande expressie van virale antigenen op het levercelopperylak. Andere theorieën met betrekking tot de ontwikkeling van chronische hepatitis B benadrukken de rol van veranderingen in de levercelmembraan of van stoffen die het immuunsysteem beïnvloeden. Onderzoekingen betreffende de activiteit van het cellulaire immuunsysteem hebben vooralsnog geen homogeen resultaat opgeleverd.

In het mechanisme van viruseliminatie zijn waarschijnlijk zowel de cellulaire als de humorale immuniteit van belang. Antilichamen hebben een belangrijke functie bij de opruiming van het virus kort na infectie. Bij andere virussen is gebleken dat antistoffen de productie van virale eiwitten kunnen remmen. Een hypothese stelt dat dit mechanisme ook voor het HBV kan gelden. Volgens deze hypothese behoeven geïnfecteerde levercellen niet te worden vernietigd om het HBV te elimineren. Dit is in tegenstelling met de meest gangbare hypothese, waarin HBV eliminatie het gevolg is van destructie van alle geïnfecteerde levercellen.

Het verrichte onderzoek

In hoofdstuk III wordt het onderzoek beschreven naar het tijdstip waarop het ziektebeloop tijdens de acute fase van hepatitis B in chronische richting wordt gestuurd en naar vroege veranderingen in virale replicatie, virale eliminatie of levercel beschadiging die gedurende de acute fase aanwijzingen voor een al dan niet chronisch beloop kunnen vormen.

Bij 63 patiënten met acute hepatitis B werd het verdwijnen van virusantigenen in verhouding tot de mate van levercelbeschadiging bestudeerd. HBV-DNA, DNA-polymerase-activiteit, HBeAg en HBsAg werden kwantitatief in het serum bepaald. De virale antigenen werden in leverbiopsieën aangetoond met immunofluorescentie. Alle meetresultaten werden in de tijd geplaatst ten opzichte van de dag waarop het alanine-aminotransferase (ALT) maximaal was, als teken van maximale levercelbeschadiging. Zes maanden na het begin van de ziekte waren 53 patiënten genezen en 10 waren chronische HBsAg-dragers geworden. In de vroege ziekteperiode werden bij deze twee groepen patiënten geen verschillen in virusantigenen waargenomen. Vanaf twee weken voorafgaande aan de maximale ALT daalden de HBsAg- en de HBeAg-spiegels significant bij die patiënten die genazen. Het verdwijnen van HBeAg en HBsAg correleerde niet met de mate van levercelbeschadiging. HBV-DNA, DNA-polymerase en virale antigenen in de leverbiopsieën waren voornamelijk aantoonbaar in de periode voor en tijdens de maximale ALT. De virusantigenen bleven constant aanwezig in serum en lever van patiënten die chronisch drager werden.

Deze resultaten vormen een aanwijzing dat tijdens de acute fase van hepatitis B het tijdstip waarop het uiteindelijke ziektebeloop wordt bepaald minstens twee weken voorafgaat aan dat van het maximale levercelverval. Kwantitatieve bepaling van HBsAg of HBeAg kan een vroege indicator zijn van de prognose. De bevinding dat virale kenmerken onafhankelijk van de mate van levercelbeschadiging

kunnen verdwijnen suggereert dat, naast destructie van geïnfecteerde levercellen, suppressie van de synthese van HBV-bestanddelen een deel van het mechanisme van HBV-eliminatie vormt.

In hoofdstuk IV wordt de relatie besproken tussen subtypen of antistoffen tegen subtypen van het HBV en de ontwikkeling van de klinische beloopsvormen, alsmede enige aspecten van de humorale immuniteit.

In een groep van 89 chronische HBV-dragers van Nederlandse afkomst en behorend tot het Kaukasische ras werd bij 32 een gelijktijdig voorkomen van HBsAg en anti-HBs vastgesteld. Bij deze HBV-dragers en bij 38 patiënten met een genezende acute hepatitis B werden het voorkomen van HBV-subtypen en de verdeling van risicofactoren om een HBV-infectie op te lopen vergeleken. Van de 32 chronische HBV-dragers met anti-HBs hadden 28 HBsAg-subtype ad (HBsAg/ad) en 4 subtype ay (HBsAg/ay).Het subtype van anti-HBs kon bij 25 patiënten worden vastgesteld. Bij 20 van hen kwam HBsAg/ad in combinatie voor met anti-HBs subtype anti-y. HBsAg/ay en anti-HBs subtype anti-d kwamen bij een patiënt gelijktijdig voor. Bij de overige 4 patiënten bestond er een complex patroon. In de groep met acute hepatitis B hadden 25 patiënten HBsAg/ay en 13 HBsAg/ad. In onze patiëntengroepen kwam dus HBsAg/ay vaker voor bij genezende acute hepatitis B en HBsAg/ad bij chronische HBV-dragers. Dit verschil werd gedeeltelijk veroorzaakt door het voorkomen van HBsAg/ay bij drug-spuiters en HBsAg/ad bij homoseksuelen. Personen die geen drugs gebruikten hadden een gelijke kans op infectie met HBsAg/ad of HBsAg/ay.

Achttien chronische HBV-dragers met anti-HBs werden gematched betreffende leeftijd, histologische leverafwijkingen en HBeAg-status met 18 dragers zonder anti-HBs. In deze groepen werd geen verschillend risico op besmetting met HBV gevonden. Dit geeft geen steun aan de hypothese dat gelijktijdig voorkomen van HBsAg en anti-HBs het gevolg is van twee opeenvolgende infecties met verschillende HBV-subtypen.

De frekwentie van gelijktijdig HBsAg en anti-HBs correleerde met de ernst van de leverziekte: anti-HBs werd gevonden bij 3 van de 23 asymptomatische HBV-dragers, bij 4 van de 20 patiënten met chronisch persisterende hepatitis B, bij 20 van de 41 patiënten met chronisch actieve hepatitis B en bij alle 5 patiënten met chronisch actieve hepatitis B en levercirrose. De relatief hoge frekwentie van anti-HBs bij patiënten met een ernstiger vorm van chronische hepatitis B is mogelijk het gevolg van een stoornis in de immunologische respons.

In hoofdstuk V wordt het onderzoek beschreven betreffende subpopulaties van T-lymfocyten bij verschillende beloopsvormen van hepatitis B.

De cellulaire immuunrespons van de gastheer wordt deels gereguleerd door de T-helper en T-suppressor subpopulaties. Bij 97 patiënten met verschillend beloop na HBV-infectie werden de perifere T-cel-subpopulaties bepaald: 23 asymptomatische HBV-dragers zonder leverziekte, 13 patiënten met chronisch persisterende hepatitis B, 19 patiënten met chronisch actieve hepatitis B (11 met HBeAg, 8 met anti-HBe), 7 patiënten met chronisch actieve hepatitis en anti-HBs, en 35 personen met anti-HBs na genezing van acute hepatitis B. De perifere mononucleaire cellen werden specifiek gemerkt met monoclonale Leu-1 (T-cellen), Leu-2a (T-suppressor

en -cytotoxische cellen), en Leu-3α (T-helper cellen) antilichamen en geteld met flow-cytometrie.

De Leu-3α/Leu-2α ratio's van chronische HBV-dragers verschilden niet van de ratio's van personen die het HBV hadden opgeruimd. Bij de patienten met voortgaande chronisch actieve hepatitis ondanks negatief HBeAg en positief anti-HBe werd een verlaagd aantal T-suppressor cellen gevonden.

Deze bevindingen duiden er op dat de HBV-eliminatie niet is gerelateerd aan de kwantitatieve verhouding van de onderzochte T-cel-subpopulaties in het perifere bloed. Gezien het lage aantal T-suppressor cellen hebben patienten met voortgaande chronisch actieve hepatitis na gedeeltelijke opruiming van het virus mogelijk een toegenomen immuno-reactiviteit ten opzichte van de patienten die een meer algemeen ziektebeloop hebben.

Hoofdstuk VI betreft de genetische basis die mogelijk het ziektebeloop na HBV-infectie bepaalt.

Ten einde een correlatie te vinden tussen het ziektebeloop en de fenotype-frequentie van HLA-determinanten werden 396 Nederlanders onderzocht. Allen behoorden tot het Noord-Europese locale Kaukasische ras. Zes groepen personen met verschillend ziektebeloop na HBV-infectie werden vergeleken met gezonden. De hepatitis B patienten werden als volgt gegroepeerd: 1) 47 waren genezen van acute icterische hepatitis B, 2) 60 waren genezen van anicterische hepatitis B, 3) 26 waren asymptomatische HBsAg-dragers, 4) 16 hadden chronisch persisterende hepatitis B, 5) 37 hadden chronisch actieve hepatitis B en 6) 10 hadden chronisch actieve hepatitis na eliminatie van de HBV-antigenen. 29 klasse I en 13 klasse II HLA-antigenen werden bepaald met behulp van micro-lymfocytotoxiciteits-testen.

De fenotype-frequentie van het klasse II-antigeen DQw1 was significant verlaagd bij patienten met chronisch actieve hepatitis B. Geen andere HLA-typen waren statistisch significant afwijkend van de controlegroep. Er werd geen aanwijzing gevonden dat de eliminatie van HBV is gerelateerd aan een HLA-fenotype. HLA-DQw1 beinvloedt mogelijk de mate van levercelbeschadiging aangezien het lijkt te beschermen tegen de ontwikkeling van chronisch actieve hepatitis.

Een algemene beschrijving van het onderzoek en een bespreking van de resultaten wordt gepresenteerd in hoofdstuk VII. De uitgangspunten, de patientenselectie, de methoden en het verrichte onderzoek worden besproken in relatie tot het doel van het onderzoek.

Conclusies

De resultaten van dit onderzoek wijzen uit dat bij de eliminatie van het HBV en de ontwikkeling van levercelnecrose verschillende immunologische mechanismen zijn betrokken op verschillende tijdstippen in het beloop van hepatitis B. De volgende conclusies worden ingedeeld ten aanzien van HBV-eliminatie en levercelbeschadiging bij acute en bij chronische hepatitis B.

Eliminatie van het HBV lijkt in de vroege fase van acute hepatitis B die geneest minstens twee weken vooraf te gaan aan het tijdstip waarop de levercel-necrose

maximaal is. Bij acute hepatitis B die overgaat in chronisch HBV-dragerschap treden in de vroege fase geen tekenen op van HBV-eliminatie. Deze bevindingen vormen een aanwijzing dat het ziektebeloop bij acute hepatitis B reeds is vastgelegd voordat de klinische verschijnselen optreden. Bij de onderzochte Nederlandse patienten is HBsAg-subtype ad mogelijk in lichte mate gerelateerd aan deficiente HBV-eliminatie.

De levercelbeschadiging tijdens de vroege fase van acute hepatitis B vertoont noch in de tijd, noch wat betreft hevigheid een correlatie met de mate van HBV-eliminatie. Dit steunt de hypothese dat virale eliminatie (gedeeltelijk) het gevolg kan zijn van suppressie van de virusproductie zonder dat hiervoor levercellen worden vernietigd. Niet-specifiek anti-HBs werd relatief vaker gevonden bij patienten met een ernstiger beloop van chronische hepatitis B en lijkt beter te correleren met de mate van leverbeschadiging dan met HBV-eliminatie. Dit verschijnsel werd geinterpreteerd als teken van een kwalitatief defect van de immuunrespons op de HBV-infectie. Het lage aantal T-suppressor cellen dat werd gevonden bij patienten met voortgaande chronisch actieve hepatitis B na verdwijnen van HBeAg kan duiden op een verhoogde immuunreactiviteit bij deze patienten. De lage fenotypefrequentie van HLA-DQw1 binnen de patientengroep met chronisch actieve hepatitis B laat zien dat genetische factoren mogelijk zijn betrokken bij de regulatie van de ernst van levercelbeschadiging.

De bevindingen van het onderzoek dat in dit proefschrift wordt beschreven kunnen theoretisch implicaties hebben voor de behandeling van hepatitis B. Antivirale middelen zouden een beter effect kunnen hebben indien zij in een vroege fase van hepatitis B worden toegediend. Herhaalde kwantitatieve bepaling van HBsAg of HBeAg kan van nut zijn bij de vroege identificatie van patienten met een potentieel chronisch beloop. De subgroep van patienten met chronisch actieve hepatitis B na verdwijnen van HBeAg heeft enige kenmerken van "auto-immuun" hepatitis en kan mogelijk zijn gebaat bij behandeling met immunosuppressiva.

WOORD VAN DANK

Mijn dank gaat uit naar allen die op enigerlei wijze hebben medegewerkt aan het tot stand komen van dit proefschrift. De opzet en uitvoering van het gehele onderzoek werd begeleid door dr. R.A. Heijtink en dr. S.W. Schalm, die ik daarvoor met nadruk dank. Ook mijn promotores, prof. J.H.P. Wilson en prof.dr. G.C. de Gast, dank ik voor hun kritische begeleiding. Mijn familie en naaste collega's komt eer toe voor hun stimulerende tolerantie.

CURRICULUM VITAE

De schrijver van dit proefschrift werd geboren op 24 juli 1946 te Rijswijk. Na het behalen van het getuigschrift Gymnasium β aan het Christelijk Lyceum te Delft, studeerde hij geneeskunde aan de Rijksuniversiteit te Leiden, alwaar in 1973 het artsexamen werd afgelegd. De militaire dienstplicht werd vervuld als reserve-eerste luitenant arts aan de afdeling Medisch Wetenschappelijk Beleid van de Inspectie Geneeskundige Dienst Koninklijke Landmacht te 's-Gravenhage. In 1974 werd de opleiding voor het specialisme Inwendige Geneeskunde aangevangen in het Westeinde Ziekenhuis te 's-Gravenhage (opleiders A. Alleman, Dr. E. van Leer), in 1979 gevolgd door inschrijving als internist in het Specialistenregister. Aansluitend was hij werkzaam als hoofdgeneeskundige bij de afdeling Inwendige Geneeskunde II van het Academisch Ziekenhuis Rotterdam Dijkzigt (hoofd: Prof. Dr. M. Frenkel), alwaar met de bewerking van dit proefschrift werd begonnen. Sedert 1981 is hij verbonden aan de Vakgroep Gastroenterologie van de Rijksuniversiteit te Utrecht (hoofd: Prof. Dr. O.J. ten Thije), aanvankelijk als wetenschappelijk hoofdmedewerker, per 1986 als universitair hoofddocent. Hij volgde de opleiding voor het specialisme Gastroenterologie aan de twee laatstgenoemde instellingen en was in 1983 als gastroenteroloog registreerbaar; in 1985 werd hij als gastroenteroloog in het Specialistenregister ingeschreven.