Analysis of Hepatitis B virus (HBV) infection mechanisms and its application to the development of antiviral agents

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Bangladesh at a glance
Area: Total: 144,000 sq km
Capital: Dhaka (Area 414 sq. km.)
Language: Bangla and English.
Population: 140 million.
Boundary: Bounded by India and Myanmar and the Bay of Bengal from the south
Climate: Tropical
**Population Statistics**

- **Population**: 140 million
- **Population growth rate**: 1.59%
- **Birth rate**: 25 per 1000/year
- **Death rate**: 8 per 1000/year
- **Infant mortality rate**: 70 per 1000/year
- **Life expectancy at birth**: 60 years
- **Literacy**: 44%
Traditionally Bangladeshis subdivide the year into six seasons:

- **Grismo** (summer)
- **Barsha** (rainy)
- **Sharat** (autumn)
- **Hemanto** (cool)
- **Sheet** (winter)
- **Bashonto** (spring)

Mainly three seasons: **summer, rainy, and winter.**
EDUCATION SYSTEM IN BANGLADESH

Four levels
- Primary: 5 years
- Secondary: 5 years
- Higher Secondary: 2 years

Tertiary/University
- Bachelor's degree: 3-4 years
- Master's degree: 1-2 years
- M.Phil course: 1-2 years
- Ph.D course: 3-4 years

Curzon Hall, Dhaka University
Introduction to cells and virus

Figure 1

Relative Sizes and Detection Devices

<table>
<thead>
<tr>
<th>Scale</th>
<th>Object</th>
</tr>
</thead>
<tbody>
<tr>
<td>1 m</td>
<td>Human Eye</td>
</tr>
<tr>
<td>1 dm</td>
<td>Apple</td>
</tr>
<tr>
<td>1 cm</td>
<td>Wasp</td>
</tr>
<tr>
<td>1 mm</td>
<td>Human</td>
</tr>
<tr>
<td>100 µm</td>
<td>Hair</td>
</tr>
<tr>
<td>10 µm</td>
<td>Bacteria</td>
</tr>
<tr>
<td>1 µm</td>
<td>Virus</td>
</tr>
<tr>
<td>100 nm</td>
<td>Small Molecule</td>
</tr>
<tr>
<td>10 nm</td>
<td>Electron Orbital</td>
</tr>
<tr>
<td>100 pm</td>
<td>DNA</td>
</tr>
<tr>
<td>10 pm</td>
<td>Atom</td>
</tr>
<tr>
<td>1 Å</td>
<td></td>
</tr>
</tbody>
</table>

Size of viruses

- Poxvirus
- Animal cells
- Bacteria
- Viruses
- Proteins
- Atoms

Detection Devices

- Light microscope
- Electron microscope
- X-ray
- NMR
Virus may be defined as a cellular organism whose genomes consist of nucleic acid and which replicate inside host cells.
VIRAL COMPONENTS

1. Essential
   a. Nucleic acid
   b. Structural proteins

2. Genetic materials
   a. Type
      RNA
      DNA
   b. Information
      Structural proteins
      Viral enzymes
## VIRUSES AND DISEASES

<table>
<thead>
<tr>
<th>(1) Skin</th>
<th>Warts, Eruption</th>
</tr>
</thead>
<tbody>
<tr>
<td>(2) Brain</td>
<td>Meningitis, Encephalitis</td>
</tr>
<tr>
<td>(3) Respiratory tract</td>
<td>Common cold, Influenza, SARS</td>
</tr>
<tr>
<td>(4) Liver</td>
<td>Hepatitis, Cirrhosis, Hepatoma</td>
</tr>
<tr>
<td>(5) Digestive tract</td>
<td>Food poisoning (Diarrhea)</td>
</tr>
<tr>
<td>(6) Cervix</td>
<td>Cervical cancer</td>
</tr>
<tr>
<td>(7) Blood</td>
<td>AIDS, Infectious mononucleosis</td>
</tr>
</tbody>
</table>
VIRUSES IN PICTURE

Hepatitis A Virus

Poliovirus

Paramyxovirus causing measles and mumps

Influenza virus

Herpes virus
Rotavirus causing diarrhea in children

Adenovirus causing common cold, conjunctivitis

Norovirus

Papilloma virus
KNOWN HEPATITIS VIRUSES

- Hepatitis A virus (HAV)
- Hepatitis B virus (HBV)
- Hepatitis C virus (HCV)
- Hepatitis D virus (HDV)
- Hepatitis E virus (HEV)
Viral Hepatitis - Historical Perspective

“Infectious” → A
Viral hepatitis
“Serum” → B D
NANB (Non A Non B)

E Enterically transmitted
C Parenterally transmitted
F, G, ? other
## Definitions

<table>
<thead>
<tr>
<th>Condition</th>
<th>Description</th>
</tr>
</thead>
<tbody>
<tr>
<td>Acute</td>
<td>Short term, sometime severe</td>
</tr>
<tr>
<td>Chronic</td>
<td>Lingering - may or may not be severe</td>
</tr>
<tr>
<td>Cirrhosis</td>
<td>Fibrosis</td>
</tr>
<tr>
<td>Jaundice</td>
<td>Yellowing of the skin</td>
</tr>
</tbody>
</table>
SYMPTOMS

- Tiredness
- Loss of appetite
- Fever
- Diarrhea
- Dark urine
- Light-colored stools
- Jaundice (yellow skin and eyes)
SYMPTOMS
Tea-colored urine of a patient suffering from viral hepatitis
LIVER CIRRHOSIS
What is hepatitis B Virus?

Hepatitis B virus attacks your liver.

Liver
HEPATITIS B VIRUS (HBV)

- 1930, 'Serum hepatitis' distinguished clinically.

- 1963, Blumberg: discovered 'Australian antigen - Au'.

- 1967: Au antigen = HBsAg (Hepatitis B virus surface antigen).

- 1970, Dane: discovered 42nm 'Dane particles'

- 1973: HBeAg discovered (endogenous antigen).
Hepatitis C Virus

- First identified in 1989
- Scientifically classified as *hepacvirus*
- Contains *ribonucleic acid (RNA)*
# Virology of HBV and HCV

<table>
<thead>
<tr>
<th>Viral features</th>
<th>HBV</th>
<th>HCV</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Structure</strong></td>
<td>42 nm</td>
<td>50 nm</td>
</tr>
<tr>
<td><strong>Family</strong></td>
<td>Hepadnaviridae</td>
<td>hepacivirus</td>
</tr>
<tr>
<td><strong>Mutation rate</strong></td>
<td>low</td>
<td>high</td>
</tr>
<tr>
<td><strong>Genotypes</strong></td>
<td>8 genotypes</td>
<td>6 main genotypes,</td>
</tr>
<tr>
<td></td>
<td>4 main subtypes</td>
<td>more than 50 subtypes</td>
</tr>
<tr>
<td><strong>Viral half-life</strong></td>
<td>2-3 days</td>
<td>3 hrs</td>
</tr>
</tbody>
</table>
Geographic Distribution of Chronic HBV Infection

HBsAg Prevalence

- ≥8% - High
- 2-7% - Intermediate
- <2% - Low
Subtype prevalence

Japan: Subtype adr, genotype C is prevalent
**PUBLIC HEALTH IMPACT**

<table>
<thead>
<tr>
<th>Location</th>
<th>No. of People (million) infected by</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>HBV</td>
</tr>
<tr>
<td>Worldwide</td>
<td>350</td>
</tr>
<tr>
<td>USA</td>
<td>1</td>
</tr>
<tr>
<td></td>
<td>5,000 deaths/year</td>
</tr>
<tr>
<td>Bangladesh</td>
<td>7.5</td>
</tr>
<tr>
<td></td>
<td>30,000 deaths/year</td>
</tr>
<tr>
<td>Japan</td>
<td>1.2% of total population</td>
</tr>
</tbody>
</table>
Will I die from hepatitis B?

Most people do not die from it.

Liver damage (cirrhosis) does not go away.

Liver cancer may lead to death.

Good medical care may make your risk less.
Will I ever get rid of the virus?

About 2 months to show up in your blood.

**Acute Hepatitis B**

90% may get rid of the virus from their bodies after a few months.

**Chronic Hepatitis B**

10% may become carriers.
Hepatitis B Virus (HBV)

Smallest DNA virus known, only 3,200 bp in its genome.
42 nm Dane particle
17-25 nm spherical particle
22 nm tubular/filamentous particle
HBV GENOME

Predominantly ds DNA
partially ssDNA

Four polypeptides
S gene encodes HBsAg
C gene encodes HBcAg, HBeAg
P (polymerase) and
X genes
Hepatitis B virus replication

Entry and uncoating

Receptor(s)? Co-receptor(s)?

Translation

Pre-core and HBx

Secreted HBeAg

Spheres and filaments containing HBsAg

Release

Cytoplasm

Golgi complex

Nucleus

cccDNA synthesis

POL

Generation of minichromosome

mRNA transcription

Pre-genomic RNA (3.5 kb)

Encapsidation and reverse transcription

RNA+

DNA+

DNA−
In vitro HBV infection: What makes it difficult?

- Lack of susceptible cell lines or cells producing HBV abundantly
- Highly species-specific
- Human serum samples and human hepatocytes are not readily available
VSV pseudotype virus

Pseudotyping:

- Genome: VSV
- Surface: different virus (envelope)

VSVΔG*(HBV) pseudotypes

HBV Surface antigen

Vesicular stomatitis virus (VSV)
Advantages of using VSV pseudotypes

Useful for those who

do not grow well in tissue culture like HBV, HCV or HTLV-I

are highly biohazardous to humans like Ebola

Assays using VSV pseudotypes are rapid and safe
Schematic representation of HBV surface antigens

PreS$_1$  PreS$_2$  S

ATG ——— ATG ——— ATG ——— TAA

Small
middle
large

Viral attachment region
Hydrophilic region
Schematic diagram of making expression vectors

- **HBV**
- **HBV**
- **pBS-adr4 X 2 (R)** Amplified by PCR
  - **PCR product** 
    - Cut by enzyme
    - **Cut by enzyme**
    - **Cut by enzyme**
    - bring together (ligation)
    - **HBsAg**
    - **pCAGGS/HBs Expression vector**
Detection of HBs by Immunofluorescence assay (IFA) in cells transfected with plasmid containing HBs L, M, or S gene

Essential terms: Indirect immunofluorescence assay, antigen and antibody
Production of VSVΔG*(HBV) pseudotype

1. VSVΔG*-G Glycoprotein

2. Transfection

3. Infection

4. Supernatants harvested

5. HepG2 cells

Expression of GFP
Detection (A) and neutralization (B) of VSVΔG*(HBV) pseudotype viruses

Infectious titer (IU/ml)

Anti-HBs MAb
- ■■■■
+ ■■■■

Transfected pCAGGS plasmids

L M S L+M+S L+M M+S L+S None
Effect of bovine lactoferrin and trypsin on the infectivity of VSVΔG*(HBs/M) pseudotype to HepG2 cells

A

VSVΔG*-G
VSVΔG*(HBs/M)

B

Relative infectivity (%) vs Lactoferrin (mg/ml)

Relative infectivity (%) vs Trypsin (%)

0 0.01 0.1 1
Summary of research work

- VSV pseudotype viruses bearing HBV surface antigens were produced

- The pseudotype virus infected human hepatoma cell lines, like HepG2 and JHH-7

- The HBV pseudotypes can be used to analyze early stages of HBV infection

- The pseudotypes can also be used for the development of antiviral agents
Viral Hepatitis
What Every Teenager Needs to Know

- Hepatitis B virus (HBV) is 100 times more infectious than HIV.

- About 20% of Japanese have been infected with HBV.
Things to know

About 15%-25% of chronic HBV hepatitis die from either cirrhosis or liver cancer.

Chronic infection occurs in:
- ~ 90% of infants infected with HBV at birth
- ~ 30% of children infected at age 1-5 years
- 2-6% of people infected after age 5 years
Things to know

HBV is found in the blood and body fluids of an infected person.

Average incubation period is about 16 weeks
Concentration of HBV in Various Body Fluids

<table>
<thead>
<tr>
<th>High</th>
<th>Moderate</th>
<th>Low/Not Detectable</th>
</tr>
</thead>
<tbody>
<tr>
<td>blood</td>
<td>semen</td>
<td>urine</td>
</tr>
<tr>
<td>serum</td>
<td>vaginal fluid</td>
<td>feces</td>
</tr>
<tr>
<td>wound exudates</td>
<td>saliva</td>
<td>sweat</td>
</tr>
<tr>
<td></td>
<td></td>
<td>tears</td>
</tr>
<tr>
<td></td>
<td></td>
<td>breast milk</td>
</tr>
</tbody>
</table>
Examples of How HBV Can be Spread

- Sexual contact
- Injection drug use
- Tattoos and body piercing
- From an infected mother to her child at birth
- Sharing personal care items with an infected person
How can HCV be spread?

Sources of Infection for Persons With Hepatitis C

- Injecting drug use: 60%
- Sexual: 15%
- Transfusion (before screening): 10%
- Occupational: 4%
- Other: 1%
- Unknown: 10%

* Nosocomial; iatrogenic; perinatal

Source: Centers for Disease Control and Prevention

USA data
Hepatitis B Vaccine
(licensed in 1982)

Three shots over a 4-6 month period

Recommended for:
- all infants
- all children and adolescents
- Adults
at increased risk
Other Ways to Prevent Hepatitis B

- Practice safer sex
- Wear gloves if you have to touch anyone’s blood
- Do not shoot drugs
- Do not share toothbrushes, razors etc.
Treatment

- No treatment for new infection
- For chronic infection, antiviral medicines are available
- Liver transplant
  - A new liver from a donor
Hepatitis B can be prevented!

If you can get 3 shots of HBV vaccine

... You can get long lasting protection.
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