REVIEW

Hepatitis B: Review of development from the discovery of the "*Australia Antigen*" to end of the twentieth Century

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Abstract

"Parenteral" or "serum" hepatitis is known to have afflicted man for centuries. However, it was not until the mid-1960s that the causative agent of this infection, the hepatitis B virus, was discovered. Since then, the biology and the replication strategy of the virus, and the clinical features and the epidemiology of the hepatitis B infection have been determined. Knowledge about the virus and the infection it causes led to the development of firstly, a plasma-derived vaccine and later a recombinant vaccine for the prevention of the infection. Integration of the hepatitis B vaccine into newborn vaccination programmes on a worldwide basis represents a major step in the effort to eliminate this infectious disease and its complications. Laboratory tests are available for the diagnosis and monitoring of the disease. Therapies have been developed to halt the progress of the chronic infection in affected individuals. While these developments have resulted in a decrease of the frequency of infection in many countries, particularly those that have implemented universal immunization of newborns, the chronic infection remains a significant global problem. Worldwide, over 300 million individuals are infected and each year, an estimated 1 million persons die from chronic complications of the disease including hepatocellular carcinoma and hepatic failure. The therapies currently available result in elimination of the virus in only a relatively small proportion of subjects and carry with it serious side effects. Geopolitical, economic and other factors hinder the vision of elimination of the infection through immunization programmes. Nevertheless, work continues to clarify further the underlying pathological mechanism of the infection, the host and viral factors that promote elimination or persistence of the virus in the human host. It is hoped that such investigations will reveal viral targets for the design of newer and potentially more effective drugs to treat the infection.

Key words : Australia antigen, HBV, chronic hepatitis, HCC.

HUMAN VIRAL HEPATITIS – HISTORICAL PERSPECTIVE

Hepatitis has been recognised since the beginning of recorded history as an ailment characterised by the distinctive yellow discoloration of its victims. Such records include the 5th century BC Babylonian Talmud, the 4th century BC writings of Hippocrates, and Chinese literature from the 10th century AD.[1-3] The infectious nature of the illness was suspected based on its occurrence as epidemics associated with crowded and unsanitary conditions. A reflection of this knowledge can be found in the recommendations of Pope Zacharias in the 8th century AD, for the isolation of hepatitis patients to prevent spread of the disease. Over the years, various other causes for hepatitis have been suggested, including exposure to toxic substances, and inflammatory affliction of the liver. That a virus may be the cause of the disease was first suggested at the beginning of the 20th century.[4] This suspicion was strengthened by reports of outbreaks of hepatitis among soldiers who had received yellow fever vaccine.[5,6] It was known then, that the serum used to culture the yellow fever virus contained a filterable agent.[5] In addition, there were reports on the occurrence of hepatitis following the reuse of unsterilised needles and surgical equipment[7,8] as well as reports of the association between hepatitis and

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the inoculation of other vaccines containing human serum.[9] It became clear that an infectious agent carried in human blood caused hepatitis. The proof for this deduction was provided by a series of studies carried out on human volunteers at around that period of time.[10,11] At that time, two distinctive clinical forms of hepatitis were recognised based on epidemiological features. The first was a short incubation, orally transmitted form that tended to occur in epidemics named "infectious hepatitis", and the second was a long incubation, parenterally transmitted form named "serum hepatitis". MacCallum (1947) suggested the names hepatitis A and hepatitis B for these 2 forms respectively,[12] but this nomenclature was not adopted for general use until its introduction by the WHO 25 years later in 1973.

DISCOVERY OF THE CAUSATIVE AGENT OF HEPATITIS B

During the next decade and a half, efforts to search for the agents responsible for hepatitis A and hepatitis B were largely unsuccessful. The breakthrough came about in the mid-1960s in a rather interesting manner. Baruch Blumberg, a medical geneticist was attempting to relate human protein polymorphisms with predisposition to certain diseases. Working together with a biochemist at the National Institute of Health (NIH) in the United States, he developed a method based on immune-precipitation to rapidly screen for novel blood proteins. He used the serum from patients who have received multiple transfusions to screen for these putative novel antigens in normal individuals from diverse backgrounds. The rationale for this was that multiply transfused individuals were most likely to have encountered foreign antigens and therefore possessed antibodies for detection of these antigens. At about the same time, Harvey Alter, who was also working at the NIH, was studying non-hemolytic transfusion reactions, which he suspected to be the result of an immune response to a foreign antigen. In collaboration with Blumberg, he started to screen for the suspected antigen in the blood of leukaemia and hemophilia patients, using the panel of serum collected by Blumberg for his polymorphism studies. In 1963, a serum derived from an Australian aborigine was found to be reactive with the serum of a hemophiliac patient from New York, which was not an unusual finding in itself. But, in that particular experiment, only 1 out of 24

hemophiliac sera tested reacted with the aboriginal serum, indicating that the antigen detected was rare or novel, and could be either an infectious agent or a genetic marker of disease susceptibility. The antigen was named the "Australian Antigen".[13] However, the significance of the antigen was not realised then.

With the description of this new antigen, various researchers started to screen the blood of different categories of patients as well as normal individuals. The results of these studies indicated that the Australian antigen (Aa) was present in high frequency among hemophilia and leukemia patients and present in low frequency among normal individuals.[14] As Down's syndrome was known to be associated with increased risk of leukemia, children with Down's syndrome were also tested. The study showed that Down's syndrome children who were institutionalised had a high frequency of Aa compared to those who were not.[15] This led to the conclusion that the Aa was more likely to be an infectious agent rather than a genetic marker of human origin. Prince and Okochi, who established that the Aa was found in most patients with serum hepatitis, and that blood seropositive for the Aa was more likely to cause post-transfusion hepatitis, provided the evidence that linked the Aa to hepatitis.[16,17] The final clue came about when a technologist working in Blumberg's laboratory, and a Down's syndrome patient, both of whom were previously negative for the Aa came down with hepatitis and become positive for the Aa. It became accepted then that the Aa was indeed the long sought for causative agent of "serum hepatitis". Dane et al, using electron microscopy subsequently identified the agent as 42nm particles in the blood of Aa positive patients.[18] The viral nature of these particles was confirmed by Kaplan et al who demonstrated the presence of DNA polymerase activity in the Dane particles.[19] It became clear then that the Aa was part of a virus that caused hepatitis B.

Following the unequivocal identification of the Hepatitis B Virus (HBV) as the cause of serum hepatitis, researchers embarked on a very active phase of hepatitis studies. The direction of these investigations was driven by 2 main considerations. Firstly, it was apparent that, in order to prevent transfusion hepatitis, there was a need to test all blood donors for the Aa, now known as the hepatitis B surface antigen (HBsAg). It was also clear that the existing detection method based on gel diffusion was

inadequate. Therefore, newer approaches for identification of the virus were required. Secondly, it was clear that there was a need to develop a protective vaccine against this infection.

KEY DEVELOPMENT IN THE NEXT DECADE

The development of a highly sensitive test for the HBsAg arose out of the curiosity of 2 researchers working at the Bronx Veterans Administration Medical Centre, Rosalyn Yalow and Solomon Berson. Their investigations on the fate of insulin in diabetic patients led to the design of a highly sensitive, radiolabel based immunoassay – the radioimmunoassay or RIA. This represented a major landmark in technological development that won them both a Nobel Prize in 1977. Using this approach, the first radioimmunoassay for HBsAg testing become available in the early 1970s.[20] With this development, the United States passed a law in 1972, making the testing of all donor blood mandatory.

The second major development in the 1970s was the successful production of the hepatitis B vaccine. Two key observations were central to this development. The first was the demonstration of viral coat protein devoid of nucleic acid in serum of infected subjects,[21] and the second was the demonstration that heat-treated HBV contaminated blood provided protection from HBV infection.[22] A patent was filed by the Fox Chase Cancer Centre (FCCC) for production of the vaccine using viral subunits (in the form of virus coat protein) in 1969. 2 years later, in 1971, Merck obtained the license from FCCC to develop the subunit vaccine, which became available 10 years later in 1981.[23] This represented the world's first licensed vaccine against a human cancer, hepatocellular carcinoma. Meantime, genetic recombinant technology and protein expression systems became established in the mid-1970s. Reasoning that the supply of human carrier plasma is a limited commodity and that the supply would not be adequate for the anticipated needs, efforts were started to develop alternative methods for vaccine production. A yeast expression system was used to express the hepatitis B surface antigen. A report of successful production of the HBs antigen in yeast appeared in Nature in 1982.[24] Two years later in 1984, McAleer et al reported the production of the recombinant hepatitis B vaccine.[25] In 1986 this vaccine was licensed, making it the first ever recombinant vaccine to be licensed.

Simultaneously, basic and applied studies were carried out to determine the biology of the virus including its antigenic and molecular structure, its genomic organisation, its life cycle and replication strategy. To facilitate these studies, various experimental models were employed including small animals naturally infectible by their respective hepatitis virus, transgenic mouse model as well as in-vitro cell culture systems. Clinical and epidemiological studies were also actively pursued to define the pathology of the infection, the global distribution of the HBV and its modes of transmission.

BIOLOGY OF THE HEPATITIS B VIRUS

Molecular Structure and Life cycle of the HBV

The virus is a spherical, enveloped particle of 42nm in diameter. It contains within its nucleocapsid, a partially double stranded (ds) DNA as well as DNA polymerase with reverse transcriptase (RT) activity. It is the first of a group of viruses to be classified within the hepadnaviridae family,[26,27] which include three rodent members (woodchuck, tree squirrel and ground squirrel hepatitis virus), and two avian members (duck hepatitis virus, heron hepatitis virus). The virus genome is a highly compact circular structure, which comprises of a negative sense DNA strand of 3.2kb and a positive sense DNA strand of 1.7 – 2.8kb. The genome encodes 4 overlapping open reading frames (orfs) designated the S, C, P and X genes which are responsible for the production of the envelope protein (HBsAg), the core protein (HBcAg), the DNA polymerase and the X-protein respectively.[28-30] The HBV is rather unique in that it is the smallest of all known ds DNA viruses to infect man, which explains the use of overlapping frames for its genes. It is also characterised by a unique replicative strategy, whereby its DNA is transcribed via an RNA intermediate using the RT activity of DNA polymerase.[31] The virus exhibits relative genetic diversity, presumably the result of the lack of proof reading ability of the RT. In addition, it is highly tissue specific, has a very limited host range and a tendency to persist in its host.

Definition of the steps in the virus life cycle was achieved largely by experimental studies using non-human hepadnaviruses in their respective hosts, HBV transfected cell lines, and HBV infected chimpanzees. The first step in the cycle involves the binding of the virus to the

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liver cell via an unknown receptor followed by intake into the cell via endocytosis. Upon entry into the cell, the virus loses its protein coat to facilitate the entry of the nucleocapsid into the host cell nucleus. Within the nucleus, the virus completes synthesis of the plus strand DNA giving rise to covalently closed circular (ccc) DNA, which serves as the template for transcription of the viral RNAs. The RNA transcripts that are produced include a 3.5kb pregenome as well as sub-genomic fragments of 2.1 and 2.4kb respectively. The pregenome directs the synthesis of the HBcAg, DNA polymerase and the negative sense DNA, whereas the sub-genomic fragments direct the synthesis of the HBsAg. The enzyme required for production of these RNA transcripts is RNA polymerase II derived from the host cell. After transcription, the RNA transcripts are polyadenylated and then transported to the cytoplasm where they are translated to C, pre-C, P, envelope and X proteins. The next step involves the packaging of the RNA pregenome and the Pol within the core particles. Negative sense DNA synthesis then proceeds utilising the RT activity of the DNA polymerase, followed by positive sense DNA synthesis. The now mature progeny core particles then reach the endoplasmic reticulum (ER) where they associate with envelope proteins and bud into the lumen of the ER, from which they are secreted via the Golgi apparatus out of the cell, thereby completing the replication cycle. (For details, see reviews by Ganem D, 1996;[32] Nassal M and Schaller H, 1996;[33] Ganem D and Schneider, 2001[34]).

In summary, the mechanism of virus replication has been largely defined. However, there still remain questions about the life cycle of the HBV. For example, much remains unknown about virus attachment and the nature of the viral receptor, a major determinant of tissue tropism. The cis acting elements required for virus replication has also not yet been determined. Mapping of these elements is considered important as it can facilitate the design of novel vectors for identification of the viral receptor and potential targets for anti-viral therapy as well as for therapy of liver disease.

Clinical and Epidemiological Aspects of HBV Infection

Hepatitis B is among the most common persistent viral infection in man. Epidemiological studies have mapped the frequency distribution of the infection worldwide. Overall, HBV infects about 5% of the world population and exacts an annual death toll of a million due to chronic liver disease and liver cancer.[35,36] Areas of high endemicity are defined by infection rates of 8% or more; this include sub-Saharan Africa, Central and East Asia, and parts of Central America. In these regions, widespread infection commonly occurs in infancy or childhood. The level of endemicity of the infection determines the lifetime risk of acquiring the infection for people living in different geographical regions. For high endemicity areas, which represent about 45% of the global population, the risk of infection is over 60%. In areas of intermediate endemicity, the risk is estimated to be between 20% and 60%. The relative risk of developing chronic HBV infection is determined by the age of infection, and varies inversely with it. At birth and in the perinatal period, the risk is close to 90%, which is in marked contrast to that in older children and adults in whom the risk is around 10% or less. In Malaysia, the frequency of hepatitis B in asymptomatic individuals varies with ethnicity and sex from a high of around 6-7% to a low of less that 1%. It is highest among Chinese males and lowest among the Indian population. The average male to female ratio is about 2.5:1 (unpublished data).

The recognised modes of transmission of hepatitis B are sexual contact, parenteral exposure to infected blood or other infected body fluids, mother to child perinatally and close contact with an infected person. The risk factors therefore include intravenous (IV) drug use, homosexual activity, sexual contact with an infected person, multiple sexual partners, hemodialysis and HIV infection. The outcome of infection is determined to a large extent by the immune status of the individual. In a normal person, infection generally results in an acute illness that is followed by recovery and life long immunity. In immune compromised persons, the infection can be fulminant, resulting in liver failure and a high mortality. Chronic hepatitis tends to occur in persons infected during infancy or childhood when the immune function is believed to be relatively immature and the person likely to be tolerant of the virus.

The biochemical and serological profile in acute hepatitis is well defined. Briefly, acute hepatitis is characterised by the presence of anti-HBc IgM, which convert to IgG with recovery, and by the transient presence of HBsAg, HBeAg and HBV DNA. Clearance of HBsAg, HBeAg and HBV DNA is followed by seroconversion to anti-HBs and anti-HBe. In contrast, progression to chronic hepatitis is characterised

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by persistence of the HBsAg and HBeAg, and absence of the anti-HBs response. The continued presence of HBV DNA is a marker of continued virus replication in these individuals. The complications of chronic HBV infection are well known and include liver cirrhosis, liver cancer as well as liver failure.

RESEARCH ACTIVITIES OVER THE LAST DECADE

Over the past decade and a half, medical researchers have turned their focus onto fundamental investigations in their attempt to find out the underlying mechanisms that determine the course and outcome of infection. The questions that were raised included :

1. What determines the outcome of infection?
2. What are the mechanisms for viral persistence?
3. How does the virus cause tissue injury in chronic hepatitis?
4. What is the pathogenesis of HBV related hepatocellular carcinoma (HCC)?

Pathogenesis of HBV Infection
Understanding the immunobiology of HBV infection was central to some of these questions. It has been determined that clearance of the virus is dependent on both humoral and cell mediated immune responses.[37-39] Briefly, the humoral response to the infection is T-cell dependent and involves the generation of anti-HBs for the clearance of the virus from the circulation as well as for the prevention of virus attachment to the hepatocyte. The cell-mediated immune response is HLA restricted and involves both the CD4 and the CD8 T cells. The CD4 T cell response is directed against the nucleocapsid antigens, HBc and HBe, which are processed and presented by macrophages or "Antigen Presenting Cells" (APCs). The interaction between the CD4 cell and the APCs results in (1) the induction of virus specific CD8 T cells, as well as envelope specific B cells, and (2) the production of cytokines, which inhibit virus proliferation. In contrast, CD8 cells are responsive to viral peptides displayed on the infected hepatocytes. Interaction of CD8 cells with these peptides results in apoptosis of the infected cell, production of inhibitory cytokines and activation of effector cells. The interaction and cooperation between the humoral and cellular arms of the immune response appears to be essential for clearance of the virus.

The pathogenesis of liver cell injury in hepatitis remains speculative and the factors that favour persistence over clearance of the virus are not fully understood.[34,40-42] It is logical to expect that both host factors and virus factors are involved in determining the course and outcome of infection. Epidemiological and clinical data suggest that an impaired immune response, the male sex, homosexual orientation and possibly genetic or racial factors may be important host determining factors.[43,44] The outcome of infection by the virus is likely to be influenced by the host immune response; variation of the immune response is dependent on polymorphism of genes residing within the major histocompatibility complex (MHC). A study in Gambia showed that there was an association between an HLA class II allele (HLA DRB1*1302) and clearance of the virus.[45] Similarly, Hohler et al reported that HLA-DRB1*1301 and *1302 protect against chronic hepatitis B.[46]

Numerous possible virus factors that may play a role in persistent infection have been suggested including :

1. Epitope inactivation in mutant virus resulting in decreased HLA binding and/or failure of T cell receptor (TCR) recognition
2. TCR antagonism by the mutant virus leading to interference with epitope recognition
3. Down-regulation of virus gene expression and/or host cell immune regulatory molecules
4. Infection of immunologically privileged tissues
5. Selective immune suppression by infection of lymphocytes/monocytes

It is believed that these mechanisms are operative only in the context of an ineffective host immune response. It is also the consensus that tissue damage in chronic hepatitis is due to both the activated Cytotoxic T lymphcytes (CTL) and non-specific cells (such as NK cells & inflammatory cells) recruited by the CTLs. Briefly, viral persistence is dependent on evasion of the host immune surveillance system by various means. Among the different hypothesis, the selection of mutant virus that is invisible to the immune system has generated numerous studies. However, till date, the molecular and cellular basis of the different hypothesis remains to be defined.

Genetic heterogeneity of the HBV
Contrary to the tendency of most biological systems to maintain genetic stability, the HBV undergoes relatively rapid and drastic sequence

changes. The sequence heterogeneity exhibited by the virus can be divided into 3 main categories:

1. Genotype specific, geographically restricted variation
2. Spontaneous mutations arising during chronic infection
3. Variants with selective survival advantage

Genotype-specific, geographically restricted sequence variations are stably transmitted in the host population and may result from neutral evolutionary drift of the virus genome. Such variation may also be secondary to long-term adaptation of the virus to genetic determinants of the specific host population. Of this type of variation, that related to the HBsAg is well recognised. S gene heterogeneity allows the definition of 4 serotypes due to a common "a" determinant and 2 mutually exclusive *d/y* & *w/r* determinants, which define lysine/arginine at positions 122 and 160 respectively.[47,48] Nine subtypes can also be defined based on the t/i or threonine/isoleu determinants at position 126.[49]

Virus heterogeneity can also be defined genotypically. Currently, 7 genotypes, A to G, are described on the basis of genome wide variability.[50-54] These genotypes have been found to cluster geographically.[55] Genotype A is pandemic but most prevalent in NW Europe, North America and Central Africa. Genotypes B and C are mostly found in Asia, while genotype D is predominant in the Mediterranean region and the Middle East extending into India. Type E is typical for Africa and F is described in American natives and Polynesians. Type G is most recently defined and is found in samples from the United States and France. However, the possible association between genotype and clinical course of the infection is still being investigated.

The prevalent genotypes in the East and South East Asian regions are genotypes B and C. The predominant genotype among our patients in the University Malaya Medical Centre (UMMC) is type B (66.7%) followed by type C (22.2%). A small number of type D isolates (3.7%) are also found. It is noted that for 7.4% of these isolates, the genotype was indeterminate. This may be due to either mixed infections or new genotypes not previously described. Additional studies including sequencing and phylogenetic analysis are necessary to determine the nature of these isolates. Preliminary data of our study indicate that genotype C is significantly associated with chronic complications of hepatitis B.

Genotypic variants believed to provide a selective advantage to the survival of the virus are precore mutants, core promoter mutants, pre-S deletion mutants and "a" determinant mutants. The most widely studied of these mutants is the precore mutant, which is unable to synthesis the HBeAg.[56-58] The HBe minus phenotype, which characterises this group of mutants, has been shown to be due to the introduction of a stop codon in the precore region in the majority of cases.[59] Other less common mutations that can cause a similar phenotype include the inactivation of the pre-C start codon and deletions or insertions that result in frame shift. It has been speculated that the loss of this epitope may result in the failure of recognition by the immune system, thereby allowing the virus to evade immune clearance. However, the HBe antigen is not an integral component of the virion; further, the HBe and HBc antigens cross react at the CD4+ T-helper (Th) cell and the CTL level. Therefore, it is unlikely that this represents a major advantage to the virus population in terms of immune recognition. Nevertheless, it is speculated that over the long run this may be sufficient to drive selection of the pre-C defective variant. There is no apparent association between the precore mutant strain and the course and outcome of infection.[60,61] Both the wild type and the mutant virus are equally frequent in patients with end stage liver disease and hepatocellular carcinoma.[62,63] However, the mutant has been reported in many studies to have a higher potential to induce fulminant hepatitis.[64-68] It is suggested that this may be related to an imbalance in the Th1 and Th2 cell populations induced by the wild type and the mutant strains.

The other region that is quite frequently mutated is the core promoter region.[69] This part of the viral genome comprises of the basal core promoter (BCP) and an upstream regulatory sequence (URS). The promoter sequence appears to be important for regulation of virus replication. Numerous different mutations have been found although the most important ones are believed to be those clustered within the BCP. Of these, a pair of mutations involving nucleotides 1762 and 1764 simultaneously is reported to be associated with decrease HBeAg synthesis although virus replication appears to be unaffected.[70,71] The paired mutations are commonly found in active hepatitis, and in patients with chronic hepatitis who develop end stage liver disease (ESLD) and HCC, although the clinical relevance of this association is unclear.

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We have studied the HBe minus phenotype among local isolates and determined the types and distribution of mutations that may account for this phenotype. In summary, the pre-C stop codon mutation accounted for the majority of the e minus phenotype (67%), similar to that reported in the literature.[72] Other mutations involve the core promoter, the most common being the 1762/1764 paired mutation (25%).

S mutants have also been an important area of study. The reason for this is obvious as immunity to the infection is dependent on production of antibodies to the S antigen. Mutations that affect the B cell epitope involve the pre-S 2 region; these mutations are largely in frame deletions, and are believed to allow immune escape, by interfering with immune recognition.[73-75] Mutations involving the "a" determinant also affect the immune response to the virus. The "a" determinant is part of the S domain and is exposed on the surface of virions as well as subviral particles. It is believed to react with protective or neutralizing anti-HBs antibodies elicited during infection. Mutation of the "a" determinant that affects the binding of the epitope by neutralising antibodies will therefore be expected to facilitate immune escape.[76-78] Indeed, these mutations were discovered from studies of breakthrough infection among vaccinated people,[79-81] liver transplant recipients treated with HBs antibodies,[82,83] and chronic carriers with "occult" infection.[84,85] Results of longitudinal studies also indicate that accumulation of "a" determinant variants occur during the course of chronic hepatitis particularly during seroconversion to anti-HBs, and in chronic hepatitis patients with ESLD or HCC.

The Pol gene is important for encapsidation of the pregenomic RNA into core particles. The gene is also responsible for the synthesis of DNA polymerase, which has multiple activities required for virus replication. Within the gene are conserved YMDD residues located at the active site of the RT domain. Mutations involving the Pol gene were discovered with the introduction of nucleoside analogs such as lamivudine and famciclovir for the treatment of hepatitis B.[86,87] It was found that in patients with breakthrough during treatment, the Pol gene acquired mutations in the YMDD locus (methionine to valine or isoleucine). The YMDD mutant strains were resistant to the drug; however, the mutation also resulted in impaired activity of the DNA polymerase making the virus less replication competent.[88,89]

Hepatocarcinogenesis

Hepatocellular carcinoma is among the most serious complications of chronic HBV infection.[90] Evidence for the role of chronic HBV infection in the development of HCC has been provided by both epidemiological and experimental studies. The most well known of the epidemiological studies is that carried out by Beasley et al which involved over 20,000 male civil servants in Taiwan.[91] In this study, it was shown that chronic HBV carriers had a 100 fold greater risk of development of HCC compared to uninfected individuals. In addition, a striking geographical correlation exists between the prevalence of HBV chronic carriers and the annual incidence of hepatocellular carcinoma.

Experimental evidence for the role of HBV in HCC came from several lines of studies. Suspicion of the involvement of the virus in hepatocarcinogenesis first arose out of the high frequency of integration of HBV DNA sequence into the host genome.[92,93] Subsequent studies using animal models and in vitro systems demonstrated that hepadnaviruses can indeed induce malignant transformation in the liver cells. The results of studies gave rise to 2 main models regarding the pathogenesis of HCC – the direct and the indirect models.[94-96] In the indirect model, the HBV genes and their products do not make any direct genetic contribution to the transforming event. Instead, the virus induces liver cell injury, which in turn triggers a series of host responses. This results in repeated cycles of liver cell necrosis followed by regeneration, the hallmark of chronic active hepatitis. The increased cellular turnover would then lead to an increased risk of acquiring genetic mutations and malignant transformation.[95] Therefore, the chronic infection, by inducing cellular necrosis and inflammation, acts in an indirect manner to cause cellular transformation. In the direct model of hepatocarcinogenesis, it is proposed that the virus makes direct genetic contribution to the lesion by either providing cis acting sequences which cause deregulation of host growth genes, or by providing trans-acting factors that interfere with cellular growth control. The candidate gene that could operate to induce such changes in the host is the X gene of the virus.

The X gene and hepatocarcinogenesis

The X gene directs the synthesis of a trans-activating protein that is believed to be important for replication of the HBV. This protein possesses multiple trans-activating activity and is able to act on both viral and cellular genes, causing

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upregulation of promoter sequences.[97-100] Suspicion of its possible role in hepatocarcinogenesis arose out of the fact that the gene is conserved among tumorigenic mammalian hepadnaviruses and the promiscuous transactivating activity of the X protein. Evidence for a possible role of the HBx in malignant transformation came from different lines of investigations (detailed in paper by Buendia MA et al, 2002).[101] Some of the results of these investigations are summarized as follows:

1. The HBx is expressed through all stages of the tumorigenic process and in HCCs
2. Integrated HBV sequences frequently contain X gene sequences
3. Expression of X in transgenic mice induces frequent liver tumours
4. Expression of X in SV40 T-Ag immortalised cell lines can induce cellular transformation
5. The protein is able to activate several oncogenes through its influence on the Ras/Raf pathway
6. It is also capable of influencing the apoptotic pathway in association the P53

The evidence available suggests that the HBx protein is involved in the pathogenesis of hepatocellular carcinoma, perhaps through the deregulation of the cell cycle and of apoptosis.

CONCLUSION

The discovery of the causative agent of "parenteral" hepatitis (hepatitis B) was the culmination of a series of observations and findings of several groups of researchers working in different fields looking for answers to questions seemingly unrelated to the hepatitis question. This landmark in the history of hepatitis B, which led the award of the Nobel Prize in Physiology and Medicine for Blumberg (1976), was the beginning of four decades of active and productive hepatitis research. Studies, both fundamental and applied, have resulted in a wealth of knowledge and understanding of the basic processes of this infection that has plagued human kind for centuries. We now have tools for the easy diagnosis and monitoring of hepatitis B; vaccines have been developed for the prevention of the infection; drugs are available for the treatment of infected individuals. The hepatitis B infection has provided researchers with a model and the impetus to look at host-virus interaction, latency of infection, and viral carcinogenesis. The "hepatitis B story" also illustrates the unexpected path that research can take; and the way in which development in

several different scientific disciplines can come together to provide the tools for unraveling the mystery of this infectious disease.

We have indeed come a long way in such a short time, but the quest is far from over. Although the availability of a vaccine against hepatitis B provides the promise of ultimate control of the infection, much remains to be done to reach this goal. The WHO has highlighted some important target areas that need the consideration of health care professionals and researchers. These are :

1. Global immunisation coverage and integration of hepatitis B vaccine into national immunisation programmes worldwide
2. More effective and less costly anti-viral therapeutic agents
3. Activation of appropriate immune response during chronic infection

To achieve the first of these targets, a concerted cooperative effort involving all sectors of the health care community is required. To achieve the latter two targets, fundamental questions regarding the virus-host interaction, the mechanisms involved in virus persistence and the manner in which the virus interferes with the host cell machinery would need to be answered first. It is envisaged that continued progress will be made, particularly with the completion of the "Human Genome Sequence" and the parallel development of genomics and proteomics, and microarray (chip) technology that allow the large-scale, simultaneous and rapid interrogation of genomic DNA, mRNA and proteins. For example, this approach has already been used for expression profiling of hepatocellular carcinomas with the view to identifying genes important in carcinogenesis and tumour progression. This could open up avenues for development of novel and more effective therapies for the chronic infection and hepatocellular carcinoma. Conversely, the same approach can be used to identify host factors that predispose a person to virus latency and chronic complications of the disease.

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