

Viruses Causing Hepatitis

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INTRODUCTION

Viral hepatitis is a major public health problem throughout the world affecting several hundreds of millions of people. It was first described by Hippocrates in the 5th C BC (Table 1, Purcell, 1993). The six hepatitis viruses, named A, B, C, D, E and G, include a range of unrelated and unique human pathogens. The routes of transmission may be faeco-oral, as for hepatitis A and E or parenteral, as in hepatitis, B, C, D and G infections.

Viral hepatitis is a cause of considerable morbidity and mortality in the human population, both from acute infection and chronic sequelae which include, chronic active hepatitis and cirrhosis. Hepatocellular carcinoma which is one of the ten most common cancers worldwide, is closely associated with hepatitis B, and in some regions of the world with hepatitis C virus.

Table 1 History of hepatitis and virus discovery

<i>Time</i>	<i>Event</i>
5 th C BC	Hippocrates describes jaundice, advocates honey and water diet
8 th C AD	Infectious nature of viral hepatitis described
17 th & 19 th C AD	Epidemic jaundice in military and civilian populations during wars Lurman reports post-vaccination outbreaks in dockworkers WWII, outbreaks after vaccination for measles and yellow fever
1947	Mc Callum classifies serum hepatitis and infectious hepatitis
1965	Blumberg discovers Australia antigen (HBsAg) in aborigines
1970	Dane discovers complete HBV particle
1972	Magnius identifies HBeAg
1973	Feinstone, Kapikian and Purcell identify HAV
1977	Rizzetto describes delta antigen (HDV)
1983	Balayan identifies HEV from Non-A, non-B hepatitis specimens
1988	Chiron group clones and identifies HCV
1995-96	Abbott and Genelab reported discovery of GBV-C and HGV respectively, later proved to be the same

This is a brief review of the structure and replication of viruses causing hepatitis, since epidemiology, pathogenesis, diagnosis and management are reviewed by other authors.

Hepatitis A virus

Hepatitis A virus (HAV), of the genus *Hepatovirus* in the family *Picornaviridae*, is a 27nm, unenveloped, icosahedral RNA virus which is the cause of infectious or epidemic hepatitis transmitted by the fecal-oral route (Regenmortel *et al*, 2000). There are 12 capsomers per nucleocapsid and only one serotype. There are two major human genotypes, I and III, the sequences of which differ at greater than 15% of the bases studied. Genotypes II and VII have also been described from humans, and genotypes IV, V and VI from non-human primates (Lemon and Martin, 2004).

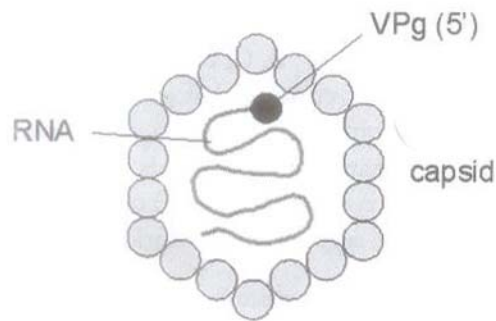


Fig. 1 Schematic structure of hepatitis A virus

The HAV genome comprises about 7,500 nucleotides (nt) of positive sense RNA which is polyadenylated at the 3' end. It has a polypeptide (VPg) attached to the 5' end, which has a hairpin structure. A single, large open reading frame (ORF) occupies most of the genome and encodes a polyprotein with a theoretical molecular mass of Mr 252,000 (Figure 1). The HAV polyprotein is processed to yield the structural (located at the amino-terminal end) and non-structural viral polypeptides. Many of the features of replication of the picornaviruses have been deduced from studies of prototype enteroviruses and rhinoviruses.

Viral replication takes place in the cytoplasm of the infected cell and most likely occurs by a protein primed mechanism similar to that which has been proposed for poliovirus (Paul *et al*, 1998). The first step in replication is likely to be the binding to a specific cellular receptor. In cultured cells, a candidate receptor, HAVcr-1, has been identified. Uncoating and release of viral RNA into the cytoplasm occurs within 4 hours after infection (Bishop and Anderson, 2000). Subsequent steps in the viral life cycle include i) the translation of the large open reading frame leading to the synthesis of a giant

polyprotein, ii) the transcription of a negative strand copy of the viral genome, iii) the synthesis of new positive-strand RNA molecules from the negative strand intermediate, iv) the packaging of these new viral RNAs into assembled viral particles and v) the release of virus from the infected hepatocyte into the biliary canaliculi. The positive sense virion RNA acts directly as messenger RNA for syntheses of the large polyprotein following its release into the cytoplasm and elimination of the virally encoded peptide, VPg. Protein translation is initiated under the control of the internal ribosomal entry site (IRES) located within the 5' untranslated region. The polyprotein is co- and post-translationally processed into both structural and non-structural proteins. The amino-terminal 40% of the polyprotein contains the structural proteins that form the capsid, which result from further processing and a 'maturation cleavage' of precursor proteins. The assembly of new viral particles occurs within membranous vesicles, which appear to be transported to the plasma membrane where it interfaces with the hepatic canaliculi, resulting ultimately in the secretion of virus into the biliary system (Martin and Lemon, 2002).

Hepatitis E virus

Hepatitis E virus (HEV), now provisionally assigned to the genus *Hepevirus*, is the cause of enterically-transmitted non-A, non-B hepatitis. It is another non-enveloped, single-stranded RNA virus, which shares many biophysical and biochemical features with caliciviruses and togaviruses. Although there is a single serotype, there are multiple genotypes. Hepatitis E virus is an important cause of large epidemics of acute hepatitis in the subcontinent of India, Central and Southeast Asia, the Middle East, parts of Africa and elsewhere. This virus is responsible for high mortality during pregnancy, particularly during the third trimester.

Hepatitis E viral particles are non-enveloped, spherical, 27-34 nm, with icosahedral symmetry and contain a positive sense, single-stranded RNA genome of about 7.2 kb. Transfection studies indicate that the full-length RNA genome is infectious (Panda *et al*, 2000). The genome has a capped untranslated region at the 5' end and a poly (A) tail at the 3' end. The 3' end of the genome appears to interact with the RNA-dependent RNA polymerase and may serve as an initiation site for replication. There are three open reading frames. The first, ORF 1, encodes 1690 amino acid (aa) protein that is cleaved into a number of nonstructural proteins including an RNA dependent RNA polymerase, a helicase and a cysteine protease. The ORF2 encodes a 660 aa protein which is the major capsid protein, which is expressed intracellularly and contains the immunodominant epitopes

(Khudyakov *et al*, 1994). The ORF-2 protein has been shown to self-assemble into virus-like particles in the absence of either ORF-1 or ORF-3 (Xing *et al*, 1999). The third ORF encodes a structural phosphoprotein of 123 aminoacids which also contains epitopes and may serve as a membrane anchor and play a role in signal transduction (Tyagi *et al*, 2001).

The replication of HEV is probably limited to the hepatocyte and the bile duct epithelium. It has been postulated that after HEV enters the hepatocyte, the positive sense HEV RNA is translated to produce the nonstructural proteins. Either by itself, or in association with cellular proteins, the RNA dependent RNA polymerase directs the generation of negative stranded pregenomic RNA from the 3' end of the genome. Subsequently, positive strand RNA synthesis takes place with the negative strand RNA serving as a template. The positive strand RNA and subgenomic RNAs produce the structural proteins that encapsidate the positive strand to form nascent viral particles (Jameel, 1999).

Hepatitis B virus

Hepatitis B virus (HBV), a member of the *Hepadnaviridae* family, is a double-stranded DNA virus which replicates, unusually, by reverse transcription. Hepatitis B virus is endemic in the human population and hyperendemic in many parts of the world. A number of variants of this virus have been described. Natural hepadnavirus infections also occur in other mammals including woodchucks, ground squirrels and ducks (Koff, 2004).

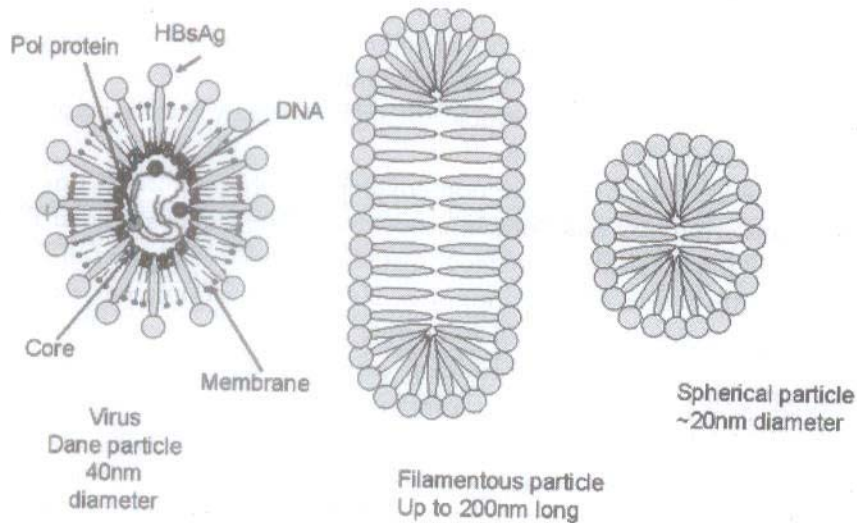


Fig. 2 Schematic representation of circulating Hepatitis B virus particles

Complete HBV particles have a double shell with a diameter of about 42 nm, consisting of a 27 nm spherical, icosahedral nucleocapsid core and 7 nm wide surrounding envelope. HBV is associated with three circulating particles, the 42 nm complete particle, smaller spherical particles of 22 nm diameter and filamentous particles which are 22nm wide and may be up to 200 nm in length (Figure 2).

The 22 nm particles are present far in excess of the 42 nm particles and are composed of the surface protein, carbohydrate and lipids (Tiollais *et al*, 1985). The principal protein of the envelope is HBsAg, which has 226 amino acids, encoded by the *S* open reading frame. Group specific determinant *a* and subtype determinants *d*, *y*, *w* and *r* are also encoded by ORF *S* and are present on the hepatitis B surface antigen (HBsAg). The nucleocapsid consists of 180 repeating subunits of its core protein which contains C-terminal packaging signals and nuclear localization signals. The nucleocapsid contains the circular DNA, a covalently attached primer protein, HBV DNA polymerase, reverse transcriptase and protein kinase activity. The core protein expresses a major antigenic reactivity, the hepatitis B core antigen and self assembles into a capsid-like structure. A related antigenic reactivity known as the hepatitis B e antigen is a non-particulate, soluble antigen derived from HBcAg by proteolytic cleavage. Each nucleocapsid contains a circular partially double stranded DNA genome. The length is 3020 to 3320 nucleotides for the long minus strand and 1700 to 2800 nucleotides for the short strand. The long strand is organized into four overlapping ORFs, S, C (core), X and P (Pol). S and C have associated upstream regions termed pre-S and pre-C, respectively. Table 2 lists the gene products of each open reading frame.

Table 2 Hepatitis B virus open reading frames, gene products and amino acid residues of identified proteins

ORF	Gene product	Amino acid residues
Pre-S1, pre-S2, S	Large HBsAg protein	400
Pre-S2, S	Middle HBsAg protein	281
S	Small (major) HBsAg protein	226
Pre-C, C	HBcAg	189
C	HBcAg	212
P	DNA polymerase	844
X	HBxAg	154

The liver is the major target organ and the principle site of HBV replication. The initiating events of hepatitis B virus infection are poorly understood. HBV attachment to a specific cell surface hepatocyte specific viral receptors has been postulated to involve binding affinity sites on the

pre-S1 and pre-S2 proteins of the HBV envelope. Regardless of the exact mechanism of initial attachment, HBV entry into the hepatocytes probably results from fusion of the viral and host cell membranes. The nucleocapsid is transferred to the cell nucleus and HBV DNA is released in an open relaxed circular form. Integration of subgenomic fragments of HBV DNA or the intact HBV genome into the host genome of some hepatocytes may occur during HBV infections, but is not a required step in replication. In the hepatocyte nucleus, the relaxed circular HBV DNA is converted to a covalently closed circular DNA by repair of the positive DNA strand. This fully double stranded DNA serves as the template for the synthesis of a series of RNA transcripts that include a pre-genomic RNA with about 3,500 nucleotides and smaller RNA transcripts (Ganum and Varmus, 1987). After translation, the HBV reverse transcriptase is bound to the pre-genomic RNA which serves as a template for reverse transcription, leading to the production of full length minus DNA strands as well as messenger RNA for the core and polymerase proteins. The pre-genomic RNA transcript and the HBV reverse transcriptase are encapsidated in core particles. Phosphorylation of the core proteins occurs within the core particles and simultaneously the RNA template is degraded by RNase H. The structural and non-structural viral proteins are translated from the smaller RNA transcript, which vary in size from 700 to 2400 nucleotides. In contrast to reverse transcription of the long strand, the short positive sense strands are believed to be generated from templates of negative stranded DNA. Current evidence suggests that HBsAg envelope is formed, at least in part, as a transmembrane polypeptide of the endoplasmic reticulum and is added to the core particle in the endoplasmic reticulum. The intact nascent HBV particle is then exported from the hepatocyte by a poorly understood process.

Hepatitis C virus

Hepatitis C is a lipoprotein enveloped RNA containing virus that has been classified in the genus *Hepacivirus*, in the family *Flaviviridae*. Several genotypes have been identified. A high degree of HCV variability which may favour viral persistence has been attributed to a high rate of viral replication coupled with poor or absent proof-reading ability of the RNA polymerase leading to failure to detect transcriptional errors. Infection with this more recently identified virus is common in many countries. Hepatitis C virus is associated with chronic liver disease and also with primary liver cancer.

Classic filtration studies suggested a size between 35 and 60 nm particles, and electron microscopic studies on an enriched plasma sample show particles of 55-65 nms which may be the actual virions. Treatment with detergent liberates 30 to 35 nms icosahedral particles, which may associated with

nucleocapsids (Takahashi *et al*, 1992). HCV is a linear particle single stranded RNA genome which has 9400 to 9600 nucleotides. The genome has a 5' untranslated region of about 340 nucleotides which is well conserved and a single large open reading frame that encodes a polyprotein of approximately 3000 aminoacids. The conserved 5' non translated region contains an Internal Ribosome Entry Site (IRES) element at which HCV polyprotein translation is initiated. Small hairpin structures and multiple stem loops may be involved in modulating viral transcription. After the large ORF are short but variable length 3' non-translated regions containing poly (A) or poly (U) sequences which are thought to be involved in RNA replication, packaging and infectivity (Yanagi *et al*, 1999). The large polyprotein is subjected to co-translational and post-translational cleavage through the action of cellular signal peptidases and virus specific proteinases (Bartenschlager and Lohmann, 2000). Approximately one third of the polyprotein comprises of structural proteins, which are an internal protein called the core or C-protein, as well as two glycosylated proteins, called E-1 and E-2, which are present in the envelope (Figure 3). The remaining two-thirds of the polyprotein is cleaved into distinct non-structural proteins that are involved in the replication of HCV and have multiple enzymatic activities and also function as transcriptional modulators (Lin *et al*, 1994). The non-structural proteins may form complex interactions with each other and recruit cellular proteins for some components of viral replication and production of virions.

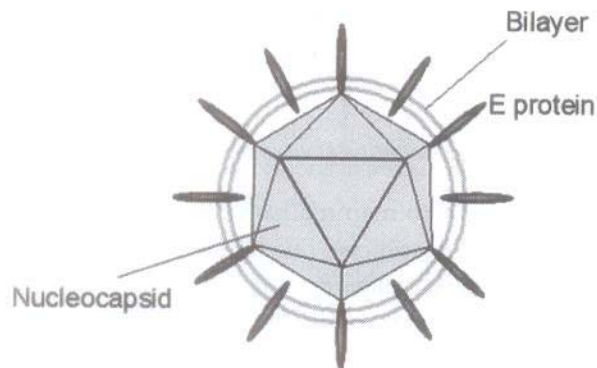


Fig. 3 Proposed structure of Hepatitis C virus

Hepatitis D virus

Hepatitis D virus (HDV) is an unusual, single-stranded, circular RNA virus with a number of similarities to certain plant viral satellites and viroids.

This virus requires hepadna virus helper functions for propagation in hepatocytes, and is an important cause of acute and severe chronic liver damage in some regions of the world.

The HDV particle is 35 to 37 nm in diameter and is coated with Hepatitis B surface antigens and small amounts of the pre-S proteins similar in composition to the small 22 nm Hepatitis B surface antigen particles. It acquires this coat while budding out of the cell (Figure 4). Within the virus particle, ribonucleoprotein cores, consisting of HDV RNA cores complexed to about 70 copies of the HDV antigen, appear to have a diameter of 19 nm (Ryu *et al*, 1993). The RNA genome of hepatitis D virus is approximately 1680 nucleotides and is similar to a plant viroid (Taylor, 1999). The HDV antigens are nuclear phosphoproteins that exist in two isoforms of 195 and 214 amino acids. The HDV antigens are produced from an 800 nucleotides polyadenylated mRNA of antigenomic sense. They have the ability to bind RNA and also possess a nuclear localization signal and the ability to self associate into multimers. The larger antigen undergoes post translational modification that enhances targeting to the nucleus. The smaller antigen participates in the transport of RNA into the nucleus (Shih and Lo, 2001).

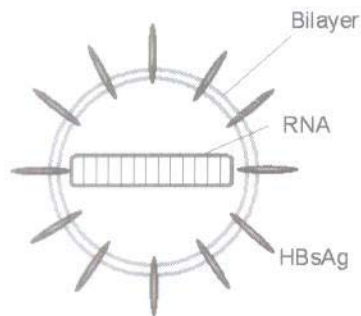


Fig. 4 Proposed structure for hepatitis D virus particle

HDV replication is limited to mammalian cells and it is presumed to occur only in the hepatocyte. The precise mechanism of replication is not clear but genomic replication is believed to involve redirection of host RNA polymerase II. HDV antigen binds to this polymerase, promotes its elongation and thereby regulates mRNA synthesis and HDV genomic RNA replication (Yamaguchi *et al*, 2001). The synthesis of antigenomic RNA is independent of host RNA polymerase suggesting that other host polymerase may be responsible (Modahl *et al*, 2000). HDV is thought to replicate by a 'double rolling circle' mechanism in which RNA dependent RNA transcription from the circular RNA genome leads to the formation of an RNA intermediate which is then self-cleaved into monomers. The

monomers are ligated to form an antigenome which through another cycle of the rolling circle mechanism produces nascent genomic RNA (Taylor, 1999).

Hepatitis G virus

The GB hepatitis or hepatitis G viruses (GBV-A, GBV-B and GBV-C, HGV) were cloned and genomic characterization shows that they are related to other positive-stranded RNA viruses with local regions of sequence identity with various flaviviruses. Infections are often asymptomatic and it is interesting to note that HGV and HIV co-infections has been reported to result in decreased HIV replication (Jiang *et al*, 2001), although other reports dispute those findings (Kaye *et al*, 2005).

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