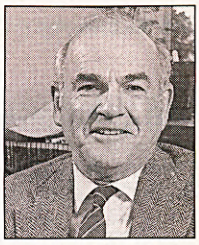
Complex interactions of hepatitis B virus with its host and environment

The Croonian Lecture 1994



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Although one can say that most (80–90% or more) hepatocellular carcinoma (HCC) is caused by hepatitis B virus (HBV), hepatitis C virus (HCV) or both, such a bold statement misses the richness of the interactions of the virus with its hosts, the environment, other viruses and time. An understanding of the complexity reveals many places where intervention can take place to prevent or treat a disease. The more details of the life of the virus that are known, the more the interventional approaches that can be taken for the benefit of public and personal health. Despite the complexity, it is reassuring to know that it is not necessary to understand everything in order to effect changes. The history of medicine is filled with applications developed before all that could be known about a disease had been revealed.

Classification of the hepatitis viruses

There are five known and lettered hepatitis viruses (A, B, C, D, E), probably a sixth (?F), and undoubtedly other viruses yet to be discovered. In 1967, after we identified HBV (the first of the viruses to be discovered) there was a tacit assumption that the other viruses which were known to exist but had not yet been identified, would be similar to HBV. The symptoms and signs of the various forms of hepatitis have a large overlap and it was thought that the viruses would also be similar. However, the viruses are all in different families (Table 1). HAV is a *picornavirus,* initially classified in the genus *enterovirus* but now placed in a genus of its own, *hepatovirus.* HBV has characteristics of a *retrovirus,* but is a double- and single-stranded DNA virus rather than an RNA virus. A new family, *hepadnavirus* was created to include HBV and related animal viruses. HCV is a *flavivirus* or *pestivirus.* HDV, which can infect only those who are also infected with HBV, is akin to plant viruses (viroids, virusoids and plant satellite RNA). HEV is a *calicivirus,* again another family.

The A and E viruses are spread by the faecal-oral route, B, C and D by the parenteral route (needle stick, transfusion, etc) as well as by sexual contact, and from mother to child (Table 2). It appears that these viruses, all with a tropism for the liver, developed in different forms rather than evolving towards similarity.

Clinical aspects

Viral hepatitis is an infection of the liver by one or more of the hepatitis viruses and results in inflammation accompanied by a variety of symptoms, the most striking and pathognomonic being yellow jaundice. In the acute form, seen with HAV, HBV and HEV infection, there is a prodromal period of malaise, loss of appetite and sometimes flu-like symptoms. The urine darkens, the faeces may become light coloured, pruritus may be troublesome and fever develop. When jaundice appears, the symptoms usually abate and resolution can occur in weeks or months, but fatigue and malaise may persist for longer. The usual outcome is the development of protective antibody. Rarely, acute hepatitis may lead to fulminant hepatitis which is often deadly.

Acute infections with HBV, HCV and HDV may become chronic. However, more commonly, chronic infection with HBV will commence with an initial infection (particularly in the newborn and young) which is asymptomatic but persists for years. Many of those infected will, during the course of several decades, develop a life-shortening chronic liver disease and cirrhosis, and also HCC (which is deadly).

Epidemiology of the hepatitis viruses

Taken together, hepatitis viruses may constitute the most common viral infections of humans associated with disease states. For example, in the USA which by world standards is a relatively low endemicity area, viral hepatitis was the second most common infectious disease reported in 1990.

HAV

In high endemicity areas essentially all the population becomes infected with HAV by the time adulthood is reached. Even in relatively low endemicity areas such as the USA, 75% of adults will have been infected at some time in their lives.

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Table 1. The hepatitis viruses

Name	Virus family	Particles, shape	Virion size (nm)	Nucleic acid	Nucleotides
HAV	Picornavirus	Naked, spherical, icosahedral	~27	Linear, single-stranded RNA Does not integrate	7,480
HBV	Hepadnavirus ('para-retro virus')	Spherical	42	Circular, partially double-stranded, small portion triple-stranded DNA, RNA Integrates	3,200
HCV	Flavivirus, pestivirus	Spherical	55	Positive strand RNA intermediate Does not integrate	9,500
HDV	Plant viroid, virusoids or plant satellite RNA (?)	Spherical, covered with HBsAg	36 (some 40)	Single-stranded circular RNA	~1,700
HEV	Calicivirus	Spherical, non-enveloped	32–34	Single-stranded, polyadenylated RNA	~7,500

HBV

HBV infection is a much more serious cause of long-term mortality and morbidity; it leads to chronic infection, which markedly increases the risk of life-shortening chronic liver disease and HCC. There are probably more than 350 million carriers in the world, about 25% of whom are likely to develop the late sequelae of the disease. Endemicity is high in sub-Saharan Africa, in Asia and Oceania and, strangely, among Eskimos. It has been estimated that there are several million deaths per year as a consequence of HBV. A dramatic example of mortality in a high endemicity country comes from Taiwan where death from primary cancer of the liver, which is mostly due to chronic HBV infection, is the second most common cause of death in middle-aged men.

HCV

The frequency of HCV is low in most European countries, roughly similar to the prevalence of HBV carriers, but it is extraordinarily high in Zaire, Egypt and elsewhere in Africa and Asia. In Japan, HCV is now the most common cause of primary cancer of the liver and, until the introduction of donor screening programmes for HCV, was the major cause of post-transfusion hepatitis.

HDV

HDV occurs only in individuals who are also infected with HBV and is common worldwide among HBV carriers. The frequency varies from about 4% in parts of Europe to over 10% in southern Europe, Africa and North America. It is often associated with deadly fulminant hepatitis and primary cancer of the liver. It has been estimated that worldwide about 15 million people are infected with HDV. In the USA about 7,500 cases of acute HDV infection occur annually and there are about 70,000 carriers. About 1,000 persons die each year as a consequence of HDV infection.

HEV

HEV can cause enormous epidemics when water supplies become contaminated with the faeces of infected persons. It is probably responsible for many of the widespread epidemics of hepatitis in India and other regions where it is common. A particularly tragic feature of these epidemics is the high mortality among pregnant women (which may average about 20%). The reason for this unusual morbidity pattern is unknown, and its unravelling may teach us about viruses and both female and fetal physiology. Epidemics occur in Asia and Africa but have also been reported from Mexico and Borneo. Only sporadic cases occur in the USA and these appear to be imported.

It is clear that the hepatitis viruses are common and widespread, and that in many regions of the world two or more of them may occur in the same individual. This makes it likely that interactions will occur frequently.

Virology and molecular biology: the vaccine

HBV was the first human pathogen to be sequenced, possibly because of its very short genome (for review, see Ref 1). Following our discovery of HBV in 1967, applications of immunodiffusion, electrodiffusion and, later, radioimmunoassay and enzyme immuno-

Table 2. Modes of transmission of the hepatitis viruses

Name	Method of transmission	Form of hepatitis
HAV	Faecal-oral, enteric, water-borne	Acute
HBV	Blood-borne, venereal, mother to child, transfusion, needle injection, parenteral	Acute, chronic, carrier, primary cancer of the liver
HCV	Blood-borne, parenteral	As HBV
HDV	Same as HBV	Chronic, carrier-associated after HBV infection
HEV	Same as HAV	Acute, high mortality in pregnant women

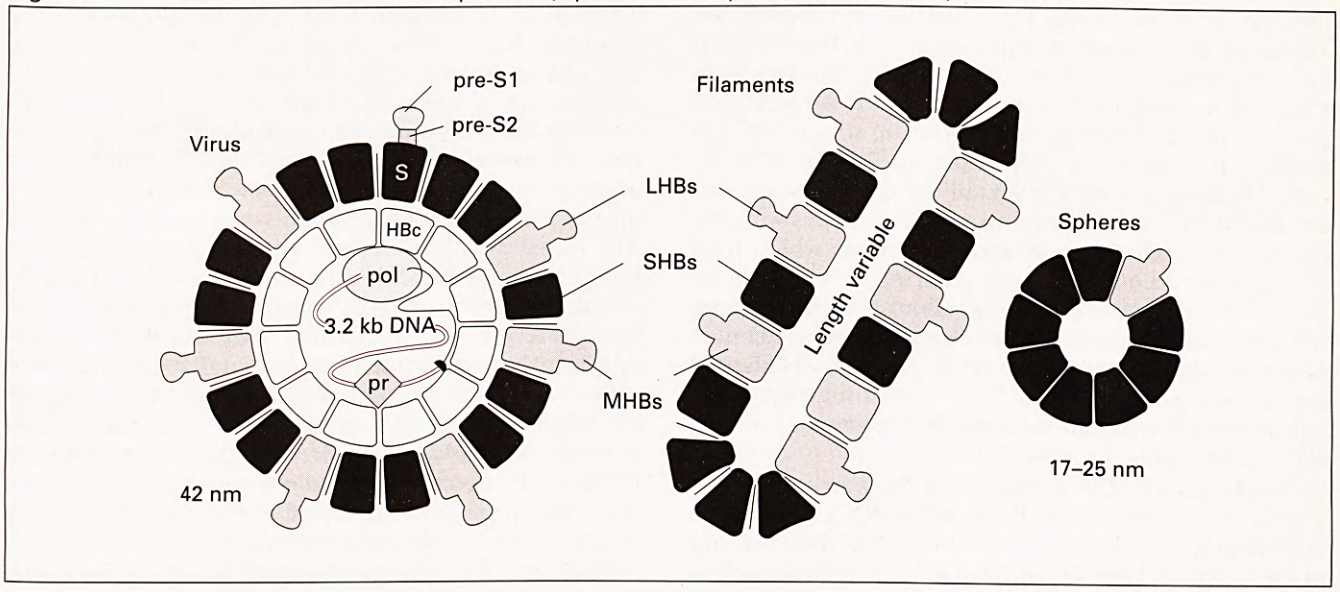
assay, resulted in the establishment of excellent diagnostic techniques. Screening of donor blood almost completey eliminated post-transfusion hepatitis due to HBV.

The diameter of the HBV virion is about 42 nm. The surface antigen (HBsAg) which is glycosylated and contains lipid, coats the virus and encloses the core. The core itself is encircled by the core antigen (HBcAg), which contains the DNA genome and the proteins involved in replication and other activities. In addition to the whole virion there are small spherical particles 17–25 nm in diameter and filaments of the same width but of variable length which contain only the surface antigen (Fig 1). These surface antigen particles were identified in our laboratory in 1968 and were used in the preparation of blood-derived vaccine.

The vaccine was invented before molecular techniques were available using simple epidemiological, clinical and biochemical methods. It had been observed that the presence of the antibody against HBsAg was rarely accompanied by the virus itself. A vaccine was therefore produced from the HBsAg particles which were present in enormous quantities in the blood of HBV carriers. This was a unique method for producing a vaccine which has been used safely and effectively in millions of people. Later, when recombinant methods were developed, the S gene was used to prepare a vaccine nearly identical to that produced by the blood extraction technique. The recombinant vaccine has the merit of easy and uniform production and the psychological benefit of *not* being blood-derived, but it is no more effective or safe than its naturally-derived predecessor.

The genome consists of 3,200 base pairs (Fig 2) and is double-stranded. Open reading frames (ORFs), of which there are four (S: surface antigen; C: core antigen; P: polymerase; X: function originally unknown), are defined as the bases between the first start and stop codons. The HBV genome has evolved towards minimal length; the total genome is only slightly larger

Fig 1. Schematic model of the HBV and HBs particles (reproduced with permission from Ref 2).



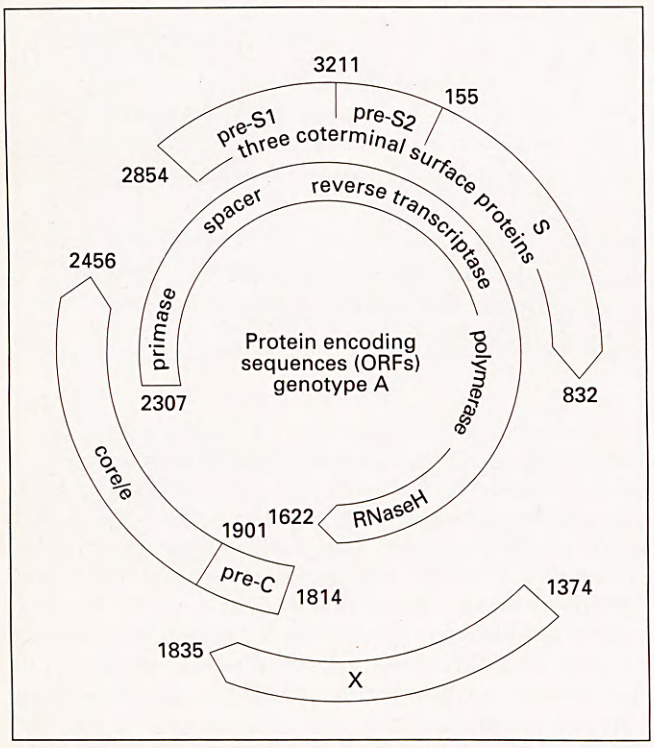


Fig 2. Hepatitis virus genome open reading frames (ORF) (reproduced with permission from Ref 2).

than the P genome, leaving a small region where there is no overlap.

The S ORF includes pre-S1, pre-S2 and S. The products of these three form a small (S product only), middle (S, pre-S2), and large HBs (S, pre-S1, pre-S2) protein. They occupy different positions on the virion and on the smaller particles which contain only HBsAg (Fig 1). S is the predominant surface antigen protein; the others are represented in different proportions in the several particles. We were the first to describe the existence of antigen subtypes on the surface antigen [3] which are manifested in the S protein; they have since been described in greater detail. All subtypes have a common antigenic portion, *a*, which renders them cross-antigenic. Therefore, a single vaccine, usually composed of a 'cocktail' of the subtypes, can be used for protection against all the subtypes with the exception of what may be escape mutants which have emerged in recent years. The sharing of this antigen portion of the molecule may reflect a further evolutionary economy by HBV, but it has proven detrimental to the virus in that it has made the manufacture of the vaccine much simpler. There are at least six genotypes of the S antigen but these do not correspond to the antigen subtypes.

The HBc and HBe proteins are the products of the C ORF. It packages its own messenger RNA (mRNA) as well as that of the viral polymerase, and also includes protein kinase, probably of host origin. This particle is encapsulated by the HBs protein before export. The ORF includes the pre-C region of 29 codons which, when translated along with the C region, produces HBe protein which can be secreted from the cells. Variants due to mutations in the pre-C sequence prevent the production of HBe but do not inhibit replication of the virus. (The possible reasons for this will be discussed in the strategies section).

The products of the P gene are not well defined but are necessary for the replication of the virus. It appears to produce a primase, necessary for priming minus strand synthesis, RNA- or DNA-dependent polymerase (reverse transcriptase), and an RNAaseH which can cleave the RNA in RNA/DNA hybrids. The polymerase protein is packaged in the core.

The X sequence is absent or displaced in at least one of the hepadnaviruses. The nature of the X codon suggests that it has been introduced only recently into the virus from the eukaryotic genome, and homologous sequences are found in primate genomes. The product of this ORF is involved in the interaction of the virus with the host cells and therefore may be involved in the evolutionary relations between HBV and its human host. In *in vitro* experiments it transactivates a variety of genes. It may also be important in the carcinogenic potential of HBV and has been shown to have a tumorigenic effect in immortalised mouse hepatocyte cultures and transgenic mice.

Life cycle of HBV

The sequence of events in the life cycle of HBV is based on experimental studies and inferences from the life cycle of other viruses (Fig 3). There is no agreement on the nature of the attachment site for HBV; in fact, there may be several, relatively weak attachments. The pre-S1 sequence 21–47 appears to attach to a membrane-bound interleukin-6, but it does not appear to be a major site. Pre-S1 may also be involved in attachment to an immunoglobulin (IgA) receptor in a process involving molecular mimicry. The glycan linked to pre-S2 may be a site of attachment since it has a weak affinity for HepG2 cells (derived from a human cancer patient), but again this does not seem to be a sufficient site. The attachment of pre-S2 to polymerised serum albumin may serve as a means for attachment, but this too is not sufficient. The transferrin binding site on cells may mediate the attachment of pre-S2, and could be related to the unusual relation of HBV replication to serum iron storage levels (see section on body iron stores). The apparently weak attachments of HBV to liver (and other) cells may be part of the evolutionary strategy designed to maintain a high level of viraemia for long periods. If affinities were strong, the viraemia would be less and the cells become overburdened with virus, decreasing the probability of chronicity because the cells would be more readily eliminated.

How does the virus get into the cell? It has been sug-

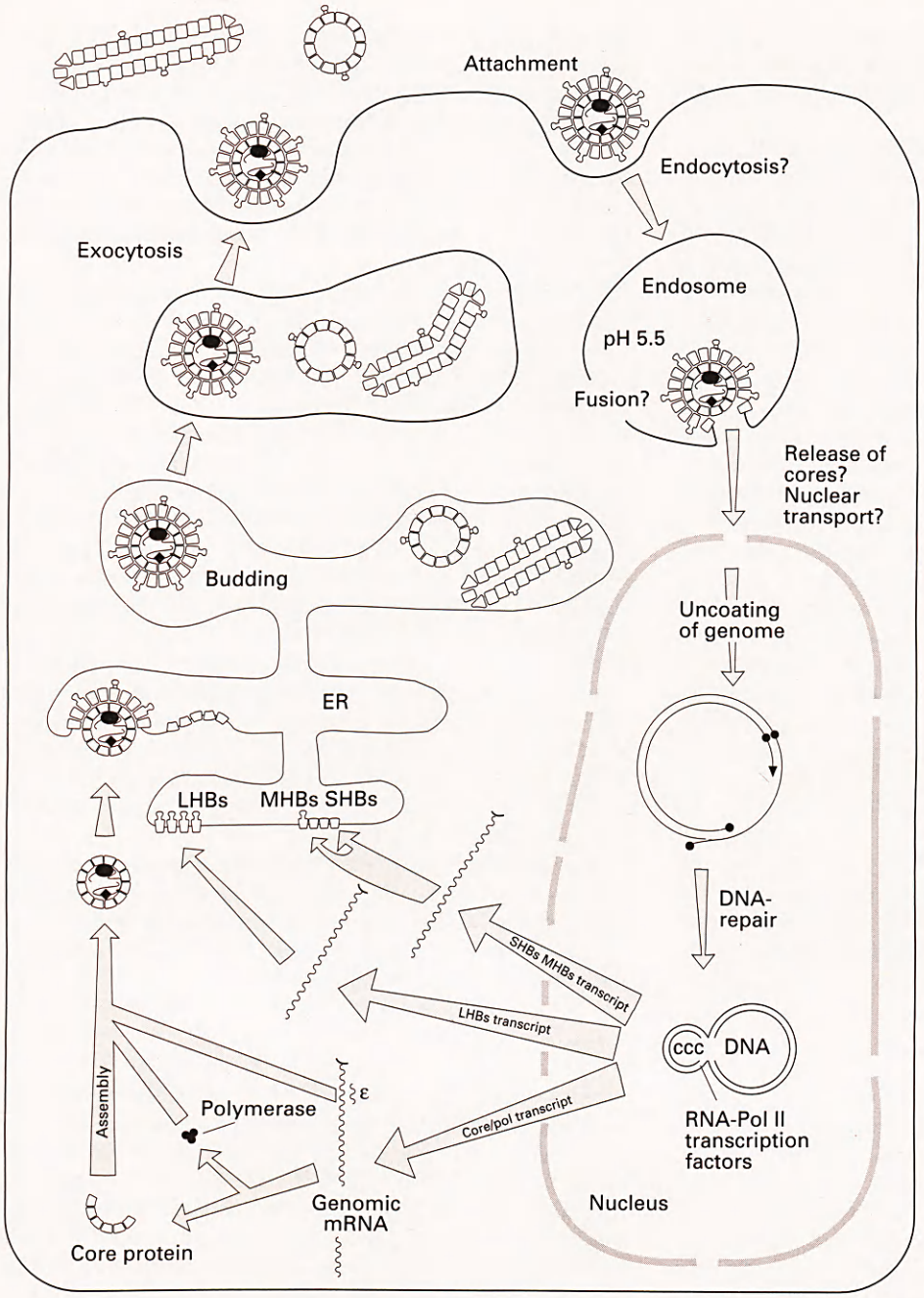


Fig 3. Schematic view of the life cycle of HBV (reproduced with permission from Ref 2).

gested that endocytosis, mediated by the transferrin receptor mechanism, may effect entry. The viral genome is then released into the nucleus and forms a double-stranded covalently closed circle. Viral polymerase helps to close the gap, though other proteins may also be utilised. The HBV DNA can integrate into the host DNA, a characteristic it shares with retroviruses. In contrast to retroviruses, though, it is not essential to the replication process and may, in fact, hinder it since the linearisation will interrupt at least one of the HBV genes. The integrated HBV DNA does not appear to replicate, but occasionally fuses with the host DNA. These fusions are found in patients with HBV-induced cancer of the liver.

It is interesting to conjecture how viruses containing portions of human genome might behave if they could replicate and be transmitted. They could bring DNA sequences from one human host into another, and integration could then occur. If this took place in human germ cells and the genes were transmitted to

the next generation, it would represent a method of acquiring heritable genetic material after birth—a fascinating technique for altering the evolutionary process.

After the covalently closed DNA is formed, the episomal DNA is transcribed to RNAs which serve as templates for their respective proteins (eg polymerase, core). These proteins combine with their mRNA in the replication complex. The primase portion of the polymerase serves as a primer for the reverse transcriptase which then proceeds to produce the negative strand DNA. An RNAaseH associated with the transcription removes the RNA template with the exception of an 18-base RNA fragment. This serves as a primer for the generation of the positive strand DNA, and the structure of the virion DNA is reproduced.

The transcripts for the several viral proteins are excreted from the nucleus into the cytosol. The core protein and polymerase are assembled into the core particle which contains the replicated DNA, and enter the rough endoplasmic reticulum where they are covered with the surface protein assembled from the S antigen component transcripts. The whole virions as well as the particles containing only HBsAg are manufactured in the endothelial reticulum and then exported from the cell, presumably by exocytosis. They are then free to enter other cells or to be transmitted to other individuals by the mechanisms already discussed. As Gerlich has noted [1], this complicated process of replication can be seen as an effort by the virus to persist at reasonably high levels in the blood so that it can be transmitted sexually, from mother to child, and by other mechanisms of blood transmission from one person to the next.

The recombinant vaccine

Using recombinant techniques, several investigators isolated clones of the S reading frame and inserted them in a variety of cells (*Escherichia coli*, yeast, human, etc). Vast quantities of vaccine are now manufactured by the recombinant process. It is the first (and, so far, only) commercial recombinant vaccine and one of the financially most successful recombinant products. It is now being used in population-based vaccination programmes involving millions of individuals.

Hepatocellular carcinoma

The earliest evidence for a relationship between HBV and HCC was discovered during field and epidemiological studies in Africa and Asia. Chronic HBV infection was much more common in the HCC cases than in controls, particularly in regions where the HBV carrier rate was high. Prospective studies in Taiwan, and later in Alaska and elsewhere, showed that individuals who were carriers at the start of a study were much more likely to develop HCC than those in the same environment who were not carriers. In Taiwan, the risk was more than 200-fold (much greater than that of heavy cigarette smokers for cancer of the lung).

Integrated sequences of HBV are found in the DNA of host liver cells. Integration is also seen in HBV carriers, and several explanatory models for the role of integration in the pathogenesis of HCC have now emerged. It is likely that a number of mechanisms are operative and may provide concurrent or alternative pathways to disease.

The host's immune response results in the slow but continuous death of liver cells infected with HBV. This, in turn, promotes cell division in the remaining cells and could increase the likelihood of mutational carcinogenic events occurring over time. The integration sites do not appear to be specific. It was suggested that insertional activation of potential oncogenes might be the explanation. This is rarely seen in humans, but quite commonly in the HCC which occurs in woodchucks infected with woodchuck hepatitis virus (WHV). Experimental evidence from studies on transgenic mice indicates that unregulated expression of either the X protein, a transcriptional activator, or the large surface antigen might be carcinogenic. Integrated HBV sequences may alter host cell growth in transgenic mice by unregulated expression of proteins. Loss of heterozygosity on chromosomes 1p, 4q, 11p, 13q, and 16q in patients with HCC is consistent with the loss of 'anti-oncogenes', and so increases the risk of cancer.

The existence of many models increases the opportunities for identifying medications that could interfere with the pathogenic processes. If taken in the appropriate combinations they could improve treatment of a deadly cancer.

Adaptations

HBV is one of the simplest viruses, yet it has developed remarkable adaptive and evolutionary techniques to perpetuate its existence. It can be argued, for example, that it has adapted to the computer age. In the mid-1970s an HBV epidemic occured among laboratory and hospital staff in an institution where computer techniques had been installed for the recording and transmission of laboratory data. Data were recorded on computer cards which were despatched from laboratory to clinic with the necessary information. Some of these computer cards became soaked with the blood of an HBV carrier, and the virus was transmitted to individuals who cut their fingers on the sharp edge.

An epidemic of acute hepatitis B involving several hundred cases developed in Swedish orienteerers. Its cause and remedy were soon found: running through the brush, the runners, wearing only shorts, suffered multiple scratches on their legs and drops of blood were left on the low-lying brush. Contestants following them could be infected with HBV if a previous runner was a carrier. In addition, there were check-points where runners could wash the blood from their legs

using a common bucket and/or towel, which provided mechanisms for transmission. When the sportsmen were required to wear long pants, the epidemic ceased, a simple remedy to a complicated pathogenic process.

Interactions between the viruses

Hepatitis viruses A, B, C, D, E and possibly F have evolved different strategies for survival, transmission, replication and probably pathogenesis. Interactions with human immunodeficiency virus (HIV) may be included here because co-infection often occurs and, in the past few decades, HIV may have affected the evolution of some of the hepatitis viruses.

In general, superinfection with other hepatitis viruses will decrease the replication of HBV. This is particularly true with delta virus (HDV); it suggests that an attenuated delta-like virus might be used to decrease HBV replication. In chimpanzees with chronic HCV infection, the intensity of acute hepatitis due to HBV or HAV is attenuated. Also, co-infection with HBV appears to decrease the replication of HCV.

During the great Shanghai epidemic of HAV in 1988 when thousands became infected as a consequence of contaminated shellfish, 15 of the 47 deaths were associated with chronic HBV carriage and in 11 of them there was active HBV replication. However, this effect has not been seen in regions of lower endemicity for HBV.

Interactions between HIV and HCV may have had a profound effect on both viruses. The spread of HIV started some time in the late 1970s (probably mostly due to changes in human behaviours). The spread of HCV may also be recent, possibly starting at the same time and for the same reasons. This could be a consequence of mutual 'support' between the two viruses. The transmission of HCV from mother to child is ordinarily not very effective, but if the mother is also infected with HIV, perinatal infection with HCV is more likely to occur. Infection of HCV carriers with HIV results in a more severe clinical course, probably associated with increased viral replication. This, in turn, might increase the probability of subsequent HCV transmission.

There are undoubtedly many interactions that affect the mutual evolution of these viruses, and continuing studies of all of them together (not an easy task) are likely to yield fascinating insights into general principles of viral evolution.

Molecular and immunological evolutionary strategies

A small compact genome may contribute to the hardiness of a virus. All the hepadnaviruses appear to have evolved toward minimal genome length, and the whole genome is only slightly larger than P, its largest ORF, and much overlapping has occurred. This can decrease the probability of mutation since a change in one gene will, in most cases, be accompanied by a mutation in another (usually P), which could be lethal to the virus.

Perhaps because of its limited genetic resources HBV has learnt to use the host extensively. The overall strategy appears to be the maintenance of a high level of virus in the blood and other body fluids to allow efficient transmission if even small amounts of blood and/or fluid are transmitted from one individual to the next. As noted, the absence of high-affinity receptors for HBV on hepatocytes and other cells helps to maintain a high level of circulating virus and HBs particles. A second major strategy is the development of chronic carriage infectious virus; for this, it must evade the immune system at least during the period when natural transmission is most effective.

HBV has seized on the most necessary biological functions of humans to ensure its own perpetuation, namely, sexual intercourse and childbirth. In premodern times, together with folk practices such as ritual circumcision, tattooing, skin cicatrix decoration, ceremonial blood exchange ('blood brotherhood') and others by which blood could be transferred from one individual to the next, these were the main methods of transmission. Before the availability of disposable equipment, the re-use of needles and other medical instruments has been a major mechanism for transmission. Until the development of blood screening programmes in the past decade, blood transfusion was an important method of transmission, but this has now been controlled.

The mechanisms by which the immune system is evaded are complex and, in some cases, unique. HIV uses a direct and brutal frontal attack on the cells which affect the immune responses of the body, thereby wreaking a tragic toll on those it has infected, whereas HBV is more subtle and benign. In many cases, infected individuals can remain asymptomatic carriers for many decades, thereby extending the period in which the virus can be transmitted.

Large numbers of circular and elongated particles which contain only surface antigen are produced by the infected cell. These can serve as a 'smoke screen' in the blood and body fluids which will engage the immune 'attack' systems of the host and spare the less numerous replicating whole virion. They may also block receptors on cell surfaces and maintain high levels of viraemia. Ironically, this strategy has worked to the disadvatage of the virus in that these HBsAg particles have been used to prepare a vaccine which has led to control, and could conceivably lead to elimination, of the virus.

The pathological effects of HBV are caused not by a direct destructive effect on liver cells but by the attack of the host immune system on the hepatocytes containing HBc and probably other viral antigens on their surface. To maintain chronic infection, destruction of the infected cells is mitigated by an interesting viral

mechanism involving the HBc/HBe system of protein encoded by the C reading frame. If translation starts at the second start codon, thereby not translating the pre-C region, HBc is produced. It is not excreted, but remains in the cell to coat the DNA and other constituents that form the core of the virus.

If translation begins at the first start codon, both pre-C and C are translated and HBe is produced. HBe shares most of its amino acid sequences with HBc and can be excreted, because the pre-C region peptides encode a signal sequence which probably facilitates secretion through the cell membrane. HBe appears to inhibit the destruction of hepatocytes which contain HBV and exhibit HBc on their surface by interacting and 'disarming' the host immune cells, probably by making them tolerant to HBcAg which is part of the HBe protein. This prolongs the life of the cells and allows replication to continue as an unhurried and lengthy process, thus preserving the life of the host usually through the decades of its most intensive sexual activity and the child-bearing period, so that he or she will have ample time to serve as a transmitter of HBV. When HBe is absent but replication of the virus persists, which is the case in pre-C mutant viruses, a much more severe and progressive disease may ensue.

HBe may also have an important effect on transmisson to the newborn. There is a remarkably high frequency of maternal transmission (90% or more) when the mother is HBeAg-positive. The excretable HBe present in the pregnant carrier can pass through the placenta and 'tolerise' the immune cells of the fetus. When exposed to the virus some time during or after the pregnancy and delivery, the immune systems will not reject it, and a long period of carriage can ensue.

HBV appears to have developed additional mechanisms to alter the host immune responses and perpetuate the carrier state. Interferon is effective in resolving acute HBV infection, but this appears to be altered in chronic HBV infections. Peripheral blood mononuclear cells from patients with acute and chronic HBV infection produce less interferon, apparently mediated by the core region of HBV. In addition, chronically infected cells, both hepatocytes and immune cells, are only minimally activated by interferon, which may be due to the virus itself or to interference by HBV polymerase.

HBV has been successful in invoking a series of mechanisms which perpetuate the life of the infected cells, induce long-term infection with consequent advantages to the transmission of the virus, and allow the host to live relatively symptom-free, usually for many decades and often entirely encompassing the child-bearing period. After that time, the effects of viral mutation can accumulate, the flexibility of the host immune response may decrease and finally become exhausted. Severe hepatic damage ensues and the host dies. In pre-modern times this may not have had much effect on overall human survival since life expectancy was short and most carriers would have succumbed to other causes of death before the destructive phase of HBV chronic infection could take over. In any case, the main transmission goals of the virus would have been accomplished by that time, and extended chronicity would have been of only marginal advantage to the virus.

Complex interactions with host and environment

Gender

Interactions of HBV with its host and the external environment have a significant bearing on infection with HBV and the pathogenesis of liver disease and HCC.

Gender is an important variable. Men, when infected, are more likely to become carriers while women are more likely to develop antibody to the surface antigen and remain protected. Therefore, in most populations more men than women are carriers. Male carriers are at much greater risk of developing HCC than women. Ironically, while the carrier females transmit the virus to their children, their male children are more likely to become carriers.

An even more remarkable observation is that in populations with a high frequency of carriers, the nature of the parents' response to HBV infection has a bearing on the gender of their offspring. In a series of studies in Greece, Papua New Guinea, the Philippines and Greenland, carrier families (ie families in which either parent is a carrier) were likely to have a higher sex ratio (more sons than daughters) than families in which the mother had anti-HBs. This effect is large and could account for the lower than expected number of female births in areas of high carrier endemicity such as south China. If verified, it is a strange observation that a viral infection could have a role in human sex determination; and therefore in human evolution.

Aflatoxin

Aflatoxin is a carcinogenic toxin produced by fungi which infect stored grains and other foods such as ground-nuts. Epidemiological studies have shown a high prevalence of HCC in areas where aflatoxin is common. Ross *et al* reported a study from Shanghai which involved 18,244 men with a total of 35,299 years of follow-up [4]. The risk ratio for HCC in individuals who were not carriers of HBV and were aflatoxin-negative was taken as 1.0. Those who had HBV without elevated urinary aflatoxin had a risk ratio of 4.8 (CI 1.2–19.7), for those with elevated aflatoxin who were not carriers it was 1.4 (CI 0.5–7.5), while those who were both carriers and had elevated aflatoxin had a remarkable risk ratio of 60.1 (CI 6.4–561.8). Hence, aflatoxin alone may not cause HCC, but combined with HBV infection its effect is profound. If these studies are confirmed, a potent and preventable co-

factor for the development of HCC will be revealed and will validate an important method of control in addition to HBV vaccination.

Genetic control

Some of our earliest studies appeared to show genetic control of the propensity for a person infected with HBV to become a carrier. Recent studies are consistent with this hypothesis. The gene, if it exists, has not been identified. One or more of the sites of HBV integration could be a possible location of such a gene(s).

There also appears to be genetic control of the carcinogenic and other effects of aflatoxin. The story, as described by McGlynn and her colleagues [5], is complex. A mutation in the p53 tumour suppressor gene has been identified in HCC in some populations, in particular those with a high prevalence of aflatoxin. There is a mutational hotspot in codon 249 of exon 7 of the p53 gene which is postulated to be related to aflatoxin exposure. Two gene loci, EPHX and GSTM1, have been identified in relation to aflatoxin detoxification. A mutant allele of EPHX and a null allele of GSTM1 decrease the aflatoxin detoxification capability. Both genes occur significantly more frequently in patients with aflatoxin, HCC and/or the p53 mutation. This observation, if confirmed, indicates a genetically controlled susceptibility to the carcinogenic effects of aflatoxin, and helps to explain why some people exposed to aflatoxin and HBV develop HCC while others do not.

Body iron stores

Plasma ferritin levels are directly correlated with body iron stores, and transferrin levels are inversely related. A study in Taiwan found that increased ferritin and decreased transferrin (ie increased body iron stores) determined at the start of a prospective study are associated with a higher risk of developing HCC or certain other cancers. In Korean patients with chronic liver disease who were carriers of HBV, those with elevated ferritin levels were significantly more likely to develop HCC than those with lower levels. This effect is not restricted to HCC. In Philadelphia, McGlynn *et al* [5] have shown that increased ferritin is also associated with shorter survival in cancer of the lung, breast and colon. Total body iron stores can be controlled by dietary intake, and it is possible that excess iron intake may be detrimental.

Additional evolutionary factors

HBV-like viruses in other species

Hepadnaviruses have been found in woodchucks (WHV) and ground squirrels (GSHV). Less well characterised viruses include the tree-squirrel hepatitis B virus (TSHBV), and a virus reported in kangaroos. Avian viruses have been reported in the domestic Peking duck (DHBV) and in herons. Viruses which appear to be similar to DHBV have been found in wild Mallard ducks in Michigan and Illinois. Several of these non-human hepadnaviruses have been sequenced and their phylogenetic relationships estimated using base pair comparisons.

It is interesting that the cauliflower mosaic virus family *phytopararetrovirus,* DNA plant viruses utilising reverse transcription during replication, are similar to human hepadnaviruses. It has been suggested that these two families, animal and plant, might be grouped into a superfamily to indicate their relationship.

An additional observation is that HDV is similar to several of the very small plant RNA viruses.

Is it possible to infer from these observations that these viruses have remote affinities with plants and plant viruses, and also that anti-viral strategies might be sought in the plant kingdom?

Human inheritance of HBV genomes: a conjecture

In common with the retroviruses, hepadnaviruses integrate into the DNA of the host genome. However, the integration is not essential for replication; on the contrary, it probably *decreases* replication and production of gene products, in that linearisation of the circular DNA splits at least one of the reading frames. Integration is common in cancer of the liver, but also in individuals who have been carriers for some years.

Does this unusual viral strategy serve a biological function? It may be conjectured that, with massive infections and in large epidemics, HBV DNA may infect and integrate into the DNA of the host germ cells. At the time of reproduction these could enter the gene pool of the population and segregate in the same manner as ordinary host genes; it would be analogous to mutation. Although germ cell integration may be relatively rare, in areas of massive infection such as East Asia and sub-Saharan Africa the absolute number of such integrations could be large. (Sections of the human genome have homologies with HBV, but little is known about how they relate, if at all, to this conjectural model.) It would represent a form of inheritance of acquired base pair sequences, and could lead to interesting models and observations on the effect of viruses on human population genetics. HBV DNA, so integrated, could be viewed as an 'engine' of evolution.

Conclusion

I have emphasised *complexity* in the natural history and pathogenesis of HBV infection. A traditional goal of the scientific process has been simplicity, unitary aetiological hypothesis and linear relationships between cause and effect. But in the natural world, interaction

and involvement with the external and internal environments are the norm. To describe the world as it is, we have to attempt to embrace complexity. Furthermore, the more we learn of the constituents of this complex system, the more possibilities there are to intervene for prevention and treatment.

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