General Virology

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General Virology

How to distinguish viruses from other microorganisms ???

- **Small Size –**
- **Genome –**
- **Metabolically Inert –.**

Structure of Viruses

Give an account on viral structure?

Viruses consist basically of :

- **1. Nucleic Acid**
- **2. Protein Coat (Capsid)**
- **3. Envelope (some)**

Nucleic Acid

It is either DNA or RNA. ***NEVER BOTH***

It may be double or single-stranded

All DNA viruses are double stranded EXCEPT ….

All RNA viruses are single stranded EXCEPT ……

All RNA viruses have non segmented genomes EXCEPT…

Nucleic Acid

Single stranded RNA viruses are divided into

positive sense and negative sense

The RNA of the positive sense genome is infectious,

The RNA of the negative sense genome is not infectious

Nucleic Acid

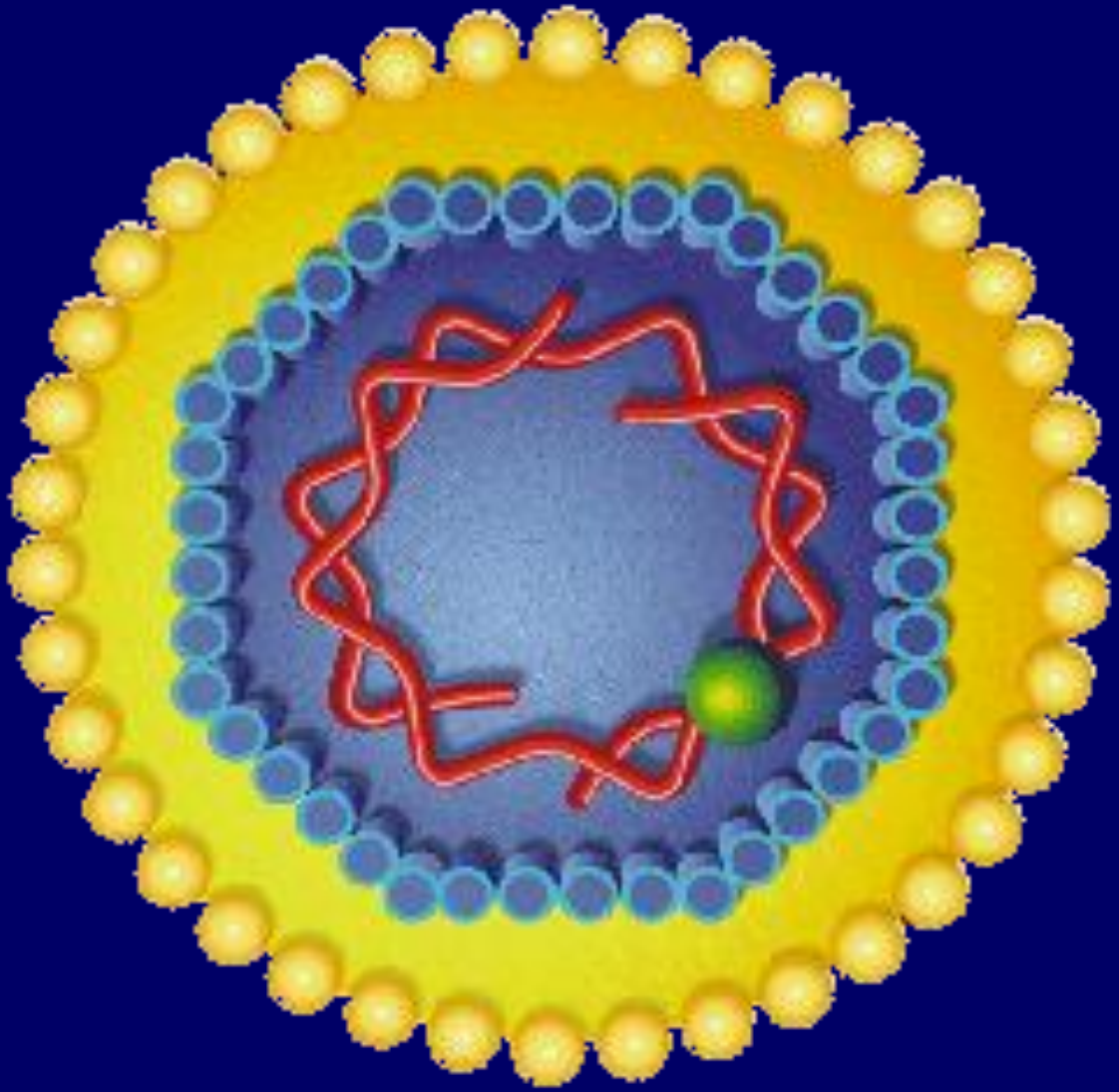
It is responsible for infectivity.

. Protein Coat (Capsid)

- It protects the genetic material.
- It includes important antigens
- It mediates the attachment of the virus to specific receptors on the host cell surface.
- It is responsible for viral symmetry
- **Capsomeres** – the protein structural units of which the capsid is composed

Envelope (some)

- Some viruses are enveloped, others are not.
- It is lipoprotein in nature, composed of lipid derived from the host cell membrane and protein that is virus specific.
- It confers instability on the virus i.e. enveloped viruses are more sensitive to heat, detergents and lipid solvents (as alcohol or ether) than non enveloped viruses



Effect of Physical & Chemical Agents

Viruses are sensitive to

- **Heat** - most viruses are inactivated at 56 C for 30 minutes or at 100 C for a few seconds. EXCEPT HBV
- **Ultraviolet radiation**
- **Oxidizing and Reducing agents**
- **Ether and detergents** destroy enveloped viruses

Viruses are resistant to

- Cold
- Phenols
- Glycerol

Effect of virus on cells

What is the effect of viruses on the infected host cell??

- **Cell death**
- **Cell transformation**
- **Latent infection**

What is the meaning of latency ? Give an example

Fate of the virus after clinical recovery

What is the end result of viral infection?

- 1.**Complete resolution**
- 2. **Persistent viral infections**:

  Chronic infection (carrier)

  Latent infection

Laboratory Diagnosis of Viral Infections

How to diagnose viral infection?

Viral diseases are diagnosed by :

- Isolation
- Direct demonstration.
- Serology

Mention rapid methods for diagnosis of viral diseases?

Isolation

Viral isolation requires the use of living cells, as viruses cannot grow on inanimate media. There are three main systems :

- Tissue culture.
- Chick embryo.
- Laboratory animals.

Tissue Culture

Virus growth in tissue culture is recognized by :

- *Cytopathic Effect (CPE)*
- *Haemadsorption*
- *Immunofluorescence (Ag detection)*

Tissue Culture

- *Transformation*
- *Inclusion bodies formation*
- *Detection of virus specific nucleic acid*
- *Interference*

Direct Demonstration of Virus

The main techniques are :

- **Detection of specific viral antigen - .**
- **Detection of viral particles**
- **Detection of viral nucleic acid**
  - hybridization technique –
  - polymerase chain reaction (PCR)

- 4. **Detection of inclusion bodies**

Serology

- Means detection of antibodies. Recent infection can be diagnosed by the following criteria:
- *Rising titer* - Two samples are taken :
- *Detection of IgM*: it is the earliest antibody to appear.
- *High stationary level*

Viral Replication

Virus Growth Cycle - It can be divided into the following stages :

- **Adsorption** : Viruses adsorb to specific receptors on the host cell plasma membrane.).
- **Entry** : The entire virus (or sometimes only the genome) enters the cell by two ways :
  - Endocytosis :
  - Fusion

- **Uncoating** :
- **Transcription** : Production of virus mRNA
- **Translation**.
- **Assembly** :
- **Release** : The new particles are released from the cell by one of two ways :

Budding :

Rupture of the host cell.

Hepatitis Viruses

- **Hepatitis viruses** - in which the liver is the primary target organ. These viruses are :
  - Hepatitis A virus (HAV).
  - Hepatitis B virus (HBV).
  - Hepatitis C virus (HCV).
  - Hepatitis D virus (HDV).
  - Hepatitis E virus (HEV).
  - Hepatitis G virus (HGV).
- **Other viruses** - in which the liver is not the primary target organ. Example of these viruses are :

Epstein-Barr virus (EBV)

Cytomegalovirus (CMV).

Yellow fever virus.

Hepatitis A Virus

- **Disease** : HAV causes hepatitis A.

  Virus Properties
- HAV is a typical enterovirus classified in the picornavirus family.
- It has a single stranded RNA genome and a non-enveloped icosahedral nucleocapsid.
- It is known as enterovirus 72.
- It has one serotype.

Transmission

- HAV is transmitted by the feco-oral route.

Pathogenesis

- HAV replicates in the gastrointestinal tract and spread to the liver via the blood.
- Hepatocytes are infected but without CPE.
- Hepatocytes are damaged by its attack by cytotoxic T cells
- The infection is cleared, the damage is repaired and no chronic infection occurs.

Clinical Picture

- The clinical manifestations of hepatitis are the same regardless the causative virus. It includes fever, anorexia, vomiting, jaundice, dark urine, pale stool, and elevated transaminases.
- HAV has a short incubatory period (3-4 weeks).
- Most cases resolve spontaneously in 2-4 weeks
- No chronic infection , no carriers, and no predisposition to hepatocellular carcinoma.

Laboratory Diagnosis

- Elevated liver enzymes (transaminases particularly SGPT).
- Detection of IgM antibodies
- Rising titer of IgG antibodies (4-fold rise).
- Isolation of HAV in tissue culture (not available in clinical laboratory.).

Prevention

- Active immunization by inactivated HAV vaccine (the virus is grown in human cell culture and inactivated by formalin). Two doses are given : an initial dose followed by a booster one 6-12 months later.
- Passive immunization with immune serum globulins.
- Observation of proper hygiene. e.g. : sewage disposal , hand washing after bowel movements.

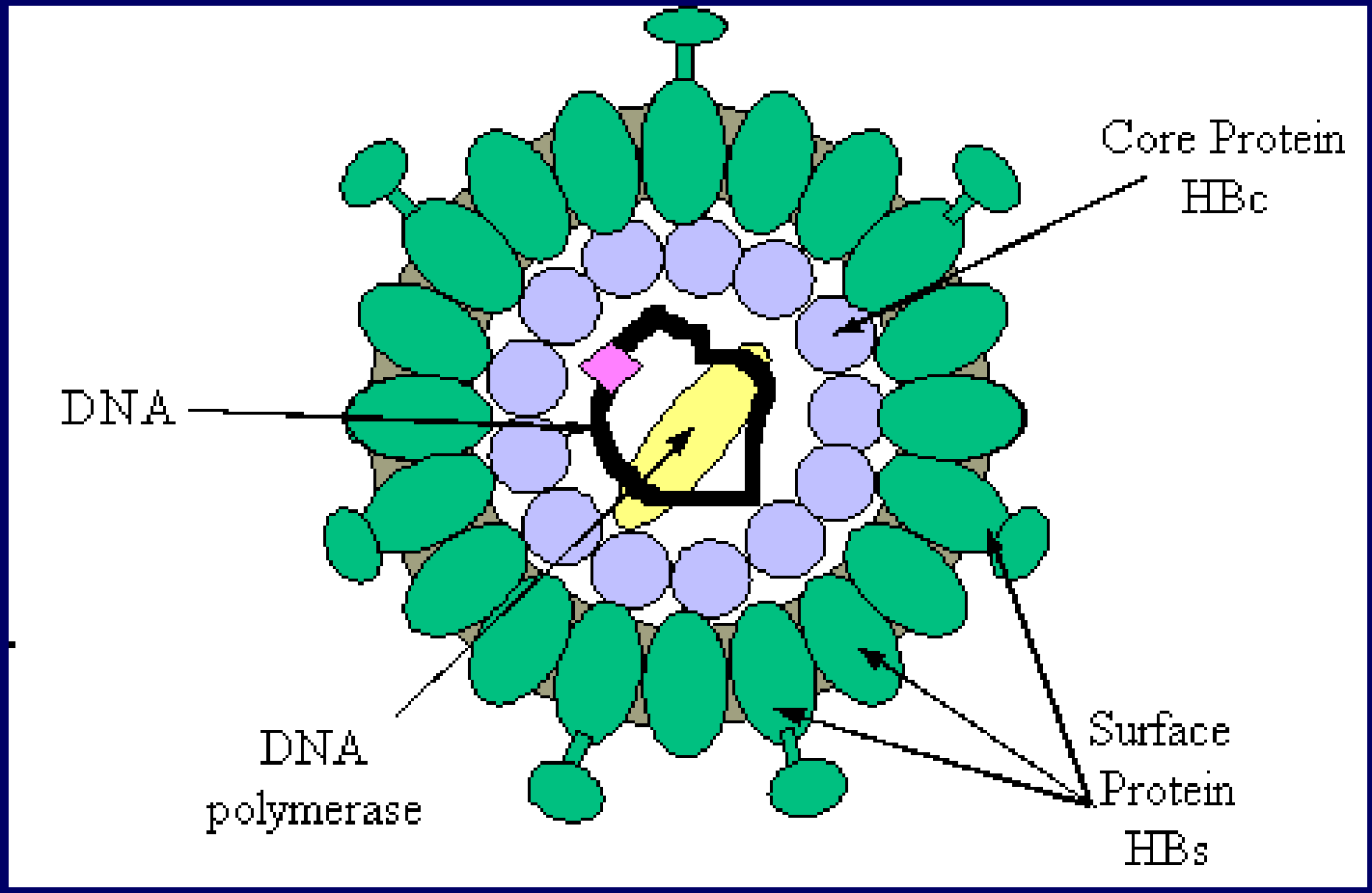
Hepatitis B Virus

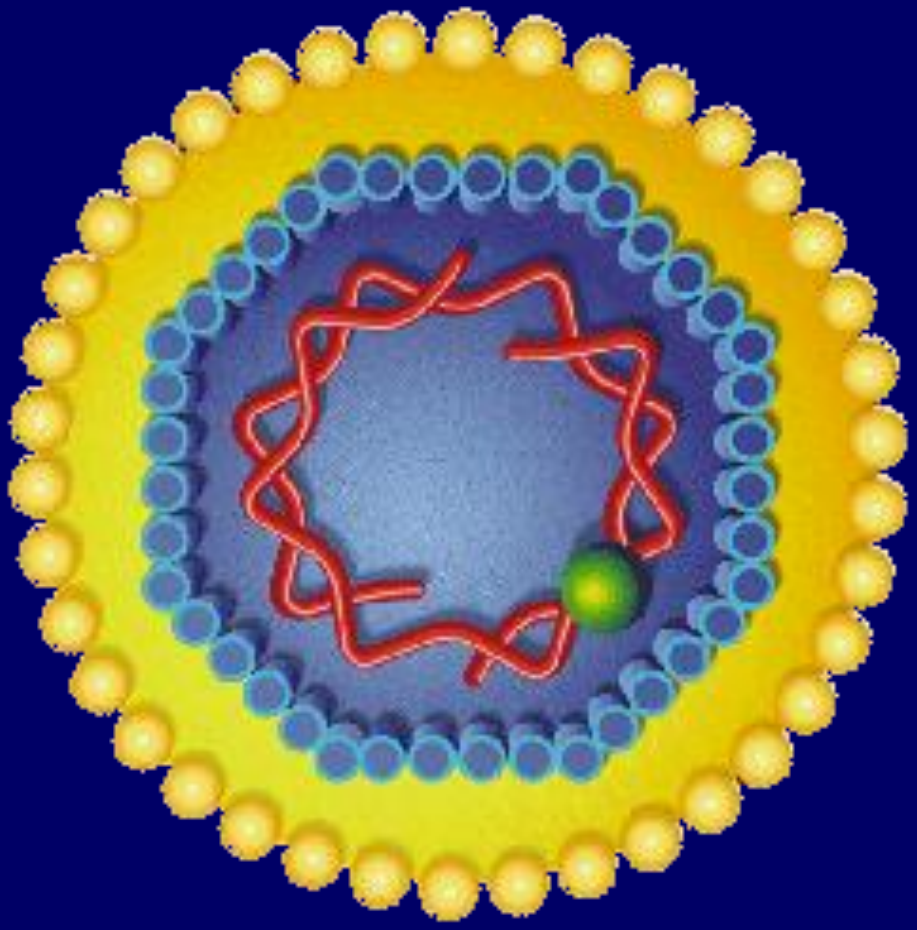
- **Disease** - HBV causes hepatitis B

Virus Properties

- HBV is a member of hepadnavirus family .
- It is enveloped virus with icosahedral core.
- It is double stranded DNA virus.
- The envelope contains a protein called the surface antigen (HBsAg) ,or Australian antigen.

In addition to HBsAg there are two other important antigens : the core antigen (HBcAg), and e antigen (HBeAg) which is important for transmissibility (infectivity).


Core Protein
HBc
DNA
DNA
polymerase
Surface
Protein
HBs



Transmission

Three main modes of transmission

- Blood (blood transfusion, blood products, IV drug abusers).
- Sexual intercourse.
- Perinatally from mother to newborn. (placenta, birth or breast feeding).

Pathogenesis

- After entering of the virus to blood, it infects the hepatocytes, but without CPE.

- Cytotoxic T-cells attack the hepatocytes causing its damage.
- 5% of HBV patients become chronic carriers of HBV (a chronic carrier is someone who has HBsAg persisting in his blood for at least 6 months).

- A high rate of hepatocellular carcinoma occurs in hepatitis B infection due to :
  - Persistent cellular regeneration that attempts to replace the dead hepatocytes.
  - Malignant transformation which occurs when HBV genome integrates into hepatocyte DNA leading to loss of growth control.
- Life long immunity occurs after natural infection and is mediated by HBs antibodies.

Clinical Findings

- Incubation period of HBV is 10-12 weeks.
- Clinical manifestations are the same as HAV, but with HBV symptoms are more severe.
- Chronic carriers are mostly asymptomatic, but some develop chronic active hepatitis, cirrhosis and death.

Laboratory Diagnosis

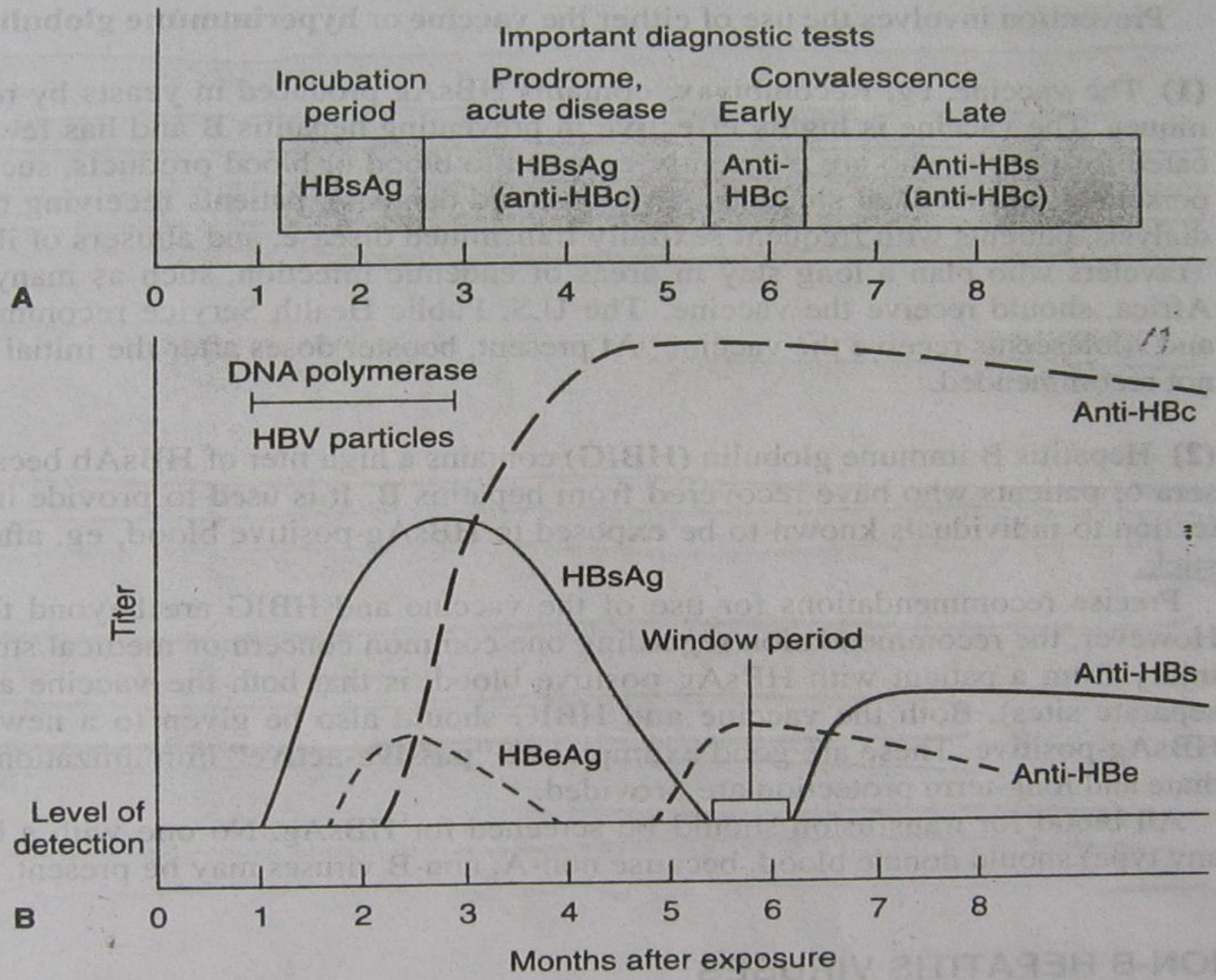
*1- **Elevated liver enzymes*** (transaminases, particularly SGPT).

*2-**Serology*** by ELISA test to detect

- HBsAg – detectable during the prodroma and acute disease, undetectable during convalescence (used for diagnosis).
- HBsAb – not detectable during the disease but detectable during convalescence (used for prognosis).
- HBcAb – present in acute and chronic infection. It is important for diagnosis during the window phase of the disease.(HBcAb IgM).

- HBcAg – it is not available in serum. It is detected in the hepatocyte.
- HBeAg – Starts to appear during the incubation period. Its presence is an important indicator of transmissibility (infectivity).
- HBeAb – indicates low transmissibility.

3- ***PCR*** **–** detection of viral DNA is a strong evidence of infection.


Important diagnostic tests
Incubation period
Prodrome, acute disease
Convalescence
Early
Late
HBsAg
HBsAg (anti-HBc)
Anti-HBc
Anti-HBs (anti-HBc)
A
0 1 2 3 4 5 6 7 8
DNA polymerase
HBV particles
Anti-HBc
HBsAg
Window period
Anti-HBs
HBeAg
Anti-HBe
Titer
Level of detection
B
0 1 2 3 4 5 6 7 8
Months after exposure

Q – What is the window phase ?

- *It is a period of several weeks when HBsAg has disappeared but HBsAb is not yet detectable. At that time, HBcAb is positive and can be used to make diagnosis (HBcAb IgM). The IgM form of HBcAb is present during acute infection and disappears 6 months after infection*

Prevention

- Active immunization (vaccination).
- Passive immunization.
- Hygienic measures.

1. Active Immunization (Vaccination)

- ***Recombinant HBV Vaccine*** – the vaccine contains HBsAg produced in yeast by recombinant DNA technique. It is highly effective.

  Indications :

- High risk groups (frequent exposure to blood or blood products)

- Newborns and adolescents – the public health services recommend routine administration of the vaccine to this population.

2. Passive Immunization

- Hepatitis B immunoglobulins (HBIG) is used. It contains high titer of HBsAb. It is prepared from serum of patients recovered from hepatitis B infection. It provides immediate passive protection, but of short duration. It is indicated in individuals exposed to HBsAg positive blood e.g. after an accidental needle stick.

- Active/Passive immunization by the use of both HBV vaccine and HBIG (given at separate sites) is recommended in :

  Individuals with needle stick injury from a patient with HBsAg positive blood.

  Newborn whose mother is HBsAg positive.

3. Hygienic Measures

- Proper routine screening of blood for transfusion.
- No one with a history of hepatitis (any type) should donate blood.

Treatment of HBV

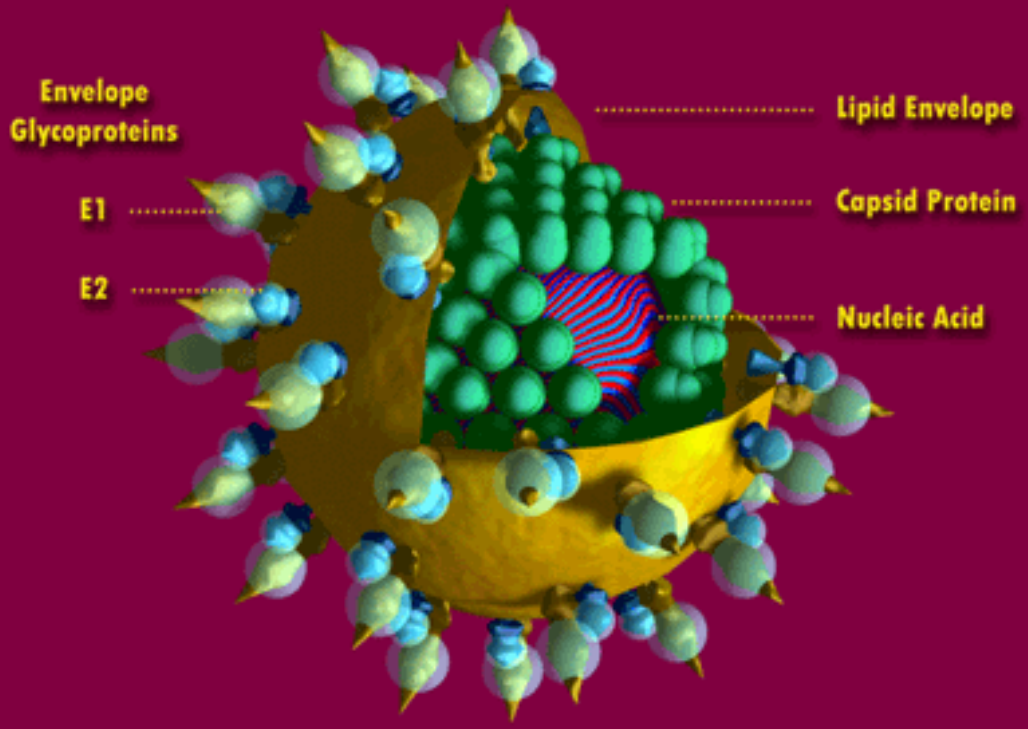
- Alpha interferon is used in chronic HBV infection.
- Antiviral drugs – nucleoside such as Lamivudine is effective against HBV.

Hepatitis C Virus

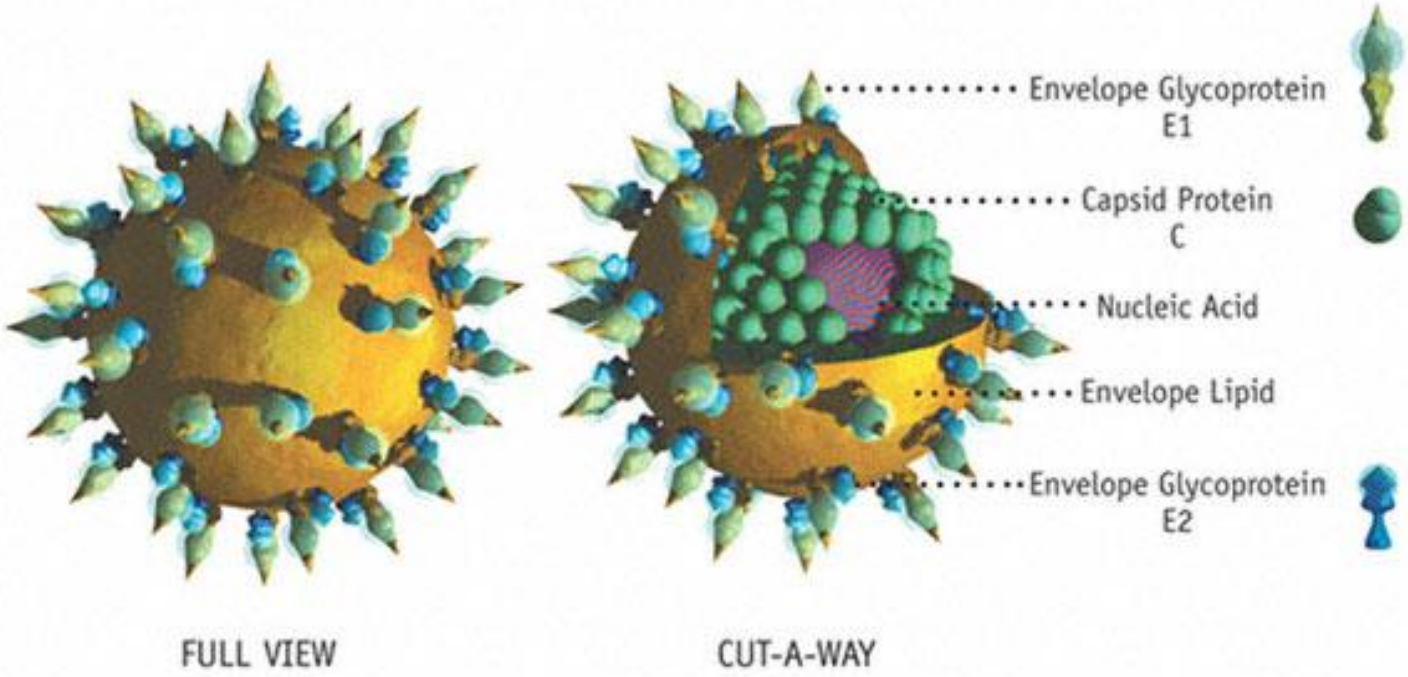
Virus Properties

- HCV is a member of the flavivirus family.
- It is an enveloped single-stranded RNA virus.
- Multiple serotypes exist.
- The gene encoding the envelope glycoprotein has hypervariable regions.

Cut-a-Way Model of Human Hepatitis C Virus



Model of the Human Hepatitis C Virus



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Published in The PRN Notebook, Volume 6, Number 1, March 2001 and The PRN Notebook Online at www.prn.org.
Three-dimensional model of HCV created by Louis E. Henderson, Ph.D., Frederick Cancer Research Center.

Transmission

- Via blood products
  - Blood transfusion.
  - Drug abusers.
- Sexual intercourse
- Perinatally (mother to new-born)
  - Transplecental.
  - Birth.
  - Breast feeding.

Pathogenesis

- HCV infects hepatocytes primarily but with no CPE.
- Hepatocyte death occurs due to immune attack by cytotoxic T-cells.
- HCV infection strongly predisposes to hepatocellular carcinoma (HCC). The virus itself is not oncogenic, with no evidence of an oncogene in the viral genome and no insertion of viral genome into the DNA of the hepatocyte. ***The high rate of HCC in HCV infection is due to prolonged liver damage and the consequent rapid growth rate of hepatocytes as cells attempts to regenerate.***
- 75% of patients are chronically infected and continue to produce virus for at least 1 year.

Clinical Findings

- HCV IP is 8 weeks.
- Clinically acute infections with HCV is mild with the same clinical manifestations of HAV and HBV .
- Many infections with HCV are asymptomatic.
- It simulates HBV in producing chronic liver disease, cirrhosis and HCC.(higher percentage)

Laboratory Diagnosis

1-Elevated liver enzymes (transaminases).

2-Detection of HCV antibodies :

By ELISA – the test does not distinguish between acute, chronic or resolved infection and false positive results can occur.

RIBA (recombinant immunoblot assay) – is done as a confirmatory test for detection of HCV antibodies.

3-*PCR is used to detect viral RNA in the patient's serum to confirm active disease.

Prevention

- Both HCV vaccine and hyperimmune globulins are not available.
- Blood found to contain HCV antibodies is discarded to prevent transfusion-acquired HCV infection.

Treatment

- A combination of alpha interferon and the antiviral drug ribavirin is the treatment of choice in chronic hepatitis C.

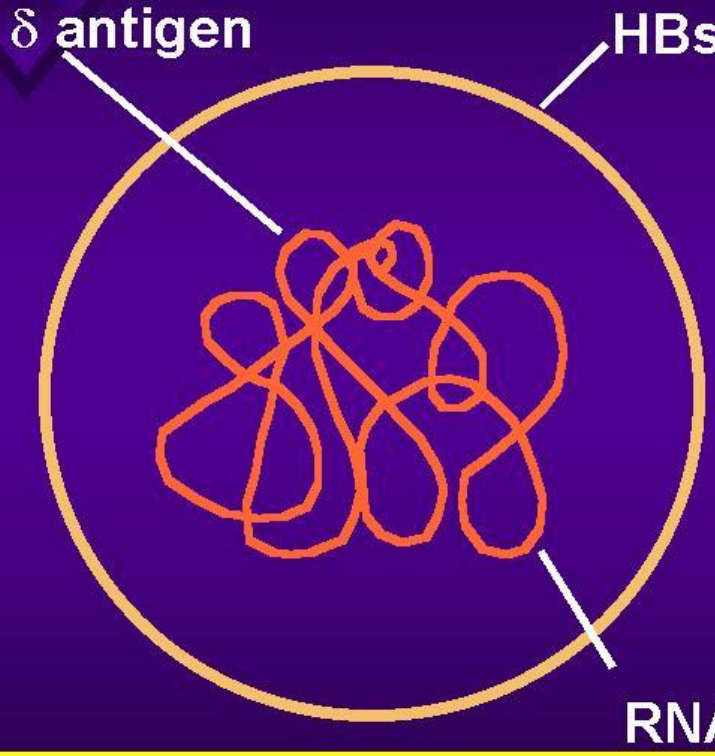
Hepatitis D Virus(Delta Virus)

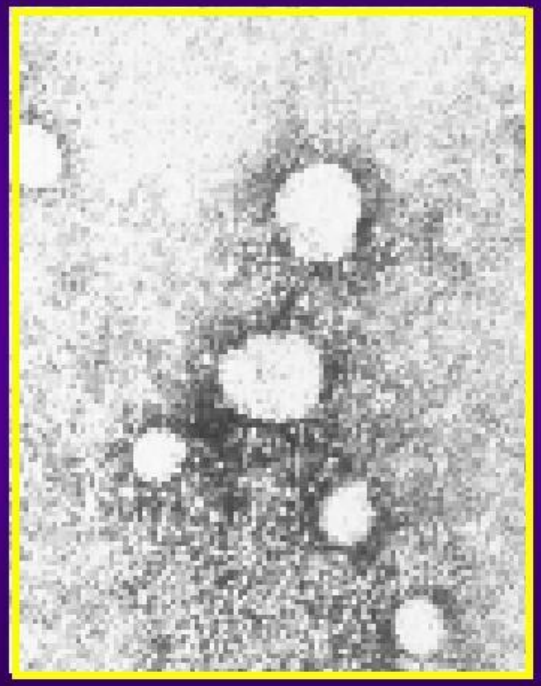
- **Disease** – HDV causes hepatitis D.

Virus Properties

- HDV is a single stranded RNA virus.
- It is a defective virus i.e. it cannot replicate by itself because it does not have genes for its envelope proteins.
- It can replicate only in cells infected with HBV to use the HBsAg of HBV as its envelope protein i.e. HBV is the helper virus for HDV.

Hepatitis D (Delta) Virus





- **Transmission**
- The same as HBV (blood, sexually, perinatally).
- **Pathogenesis**
- The same as HBV i.e. the virus-infected hepatocytes are damaged by cytotoxic T-cells.
- There is some evidence that the delta antigen is cytopathic for hepatocytes.

Clinical Findings

- Hepatitis in patients co-infected with HBV and HDV is more severe (fulminant life-threatening hepatitis) than those infected with HBV alone.
- HBV patients superinfected with HDV show higher rate of carrier state

Prevention

- There is no vaccine against HDV but a person immunized with HBV vaccine will not become infected with HDV.

Treatment

- Alpha interferon.

Hepatitis E Virus

- A major cause of enterically transmitted hepatitis.
- It is a common cause of water-borne epidemics of hepatitis in certain areas.
- HEV is a non-enveloped single stranded RNA virus.
- It is a member of Calcivirus family.

- Clinically the disease resembles HAV infection with the exception of high mortality rates in pregnant women.
- Chronic liver disease does not occur.
- No carrier state.
- Diagnosis is made by exclusion of HAV.
- No vaccine and no antiviral drugs are used for HEV.

Thank you for listening