Special Communication

AUSTRALIA ANTIGEN: THE ANTIGEN OF HEPATITIS TYPE B

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History

Identification of an antigen that is intimately related to the causative agent of viral hepatitis has given impetus to hepatitis research. Blumberg and associates (1965) found this antigen incidentally while working on precipitins of beta-lipoprotein allotypes. The precipitin was in the serum of a patient with hemophilia who had received multiple transfusions, and the antigen happened to be in the serum of an Australian aborigine. The precipitin line that formed in Ouchterlony double diffusion differed in specificity from known beta-lipoproteins and the antigen was called Australia Antigen or Au(1). The antigen was considered to be inherited as recessive trait based on statistical analysis of its occurrence in large tropical population (Blumberg et al., 1966). A relationship was gradually recognized between Australia antigen and leukemia, Down's syndrome, lepromatous leprosy and hepatitis, and the possibility was considered that the Au(1) trait might be associated with susceptibility to viruses (Blumberg et al., 1967a; Blumberg et al., 1967b). Ockochi and Murakami (1968) confirmed the association between Au(1) and hepatitis and demonstrated the appearance of the antigen during the incubation period and acute phase of classic post-transfusion viral hepatitis and the antigen became known as hepatitis associated antigen (HAA). Prince (1968) reported similar observations on patients with serum hepatitis and called the antigen serum hepatitis (S.H.) antigen. Examining the immune precipitate of Au(1) by electron microscopy Bayer and associates (1968) demonstrated aggregates of 20 nm particles, and shortly thereafter the close relationship between Australia antigen, the occurrence of viral hepatitis and the presence of virus like particles in antigenic serum was confirmed by Prince (1968), Hirschman and associates (1969), Barker and colleagues (1969), Zuckerman (1969) and Gerin and co-workers (1969).

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Nomenclature

Although several different names have been applied to the antigen, only a single well documented specificity exists and many workers have adopted the more general term, hepatitis associated antigen (HAA), rather than the genetic nomenclature, Australia antigen of Blumberg, or the more circumscript SH antigen of Prince.

Recently there have been attempts to standardize the nomenclature of the antigenic material, and of the hepatitis with which it can be associated. Designation of the serum antigen as hepatitis-B antigen (HB Ag) is receiving increasing use, notwithstanding controversy which still continues as to whether the agent is a typical virus or not. The use of the term implies that the presence of HB Ag in asymptomatic subject reflects past exposure to the agent which can cause hepatitis type B. It implies that the subject may be infectious but does note necessarily connote infection at the time of testing. It seems unlikely that the term Australia antigen will ever completely disappear but it also seems more correct to use the informative, if more mundane term hepatitis antigen. With these considerations in mind, and to conform with what is likely to be increasing usage, the term HB Ag will be used here synonymously with, and in place of Australia antigen.

Incidence

Hepatitis B antigen is extremely rare in normal Americans, but is found in high frequency in serum samples of patients with viral hepatitis (Blumberg et al., 1967b, Blumberg et al., 1968; London et al., 1969). It is found both in patients with transfusion and infectious hepatitis, but in higher frequency in the former. The connection between hepatitis B antigen (HB Ag) and acute viral hepatitis is well established (Blumberg et al., 1970a; Elling et al., 1970). It is also associated with chronic active hepatitis, persistant viral hepatitis and other forms of hepatitis (Blumberg et al., 1970b; Gerin et al., 1975a).

Hepatitis B antigen usually appears during the prodrome of the disease and disappears as symptoms and signs abate (Prince, 1968; Purcell et al., 1971) although a few patients remain chronically antigenemic after clinical recovery (Singleton et al., 1971). Some of the individuals who are repeatedly exposed to the antigen may develop antibodies to the antigen (Ockochi et al., 1970; Gocke et al., 1970).

The antigen is also found in relatively high frequency in a group of chronic diseases characterized by a prolonged and usually severe impairment of the immunity mechanism. Those with this group of diseases include patients with Down's syndrome (Blumberg et al., 1967b; Blumberg, 1966), patients with chronic renal disease who are undergoing hemodialysis (London et al., 1968; London et al., 1969). Patients with leukemia, i.e., acute and chronic lymphatic leukemia (Blumberg et al., 1965; Blumberg et al., 1967b) and patients with Lepromatous leprosy (Blumberg et al., 1967a). In two of these diseases (Down's syndrome and chronic renal disease) it has been shown that the presence of the antigen signals the presence of chronic anicteric hepatitis often of a long duration and sometimes without other laboratory evidence of chronic hepatitis (Sutnick et al., 1968; London et al., 1968; London et al., 1969).

Hepatitis B antigen can be transmitted by transfusion of blood, injection needles, mosquitoes, through placenta, oral-fecal route, and sexual contact. Acute post-transfusion hepatitis may develop in patients receiving the antigen-contaminated blood and the antigen can be detected in their blood. However, hepatitis does not develop in some patients receiving the blood but antibodies against hepatitis B antigen do develop (Ockochi and Murakami, 1968).

The antigen has been isolated from blood (Kattamis et al., 1974) and other body fluids e.g., urine (Tripatzis and Horst, 1971), saliva (Ward et al., 1972), semen (Heathcote et al., 1974) and bile (Akdamar et al., 1971). Bayer and associates (1968) have shown under the electron microscope that virus like particles are seen in the nuclei of liver cells from patients with acute and chronic hepatitis.

In addition to these disease associations the antigen is common in healthy normal people living in portions of the tropics, particularly South East Asia and Oceania (i.e., Ghana 10%, Philippine 4%, Vietnam 6%, Bongainville 4% to 20%, South India 3%, etc.) despite its great rarity in the normal United States population (Blumberg et al., 1965; and Blumberg et al., 1968). In two tropical areas (India and Philippines) the antigen is present in higher frequency in patients with lepromatous leprosy than in patients with tuberculoid leprosy or in non-leprosy controls from the same areas (Blumberg et al., 1967a). In Vellore, India, the highest prevalence was found among professional donors (Hill et al., 1973), whereas in Delhi, India, antigenemia was found frequently in males and more in younger age groups (Sama et al., 1973). In a study of 1,111 professional donors from the Blood Bank of Jinnah Postgraduate Medical Centre, Karachi, Zuberi and Lodhi (1974) found that thirty eight (3.42%) were positive for HB Ag and that poor personal hygiene and perhaps an abnormal immunological response to hepatitis virus infection were to be more significant factors in acquiring and persistence of the antigen.

In most cases of acute viral hepatitis the HB antigen disappears four to five weeks after the onset of symptoms. Persistence of the antigen for 13 weeks in the serum of a patient has also been described (Elling et al., 1970).

Hepatitis B antigen can be detected with the use of anti-serum samples from individuals who have received large number of transfusions. A number of tests have been developed to test this antigen in the serum, urine, feces, saliva, semen and bile. They are:

1. Agar gel diffusion (Gust and Kaldor, 1971).
2. Counterimmuno-electrophoresis in gel (Cocke and Howe, 1970).
3. Cellulose acetate immuno-electrophoresis (Saravis et al., 1970).
4. Complement fixation (Purcell 1970).
5. Haemagglutination (Vyas and Shulman, 1970).
6. Electron microscopy (Bayer et al., 1968).
7. Radio-immunoassay (Walsh et al., 1970).
8. Immunofluorescence (Millman 1969).
9. Latex fixation (Lehman and Gust, 1973).
10. Passive haemagglutination (Taylor et al., 1974).
11. Sandwich solid phase radio-immunoassay (Sama et al., 1973).

Physical and Chemical Properties

All information to date on HB Ag suggests that it is chiefly the protein coat or capsid material of a small virus. There appears to be some associated infectious virion, but it is too sparse to characterize (Shulman 1970).

A. Protein Like Characteristics:

Alter and Blumberg (1966) found that HB Ag was a macromolecule which appeared in the first peak of serum filtrates on sephadex

200 gel, travelled as an alpha globulin in gel electrophoresis, appeared to contain a small amount of lipid on the basis of a weak reaction with Sudan black, and differed from other lipoproteins in its immuno reactions and density. Hepatitis B antigen was more dense than beta lipoproteins. The antigen was considered to be possibly an altered, partially delipidized lipoprotein, until Bayer and associates (1968) demonstrated its virus like nature, revealed by electron microscopy of precipitates of HB Ag formed with specific anti-serum. These precipitates contained conglumerates of particles approximately 20 nm in diameter with some elongated structures of the same diameter varying in length upto 23 nm. Within the next one year Hirschman and associates (1969), Barker and associates (1969), Zuckerman (1969) and Gerin and colleagues (1969) reported the presence of the same virus like particles in serum that were positive for hepatitis B antigen by immunologic tests.

B. Virus Like Characteristics:

The hypothesis that hepatitis B antigen is a hepatitis virus had been supported by a variety of findings including transmission to humans and animals (Ockóchi and Murakami, 1968; Cocke et al., 1970) by its virus like appearance (Bayer et al., 1968), identification in the nuclei of affected individuals (Millman et al., 1969) and in other ways. Extensive evidence links the HB antigen with the agent of viral hepatitis type B (Prince 1968). Hepatitis B antigen is felt to represent the viral coat protein of the hepatitis B virus (Barker et al., 1973a). Hepatitis B antigen is a particle with morphological characteristics of a virus (Blumberg et al., 1969).

Hepatitis B antigen, reported as hepatitis B surface antigen, i.e., HBsAg (Gerin et al., 1975a), circulates in the sera of chronic carriers as a variety of spherical and filamentous particles which are grouped into three categories based on their morphologic forms: (a) the approximately 22 nm spherical particles, (b) filaments of various lengths, and (c) the 43 nm sperical particles also known as Dane particles. Because of their size, Bond and Hall (1972) could separate the filamentous and large spherical forms from the more uniform spherical forms by differential rate sedimentation in cesium chloride gradients. Characterization of the filamentous forms revealed no difference between them and the 22 nm particle with regard to antigenic composition, ultraviolet absorption spectrum or number and size of constituent substance (Gerin cited by Gerin et al., 1975b). All three forms of HBsAg share common antigens on their surfaces while the core of the Dane particle (HBcAg) represents a distinct antigenic system (Almeida et al., 1971; Hoofnagle et al., 1973; Robinson and Greenman, 1974 and Gerin et al., 1975b).

Purified HBsAg is composed of a complex mixture of polypeptides (Chairez et al., 1975). As purified from sera, HBsAg has been shown to consist of at least seven polypeptides, two of which appear to be glycoproteins (Gerin et al., 1975b) and lipids, glycolipids and proteins (Kim and Bissel, 1971; Steiner et al., 1974; Burrell et al., 1973). Additionally, variable amounts of normal serum protein may be associated with the particles as purified from serum.

The Dane particles, first described by Dane and associates (1970) is a 43 nm particle which shares hepatitis B surface antigen determinants with the more common spherical and filamentous forms in the serum. It is defined as a 27 nm electron-dense core surrounded by an outer shell consisting of HBsAg (Gerin et al., 1975b).

There is a growing body of circumstantial but convincing evidence which implicates the Dane particle as the virus of type B hepatitis; such evidence, for example, as the association of anti-HBc with infection (Hoofnagle et al., 1973), and the direct isolation of a circular double stranded DNA from cores. It is also suggested that the 22 nm particles and long forms of HB antigen are surplus virus-coat material (Gerin et al., 1975b).

HBsAg can be detected free in the serum of patients acutely or chronically infected with the hepatitis B virus-HBV (Blumberg et al., 1968; Prince, 1968). Hepatitis B core antigen on the other hand is not detected free in serum, and on electron microscopy it is associated with the inner, electron dense core component of the Dane particle found in the nuclei of hepatocytes with type B hepatitis (Hoofnagle et al., 1973; Huang et al., 1972; Barker et al., 1973a).

Barker and associates (1969) have shown that organic solvents disrupt tubular form, suggesting that they may consist of spherical particles inside a common lipid containing membrane. They also showed that ether treatment reduces the diameter of the spherical particles by approximately 4 nm, suggesting removal of a 2 nm outer coat. The coat may

contain some lipid, for the density of the particles measured in cesium chloride gradients increase from approximately 1.28 after treatment with organic solvent.

The report of Gerin and colleagues (1969) suggests that treatment with mild detergent such as tween 80 or freezing and thawing, may remove lighter capsid material and increase the density of hepatitis B antigen to the range of 1.35 to 1.4 on cesium chloride gradients.

Jozwiak and associates (1971) and Loeb and associates (1973) have shown that HB antigen contains small amounts (5%) of nucleic acid, probably R.N.A. and Hirschman and associates (1971) found D.N.A. polymerase in concentrated preparation of particulate hepatitis B antigen. Kaplan and associates (1973) and Robinson and Greenman (1974) demonstrated that the core of Dane particle contains D.N.A. polymerase and a circular double stranded D.N.A. which served as the primer-template for the enzyme (Robinson et al., 1974).

HB Ag resembles some arboviruses in size, lipid content and density and resembles some enteroviruses in size and stability. However, Purcell and associates (1970) have shown that HB Ag differs serologically from the known arboviruses and other viruses, for complement fixation reagents representing 300 serotypes of arboviruses and other viruses failed to fix complement with HB Ag or anti-HB Ag antibody.

The particles are very stable. Morphologic characteristics of HB Ag and its antigenicity by immunodiffusion and complement fixation remain unchanged after heating at 60°C for one hour after storage at room temperature for at least six months, or at 20°C for more than twenty years (Hirschman et al., 1969; Barker et al., 1969). HB Ag was found to be unaltered in its reaction with specific antibody after exposure to heat, freezing and thawing, putrifaction, acid, alkali and most catabolic enzymes (Kim and Bissel, 1971).

After treatment with organic solvent minor changes are evident, but antigenicity may actually be increased, perhaps because new sites are uncovered by removal of lipid or associated antibody (Shulman and Barker, 1969).

Sub-Types

Using an immunodiffusion procedure Levene and Blumberg (1969) suggested that hepatitis B antigen (HB Ag) from different individuals may differ in their precipitation reaction. They designated a commonly shared antigenic determinant as 'a' and non-identical antigens as either 'ab' or 'ac'. Kim and Tilles (1971) reported that HB Ag may have one, two or three determinants and that antigens differed in their electrophoretic mobility depending upon which determinants were present. They also named their determinants 'a', 'b' and 'c' without employing identity to those of earlier authors. Later 'd', 'y' and 'x' were used to designate determinants which differ from the 'b' and 'c' of the previous authors. It has been suggested that 'd' and 'y' do not occur together and may reflect differences in the genotype of the virus. The studies of Bancroft and associates (1972) after absorbing the antisera with heterologous antigens indicated that the reference antisera were distinguishing two additional antigenic determinants, designated 'w' and 'r'. Primary data suggest 'w' was more common in United States, and 'r' in Thailand.

Hepatitis B antigen (HB Ag) is known to be heterogenous in its antigenic activity. The first evidence concerning the heterogenicity of hepatitis B antigen was presented by Levene and Blumberg (1969). Reports of Brzosko and colleagues (1973) provide conclusive evidence of the existence, at the cellular level (hepatocytes), of two immunologically different specificities of hepatitis B antigen, the nuclear and the cytoplasmic. Accordingly, antibodies to HB Ag in the sera may be either nuclear or cytoplasmic. Dreemsman and associates (1975) have purified from plasma of anicteric hepatitis patients two antigenically distinct subtypes 'adw'. Biophysical studies of these purified preparations revealed considerable heterogenicity in their overall surface charge, morphology and molecular weights. Particles of hepatitis B antigen are antigenically complex. Analysis of their surface antigens has so far revealed one specificity, designated as 'a' which is common to all antigen particles, and three additional determinants 'd', 'x' and 'y', which are not shared by all particles (LeBouvier, 1971; LeBouvier et al., 1972). Specificity 'x' is present in almost all HB Ag positive sera and likely represent a host component, whereas 'd' and 'y' appear to be mutually exclusive. It has been hypothesized by LeBouvier and colleagues (1972) that the determinants 'd' and 'y' reflect the activity of two distinct genotypes of hepatitis B virion,

permitting the division of antigen into two subtypes: D-HB Ab (determinant ad + y—) and y-HBAg (determinant ay + d—). HB Ag heterogenicity was originally identified as 'spur' of partial identity by agar gel diffusion, when sera with HB Ag of different subtypes in adjacent wells evaluated with antisera containing anti-a and anti-d or anti-a and anti-y antibodies. Subsequently subtyping of HB Ag has also been performed by counter-electrophoresis (Holland et al., 1972) and also recognized by radio-immunoassay (Aach et al., 1973). The age, race, sex and socio-economic status of patients with sporadic hepatitis and subtypes D-HB Ag were similar to individuals with post-transfusion hepatitis, whereas patients with subtype Y-HB Ag had epidemiological features similar to persons with needle stick hepatitis and frequently gave history of close contact with individuals using illicity (Zuckerman et al., 1974). Y-HB Ag has also been reported to occur in healthy carriers as well as hepatitis patients (Skinhoj and Kassab, 1973).

Another determinant 't' reactivity was found by Bouvier and William (1975) which was distinct and often behave in an interesting manner, described as 'cryptic'. It was found with the help of an antiserum from a rabbit which had been immunized with purified 'adw' antigen obtained from a carrier in Thailand. With this antiserum, most 'adw' — positive sera gave spurs over three other 'adw' positive sera from Japan. By graded absorption, an antibody reactant was made that was monospecific for the spur forming component, which was named 't' ——— in honour of Thailand and Tokyo.

Immunopathology

Immunologic methodology has played a large part in elucidating the association of the hepatitis B virus with hepatitis (Giles et al., 1969; Almeida and Waterson, 1969; Edginton and Ritte, 1971; Barker et al., 1973a), arthritis (Albert et al., 1971; Onion et al., 1971), glomerulonephritis (Nowoslawski et al., 1972) and vasculitis (Gocke et al., 1971; Sergent et al., 1976) indicating that immunologic mechanism may play a significant role in the pathogenosis of these disease states. In serial observations made by electron microscopy on the serum of a patient with acute hepatitis B it was found that HB Ag appears at first unaggregated, and then as the disease progresses, it is formed into aggregates by the attachment of antibodies to the surface antigen (anti-HBs). In most cases the antigen is then cleared i.e., complexes become smaller and fewer until they finally disappear altogether (Almeida 1971). Some patients fail to clear the antigen for shorter or longer periods and later become chronic carriers, although only a minority of chronic carriers give history of an acute attack of hepatitis. Discovery of the cycle of events gave rise to the speculation as to whether some at least of symptoms of hepatitis B are mediated by means of immune complexes. Shulman and Barker (1969) have reported that many HB Ag positive sera were anticomplementary, and ascribed the activity of complexes in the serum. Electron microscope studies have shown that it was a relatively simple matter to identify immune complexes in the circulation, and their presence at a particular phase of the acute form of the disease, and never long periods in chronic hepatitis, led to the suggestion that they have pathogenic importance (Almeida and Waterson, 1969). These original observations have now been extended by the work of various groups (Brzosko et al., 1974; Reed et al., 1973b) who have found that immune complexes seem to be of importance in HB Ag positive disease.

In spite of the fact that it is difficult to demonstrate anti-HBs in many individuals known to have experienced HB Ag-positive hepatitis, they nevertheless appear to be effectively immunized for a period at least of some years (Zuckerman cited by Almeida and Waterson, 1975). On the other hand, those who are immunosuppressed, either artificially, e.g., the typically anaemic and uremic haemodialysis patients tend to experience a mild illness with a prolonged course, and in particular, tend to circulate the antigen in the blood for much longer than do initially healthy subjects such as nursing or technical staff (Marmion and Tonkin, 1972). This phenomenon, in immunosuppressed patients in hospital, constitutes a potential danger to medical and nursing staff, since it may produce the dangerous combination of a patient with highly infectious blood and no obvious hepatitis. There has been a report of a patient with leukaemia who became HB Ag-positive and infected several hospital personnel (Wands et al., 1974). Children of school age, and below have more anicteric infections than do adults and the fact that they also become chronic carriers more easily, would appear to be linked with this tendency (Almeida and Waterson, 1975). Certainly, chronic symptomless carriage of HB Ag can arise from contact of a baby with an HB Ag positive mother at birth or in the first few months of life (Schwitzer et al., 1973). HB anti-

gen and antibody (HB Ag/Ab) or free antibody to hepatitis B antigen (HB Ab) as measured by positive immunofluorescent technique (IFT) were found in 90 per cent of the sera of patients with active viral hepatitis (AVH) and in 100 per cent of sera from patients with chronic active hepatitis (CAH). These findings confirm and extend previous reports on the occurrence of HB Ag/HB Ab in the sera of patients with AVH and CAH, and also support the hypothesis that the pathogenesis of these two hepatic diseases may best be explained in the terms of serum sickness (Brzosko et al., 1971). Some antigen-antibody complexes are able to induce chronic renal lesions, which might also be possible in the case of hepatitis B antigen immune complexes (Saulier et al., 1969) and that susceptibility of patients with chronic renal disease to chronic HB Ag hepatitis is probably related to impaired immunologic responsiveness (Landau et al., 1969).

Antibodies to Hepatitis-B Antigen

Transfused haemophilia patients often have precipitating antibody (isoprecipitin) in their sera which reacts with hepatitis i antigen not found in the sera of normal individual in the United States but not uncommon in the sera of patients with leukaemia (10%) and mongolism (28%) patients. It is also found in the sera of 5 per cent or more of several populations outside the United States (Blumberg and Alter, 1965).

It has been taken as a working hypothesis that the isoprecipitin is present in the blood of the haemophilics (Blumberg and Alter, 1965) and other persons receiving multiple transfusion (Ockochi and Murakami, 1968) as a result of repeated exposure to the antigen present in donor blood (Schmidt and Lennette, 1970). The higher frequency (20%) of antibody against hepatitis B antigen in haemophilic patients as opposed to the antibody frequency in other transfused patients, approximately 3 per cent (Melartin and Blumberg, 1966) is thought to be due to the fact that the former receive large amount of frozen plasma. It is postulated that freezing results in the partial denaturation of the plasma with resulting revelation of an ordinary occult hepatitis B antigen specificity site (Blumberg and Alter, 1965). Other evidence consistent with the hypothesis that the precipitin is antibody is (i) its identification as an IgG and IgM immunoglobulin (Lander et al., 1972), (ii) the fact that it forms specific precipitins by double diffusion in agar gel in the same manner as other antigen antibody system (Blumberg and Alter, 1965).

The specificity of the anti-HB Ag antibody was not confirmed until Melartin and Blumberg (1966) and Lichter (1969) produced specific anti-HB antibody in rabbits and chimpanzees by immunization with serum containing Hb antigen showing that the precipitation obtained with haemophilia sera is indeed caused by an antibody and lends considerable support to the hypothesis that this antibody is a result of immunization by transfusion with plasma containing hepatitis B antigen.

In a study of 10,000 blood donors, Banke and associates (1971) found that anti-HB Ag antibody was more prevalent among female donors and slightly more common among older individuals. No difference in frequency between males and females has, however, been reported in the study of Lander and associates (1972).

Antibody to HB Ag, measured by the radio-immunoprecipitation technique in serial samples (Lander et al., 1971b) was detected after primary exposure in three MS-2 patients with transient hepatitis B antigen, and in one of the three MS-2 patients with persistent HB Ag. Antibody persisted for at least 18 months after exposure and was found at a time when HB Ag was still detectable in all four patients in whom antibody had developed. The persistence of HB Ag appear to be related to repeated infection with type B virus and host's response (Kattamis et al., 1974). The frequency of anti-HB Ag is in general, directly related to the age and inversely related to socio-economic status of the individuals studied (Cherubin et al., 1972).

It has been suggested that pre-existing HBsAg protects against type B hepatitis (Alter et al., 1972; Hollinger et al., 1973). The effect of infusion of antibody to hepatitis antigen results in clearance of the antigen from the circulation for upto 9 days (Reed et al., 1973a). However, Barker and his colleagues (1973b) reported on 17 cases in which an early or secondary type HBsAb response was followed by development of the disease. Immunity to type B hepatitis is not necessarily complete in individuals with previous exposure to hepatitis B virus and protection seems to be directly dependent on the amount of circulating HBsAb and only those with high titers seem to be fully protected (Concha et al., 1975).

Antibody to hepatitis B antigen, measured by radio-immunoprecipitation technique in serial serum samples from patients who had HB Ag positive hepatitis after primary intramuscular inoculation with hepatitis B antigen

(HB Ag) and reinoculated with the same dose of HB Ag was detected to be IgG after primary and secondary exposure, IgM anti-HB Ag was detected after secondary exposure (Lander et al., 1971b). Lander and associates (1972) have measured specific IgG and IgM anti-HB Ag patterns following primary exposure to the antigen.

Evidence from several laboratories using several immunologic techniques has demonstrated the presence of two distinct antigen-antibody systems associated with viral hepatitis type B (HBV). The hepatitis B surface antigen ——— HBsAg, and antibody ——— anti-HBs (Blumberg et al., 1968; Prince, 1968) and the more recently characterized hepatitis B core antigen ——— HBcAg, and antibody ——— anti-HBc (Brzosko et al., 1973; Hoofnagle et al., 1973).

Anti-HBc occurs regularly during the course of hepatitis type B disease. In general highest titers of anti-HBc are seen with prolonged circulation of HBsAg in the chronic carrier state. Titers of anti-HBc begin to fall with recovery from hepatitis B virus (HBV) infection and anti-HBc testing is important in documenting the occurrence with HBV and is of great value in epidemiologic studies in evaluating the safety and efficacy of hepatitis B immune globulin and HBV vaccines (Hoofnagle et al., 1975).

Almeida and associates (1971) disrupted the outer layer of the Dane particle with detergent and showed by immuno-electromicroscopy that the free 28 nm core was precipitated by a human convalescent serum which was free of antibody against hepatitis B surface antigen (HBsAg) i.e., anti-HBs, and has reported that hepatitis B core antigen (HBcAg) antibody (Anti-HBc) system is distinct from HBsAg-anti-HBs system.

The possible outcome of exposure to HBV of a susceptible individual are schematically shown in Figure 1. Individuals immune to hepatitis B, as evidenced by the presence of high levels of antibody against the surface antigen (Anti-HBs), do not usually develop infection when re-exposed, but many respond with a significant increase in anti-HBs levels (secondary antibody response).

Exposure to the HBV may be followed by an icteric or anicteric episode of illness, an asymptomatic form of hepatitis B, or no clinical response at all. In the latter case, the proportion of individuals reveal sero-conversion from antibody negative to antibody positive (primary antibody response), while others remain either negative or have a rapid, transient antibody response (Szmuness 1975).

Antibody against core antigen (anti-HBc) is first detected 60 to 150 days after exposure and 30 to 90 days after the first appearance of HBsAg. Low titers of this antibody can still be recovered 1 to 2 years after onset of the disease (Hoofnagle et al., 1973). Anti-HBs usually appear much later, sometimes as late as 1 to 2 years after exposure (Melnick 1975). However, in contrast to anti-HBc, anti-HBs persistence is indefinite in duration and may be life long. Nearly 95 per cent of anti-HBs-positive children and adults from endemic settings

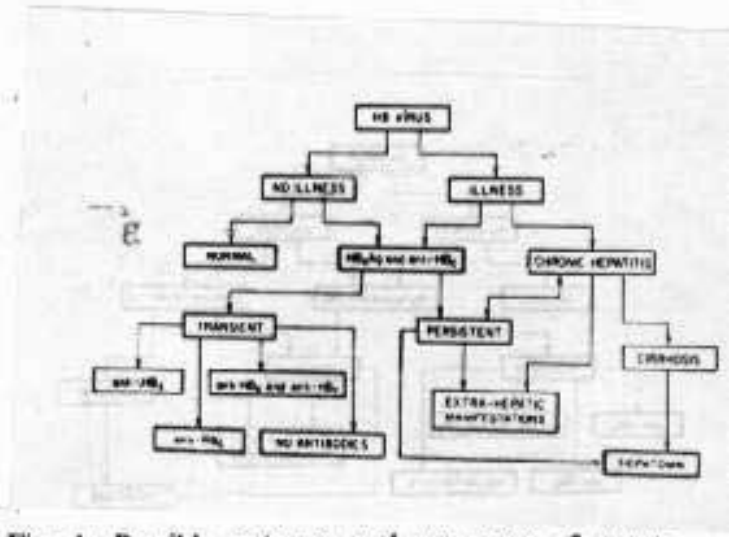

Fig. 1: Possible outcome and sequences of events after exposure to hepatitis B antigen

have been found to remain positive when retested 2 to 3 years later (Szmuness et al., 1972). Hoofnagle's (1973) studies showed that the first appearance of anti-HBs coincides with diminishing levels of anti-HBc, and therefore, both types of antibody may be detectable during a several month period. The studies of Szmuness (1975) revealed that the co-existence of anti-HBc and anti-HBs is most often observed in individuals with abnormal levels of S.G.P.T. or under conditions of continuous exposure. These data would suggest that recovery of both types of antibody may be an indicator of developing chronic active hepatitis (Szmuness et al. cited by Szmuness 1975).

On the basis of animal studies, Markenson and his colleagues (1975) reported that residual humoral immunity, demonstrable by antibody

against the group determinant 'a' and sub-determinant 'y' of HBsAg, gave protection against challenge with either heterogenous or homologuos HB virus and that anti-HBc did not appear necessary for protection against the virus.

Transmission of Hepatitis B Antigen

A. Parenteral Transmission

Hepatitis B generally is transmitted by inoculation of human blood or blood products containing hepatitis B antigen or material contaminated with the antigen (Barker et al., 1970; Gocke et al., 1970; Gocke, 1972). Although type B hepatitis can be transmitted by the oral route (Krugman et al., 1967) the most common route of infection is parenteral, mainly transfusion of infected blood (Kattamis et al., 1974). Ockochi and Murakami (1968) noted the development of HB Ag positive hepatitis in three recipients of antigen positive blood, whereas Gocke and Kavey (1969) in a retrospective study of twelve patients who had received antigen positive blood, found that hepatitis developed in nine and that serum from seven of the nine was positive for HB Ag. Similar results have been reported by Giles and associates (1969) and Krugman and Giles (1970).

Hepatitis B has increased at alarming rates in large cities where the practice of needle sharing by parenteral drug users is common. The sale of blood by this segment of the population to commercial blood banks has resulted in increased infectivity of those who receive blood products. In some cities, the incidence of clinically apparent hepatitis acquired from a blood transfusion is as high as 20 per 1,000 units of blood (Alter et al., 1970; Grady, 1970; Taswell et al., 1970). In an epidemiological survey of 49 cases of hepatitis B antigen positive acute viral hepatitis, 12 patients (24.5%) had hepatitis after blood transfusion and 13 had been given injections with non-disposable neeedles (Alkan and Fainaru, 1973).

Pooling of blood products increases the risk of transmitting the agent. For years, it was believed that storage of plasma for 6 months at room temperature was effective in inactivating the agent (Allen et al., 1950), but such treated plasma recently has been shown to transmit hepatitis (Redeker et al., 1968). Blood fibrinogen carries a particularly high risk of transmitting hepatitis B (Bove et al., 1969).

B. Non-Parenteral Transmission

Although hepatitis B is transmitted by inoculation of blood or blood products containing hepatitis B antigen, in some cases of HB Ag positive hepatitis a history of blood or blood products is lacking. Hepatitis B antigen was detected in 60 per cent of the patients with serum hepatitis and 41.9 per cent in those with sporadic hepatitis who gave no history of parenteral inoculation (Hadziyannis et al., 1972). The ratio between the cases of viral hepatitis type B due to parenteral and non-parenteral transmission in the study of Kasza and co-workers (1974) shows that the main route of transmission of viral hepatitis type B is probably the non-parenteral one.

Although several reports suggest that hepatitis type B could be transmitted by non-parenteral person-to-person contact (Koff and Isselbacher, 1968; Cossart, 1972), it was not until 1967 that Krugman and co-workers reported the experimental transmission of hepatitis B (designated as MS-2) by the oral route. The occurrence of HB antigen in both post-transfusion and infectious hepatitis suggests that it can be transmitted by either route (Blumberg et al., 1969).

The detection of hepatitis B antigen in feces (Grob and Jemelka, 1971), bile (Akdamar et al., 1971) and urine (Tripatzis and Horst, 1971), suggested that transmission occurred by the faecal-oral route. Hepatitis B antigen may be detected in the urine of patients with acute HB Ag positive hepatitis most commonly during the period of their convalescence, when the antigen was no longer present in the serum. Despite no evidence of recent hepatitis a high proportion of household contacts also had hepatitis B antigen in their urine (Heathcote et al., 1972).

The presence of the antigen in saliva with or without blood contamination raised the possibility that oral-oral transmission was potentially important (Heathcote et al., 1974; Ward et al., 1972). The significance of HB Ag is still uncertain and its presence in saliva does not necessarily indicate the presence of hepatitis B virus, but bleeding from gums would render the saliva infectious in any case. Since kissing is the most common type of intimate sexual interaction, the ingestion of blood-contaminated saliva could be one mode of non-parenteral transmission. The high risk of hepatitis among dentists may also be due to saliva dependent non-parenteral transmission (Broder-sen et al., 1974). Cacciatore and co-workers (1973) found

that hepatitis B antigen was present in the ascitic fluid of all HB Ag positive subjects at a titer similar to that in the blood.

C. *Transmission by Mosquitoes*

Much attention has been focussed on the possible role of blood sucking insect vectors in the transmission of viral hepatitis. Investigations designed to prove this hypothesis have been carried out largely in the tropics. Data supporting this hypothesis in an urban, non-tropical area were obtained during an epidemic of hepatitis in New Jersey (Levy cited by Dick et al., 1974). Persons who resided a high population density area developed a long incubation type of hepatitis following a period of unusually heavy mosquito infestation, in which each patient complained of having received multiple mosquito bites three months earlier. There was no history of drug addiction, contact with jaundiced individuals or receipt of injections.

Metselaar and associates (1973) tested pools of mosquitoes by radio-immunoassay at intervals upto 45 days after exposure to HB Ag. HB Ag was detected long after the blood itself would have been digested. The findings suggest persistence of HB in mosquitoes and that blood sucking arthropodes (Ades aegypti mosquitoes) played a role in transmission of hepatitis B antigen (Tin et al., 1973).

The observations of Dick and associates (1974) indicate that mosquitoes if abundant, may play a role in the spread of viral hepatitis in urban areas with a dense population and a high incidence of this disease. The possible role of HB Ag positive mosquitoes in transmission of viral hepatitis depends on the degree of mosquito infestation and the epidemiological characteristics of the human population involved.

D. *Sexual Transmission*

Reports of clinical cases in which HB Ag positive hepatitis was transmitted from male patients to their intimate female contacts by non-parenteral route implied that the disease may be sexually transmitted (Hersh et al., 1971; Henikst, 1973; Fass, 1974). A similar conclusion was reached in several other studies. Of 67 patients who were admitted to two London hospitals with acute HB Ag positive hepatitis and surveyed to find the source of their infection, 13 (2 were male homosexuals) had sexual contact with individuals, usually males who were either jaundiced or hepatitis B antigen positive (Heathcote and Sherlock, 1973). In a venereal disease clinic in London, positive test results for hepatitis B antigen, its antibody or both, correlated with a history of gonorrhoea, syphilis, sexual promiscuity, and in males, homosexuality (Fulford et al., 1973). In other venereal disease clinic in London prevalence of antigenemia was similarly high in male homosexual but not higher than expected in other subgroups of patients, which suggested that the disease was not spread by venereal contact except, perhaps in male homosexuals (Jeffries et al., 1973). Both studies of hepatitis B antigenemia in venereal disease clinics found that prevalence rate for non-European heterosexual males were among the highest, which indicated that factors other than sexual contact and orientation were also important (Fulford et al., 1973; Jeffries et al., 1973). However, the increased hepatitis B antigen carrier rate in European homosexual supports the proposed possibility of venereal transmission of the disease (Vahrman 1970), suggesting that virus may be transmitted perhaps from mucous membrane to mucous membrane, or from abrasions exuding infected serum.

The hypothesis that the virus could be transmitted by menstrual blood (Mazzur 1973) and the demonstration of hepatitis B antigen in the semen of 10 of 19 antigenemic men tested (Heathcote et al., 1974) indicated that hepatitis may, in some cases, be a true venereal disease. In the latter study, 9 of 24 antigenemic men were homosexual; three of them developed acute hepatitis after contact with HB Ag positive carriers, and two with chronic hepatitis were homosexual patients. Two of the 15 heterosexual males were thought to have transmitted hepatitis to 5 sexual contacts.

Papaevangelu (1973) found hepatitis B antigen in 9 (3.6%) out of 247 prostitutes who were regularly referred to them for checkup. A similar frequency (3.4%) was found in a sample of 379 pregnant women of similar age and of relatively low socio-economic level.

Although these studies suggest that sexual contact was associated with HB Ag positive hepatitis, the studies did not define a mechanism for transmission or separate the importance of sexual contact, either homosexual or heterosexual, from non-sexual intimacy that occurred between sexual partners.

E. Vertical Transmission

The term vertical transmission has come to mean transmission of hepatitis B virus (HBV) from mother to infant. The study of this mode of transmission has been made possible by the development of serologic tests for hepatitis B antigen (HB Ag) as a reliable marker of the presence of HBV. Many authors have persued investigations in areas with results being similar in some instances and divergent in other.One area of agreement has been that a high rate of neonatal infection occurs when the mother has acute hepatitis B near delivery Merrill and associates (1972) found that four of four such untreated infants became HB Ag positive. The studies of Schweitzer and Spears (1970) and Schweitzer (1975) show that seventy per cent (70%) babies became HBV infected when maternal hepatitis occurred in the third trimester or the first two post-partum months. In one case the mother developed hepatitis at six month's gestation: she became antigen negative before delivery; the cord blood was positive and the baby had been HBsAg positive since birth. These studies, therefore, indicate that transmission may occur trans-placentally or during the birth process. Once infected, the infants apparently remain hepatitis B surface antigen carrier with persistent hepatitis indefinitely (Schweitzer et al., 1973).

A number of investigators have reported on groups of maternal HB Ag carriers, discovered by screening large number of pregnant women for the surface hepatitis B antigen (HBsAg). They have then followed carrier mothers and infants with periodic antigen testing to determine the rate of transmission. It was found that the rate of transmission of the hepatitis B surface antigen from asymptomatic carrier mothers to their infants varies from 0 to 40 per cent in different areas of the world. The highest rate is in Taiwan.

F. Intra Familial Spread

In a pilot survey carried out in 1970-72 in two groups of households from New York city, one with an index donor persistent carrier of the hepatitis B surface antigen (HBsAg) and the other with an index donor free of both HBs Ag and its antibody (anti-HBs), it was found that hepatitis type B infections tend to cluster in families and that the HB Ag carrier state tends to develop much more frequently among blood relatives of the index carrier than among non-blood relatives (Szmuness et al., 1973). Evans (1972) reported that three brothers were carrier for hepatitis B antigen whose uncle had a history of short term jaundice attack in 1935.

An epidemic of HB Ag positive viral hepatitis occurred in a large family and their acquaintances during a fourth month period. Three adult members of family had clinically apparent hepatitis and asymptomatic HB Ag positive serum developed in the baby. Two other family members were found to have antibody to hepatitis B. No one in the family but the index patient used illicit drugs parenterally but all had closed contact with the index patient, the baby and each other (Mitch et al., 1974). A serologic survey for HB Ag and antibody in 17 families, each with one adult who was an asymptomatic HB Ag carrier, demonstrated an increased frequency of hepatitis B infection in family contacts of the carrier (Irwin et al., 1974). Hepatitis associated antigen (HB Ag) was detected in the serum of a 5-1/2 month old male infant with giant cell hepatitis. Three of four other members of the family were asymptomatic carriers of HB Ag with normal or nearly normal results of liver function tests and no history of liver disease (Bancroft et al., 1971).

The mechanism underlying such a familial aggregation of hepatitis B were obscure, infections or genetic factors or both could be involved. Data published by workers failed to provide evidence as to the determinants of interfamilial spread of hepatitis B. The study of Szmuness and colleagues (1975) provided evidence indicating that familial aggregation and segregation of hepatitis B are influenced by both environmental and genetic factors. Reports of familial clustering of liver disease and HB antigenemia around antigen-positive motors have suggested the possibilities of maternal fetal transmission (Ohbayashi et al., 1972; Wright et al., 1970; Bancroft et al., 1971).

Hepatitis — A Professional Hazard

Medical personnel in general and laboratory workers in particular are at risk for contracting hepatitis B (Koff and Isselbacher, 1968).

In an epidemiological survey of 49 cases of hepatitis B antigen-positive active viral hepatitis, seven were medical personnel (Alkan and Fairnaru, 1973). The fact that inoculation with as little as 0.00004 ml of blood was capable of transmitting the disease (Drake et al., 1952),

suggested that minor exposures to infected blood were adequate for transmission through minor defects in the skin or mucous membranes. In addition to patient contact, laboratory technicians also increase their exposure to potential sources of hepatitis by handling needles, blood, stools and tissue specimens from patients with clinically evidenced or occult viral hepatitis. By testing, typing and control sera routinely used in the clinical laboratory, the antigen was demonstrated in nearly two thirds of chemistry control sera as well as typing and control sera used in the haematology, serology and microbiology sections (40%, 24% and 60% respectively), suggesting that commercial typing sera and serum controls present a serious hazard of serum hepatitis for all laboratory workers (Welti et al., 1973).

During a six-month period, hepatitis developed in five employees of large, hospital based clinical laboratory. Three employees had transient hepatitis B surface antigen (HBsAg) one had antibody to surface antigen (anti-HBs) and one had neither. In the two years preceding this outbreak, only one laboratory employee had overt hepatitis. Risk factor analysis for all employees and a control group of HBs Ag and anti-HBs-native laboratory employees matched for age, length of employment and amount of exposure to blood specimen showed that only a history of sustaining cuts while handling laboratory requisitions were statistically significant i.e., P 0.005 (Pattison et al., 1974).

An antibody titre to hepatitis B antigen (Anti-HBs) was present in 55 per cent to 92 per cent of those workers who had varying degrees of contact with plasma fractionation. The degree of contact correlated with the prevalence of antibody in the population studied (Taylor et al., 1974).

By virtue of the close contact with blood products and abrasive surfaces, dentists are at a particular risk of acquiring hepatitis. The majority of dentists do not use gloves during work (Feldman and Schiff, 1973), and patients are not generally screened for history of hepatitis or illicit drug use. On the other hand oral surgeons employing procedures requiring use of wires, would particularly likely to have small cuts and abrasions on the hands and even the smallest abrasion may serve as a source of spread of hepatitis B antigen. Epidemiological aspect of 49 cases of hepatitis B antigen positive acute viral hepatitis was reviewed by Alkan and Fainaru (1973). In 17 cases the mode of transmission could not be ascertained, however, 7 of these patients had dental treatment one to six months prior to the onset of jaundice. Twelve patients treated by an HB Ag positive dentist, developed HB Ag positive hepatitis over a four-month interval. None of the hepatitis patients had blood transfusions, contacts with other jaundiced persons, or illicit drug use. This outbreak suggests inapparent non-parenteral modes of transmission by dental personnel (Levin et al., 1974).

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