Hepadnaviruses (and other reverse-transcribing DNA viruses)

LEARNING OUTCOMES:

• EXPLAIN THE IMPORTANCE OF HBV;
• DESCRIBE THE HBV VIRION AND NON-INFECTIOUS PARTICLES;
• OUTLINE THE MAIN FEATURES OF THE HBV GENOME;
• DESCRIBE THE HBV REPLICATION CYCLE;
• EVALUATE MEANS OF PREVENTING AND TREATING HBV INFECTION.
Introduction to hepadnaviruses

- Cause hepatitis and have DNA genomes
- Classified in the family Hepadnaviridae
- The best-known hepadnavirus is HBV
- The hepadnaviruses are especially fascinating for two reasons
  1. they have very small genomes
  2. their DNA genomes are replicated via an RNA intermediate
Importance of HBV
Importance of HBV

- Virus is present in the blood and semen of infected individuals
- 50 million new HBV infections each year, the majority in babies who acquire the infection from their mothers
- Many HBV infections result in mild symptoms or are asymptomatic, especially in children
- Some develop severe hepatitis, which may lead to cirrhosis and eventually to liver cancer
HBV virion

- Roughly spherical
- Diameter of about 42 nm
- Components are an envelope enclosing a capsid, inside which is the DNA and P (polymerase) protein
- Genome is made up of two strands of DNA
- One is incomplete
- DNA is partly single-stranded and partly double-stranded
- A short sequence is triple-stranded as a result of a complementary sequence at the 5' ends
- DNA has a circular conformation
- Length is about 3.2 kb
- 5' end of each of the DNA strands has a covalently linked molecule: capped RNA on the short strand and a protein (P) on the long strand
Virion contains at least one complete molecule of P (polymerase) protein

N terminus of P constitutes a ‘terminal protein’ domain

Separated by a ‘spacer’ from a domain with reverse transcriptase activity

Domain with ribonuclease H (RNase H) activity at C terminus
Capsid

- Capsid has icosahedral symmetry
- It has holes in it and short spikes protrude from its surface
- Constructed from dimers of the C (core) protein, which is largely α-helical
Virion envelope contains three protein species designated as small (S), medium (M) and large (L).

M and L proteins are longer versions of the S protein.

The surface regions of the envelope proteins constitute an antigen known as hepatitis B surface antigen (HBsAg).

The virus attachment site is near the N terminus of the L protein.

About 50 per cent of the L molecules have the N terminus on the outside of the virion.

One conformation of L protein acts as virus attachment proteins, while other binds the virion envelope to the capsid.
Non-infectious particles

- Large quantities of non-infectious particles that have been released from infected liver cells
- Composed of lipid and virus envelope proteins
- Do not contain nucleocapsids
- Spheres and filaments, diameter 22 nm, and filament can have length up to 200 nm
- All the particles more abundant in the blood than in the liver
- Non-infectious particles outnumber the virions

<table>
<thead>
<tr>
<th>Structure</th>
<th>Diameter (nm)</th>
<th>Typical concentration (μg/ml)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Virion</td>
<td>42</td>
<td>0.1</td>
</tr>
<tr>
<td>Non-infectious particles</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Sphere</td>
<td>22</td>
<td>100</td>
</tr>
<tr>
<td>Filament</td>
<td>22</td>
<td>1</td>
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</table>
Soluble virus protein

- Soluble virus protein is found in the blood of some infected individuals
- Known as hepatitis B e antigen (HBeAg)
- Function not known
HBV genome

- Four ORFs, seven proteins are translated
- The virus uses every nucleotide in the genome for protein coding
- P ORF overlaps the C and X ORFs and the entire S ORF is within the P ORF
- Virus maximizes coding capacity by expressing the L protein in two different conformations
- Genome contains direct repeats of 11 nucleotides known as DR1 and DR2
Expression of the pre-S1–pre-S2–S region gives rise to three proteins
Translation of the S region produces the S protein
Translation of pre-S2–S produces the M protein
Translation of the complete ORF produces the L protein
Expression of the pre-C–C region gives rise to two proteins
It encodes only seven proteins
Dependent on host cell
HBV replication cycle

- **Attachment**: Hepatocytes (liver cells) are the host cells for HBV in the body. Where hepatocyte receptors binds to HBV.

- **Entry**: The virion is endocytosed then the nucleocapsid is released from the endosome by fusion of the virion and endosome membranes. Once the virus genome is free in the nucleus it is converted into a circular DNA molecule. The virus DNA is not replicated in the nucleus, but more copies are brought into the nucleus later in the replication cycle.
**Transcription:** Transcription is carried out by the cell RNA polymerase II and results in the synthesis of four size classes of RNA.

**Translation:** Of the RNAs that are approximately 3.5 kb long there are two subsets that differ slightly in size. The shorter subset, which does not include the start codon for the pre-C sequence, acts as mRNA for the C and P proteins.

**Post-translational modifications:** Some of the envelope protein molecules become glycosylated and L is myristylated at the N terminus.
Nucleocapsid assembly: During DNA synthesis a nucleocapsid may either (1) move to the nucleus where its DNA boosts the pool of virus DNA or (2) bud through a membrane containing virus envelope proteins to form a virion.

Genome synthesis: Genome replication involves the synthesis of RNA in the nucleus, then copying from RNA to DNA (reverse transcription) in capsids. Stages in virion assembly include capsid construction and acquisition of the envelope by budding.

Exit: The membranes through which budding occurs are part of a compartment between the endoplasmic reticulum and the Golgi complex. As budding proceeds copies of the L protein with their N termini on the cytoplasmic surfaces of the membranes bind to capsids. Virions are transported to the cell surface, where they are released from the cell by exocytosis. Non-infectious spheres and filaments are also released from the cell.
Prevention and treatment of HBV infection

- The original HBV vaccines consisted of non-infectious spheres and filaments extracted from blood donations from HBV carriers. Now much vaccine is produced using recombinant yeast cells containing the gene for the S protein.

- The requirements to mass produce HBV vaccine cheaply and to vaccinate many more of those at risk are major challenges facing the world.

- Alpha-interferon has been used for some years to treat HBV-infected persons. This treatment does not eliminate the infection, but it results in a significant reduction in viraemia in about 20–30 per cent of cases.
The drug lamivudine, a nucleoside analogue is also used to treat HBV infection.

In many ways lamivudine is an improvement over α-interferon as it suppresses virus replication with a low incidence of side-effects, is administered by mouth rather than by injection and is cheaper.

Longterm treatment, however, results in the appearance of lamivudine-resistant HBV mutants, though they appear to be susceptible to other nucleoside analogues such as adefovir.
Treatment of chronic hepatitis B naïve patients with a therapeutic vaccine containing HBs and HBe antigens (a randomized, open and treatment controlled phase III clinical trial)

Mamun Al Mahtab, Sheikh Mohammad Fazle Akbar, Julio Cesar Aguilar, Gerardo Guillen, Euduarco Penton, Angela Turo, Osamu Yoshida, Yoichi Hiasa, Morikazu Onji

1 Department of Hepatology, Bangabandhu Sheikh Mujib Medical University, Dhaka, Bangladesh, 2 Department of Medical Sciences, Toshiba General Hospital, Tokyo, Japan, 3 Department of Biomedical Research, Center for Genetic Engineering and Biotechnology, Havana, Cuba, 4 Department of Gastroenterology and Metabolity, Ehime University Graduate School of Medicine, Ehime, Japan, 5 Department of Medicine, Sai Sei Kai Imabari Hospital, Imabari, Japan
Aim of the study:
Evaluate the safety and efficacy of NASVAC therapeutic vaccine in comparison with pegylated IFN (Peg-IFN) in patients with CHB.
Introduction

- Chronic hepatitis B virus (HBV) infection represents a major global public health problem.

- About 250 million people worldwide are chronically infected with HBV and most of them are infected at birth or during childhood.

- Epidemiological studies have shown that about 20±25% of individuals infected with HBV as children are prone to develop chronic hepatitis B (CHB), and its complications like liver cirrhosis (LC) and hepatocellular carcinoma (HCC).

- World Health Organization estimates indicate that annual deaths due to HBV are more than 0.68 million.

- A proper management of CHB patients may reduce progression to LC and HCC, and consequently HBV-related mortality.
Two types of treatments have been approved:

- **interferon (IFN)**
  - that is endowed with antiviral and immune modulatory properties

- **nucleos(t)ide analogs**
  - that directly inhibit HBV polymerase and therefore replication.

However, these drugs have a poor efficacy in terms of post-treatment sustained viral suppression and IFN generate important side effects during and after therapy.

Accordingly, various approaches are pursued to develop more effective therapy regimens.
Polyclonal immune modulators and a wide range of HBV-specific antigen-based immune therapies (vaccine therapy) have been attempted in CHB patients. Unfortunately, they did not stand the test of time and so far there has not been any valid immune therapy based on specific active immunization for CHB patients.

Recent studies have indicated that although the concept of immune therapy for CHB remains attractive further modifications in their design are needed to increase efficacy.

Both HBsAg and HBCAg-specific immunity seem warranted for control of HBV replication and liver damage.

Furthermore ad'hoc regulatory studies are requested to define dose/route of vaccine administration and duration of therapy.
For this sake, a vaccine formulation that contained both hepatitis B surface antigen (HBsAg) and hepatitis B core antigen (HBcAg) (designated NASVAC) was developed.

**NASVAC** was intended to be used via nasal route to induce a broad-based immunity at both mucosal and systemic compartments.

Pre-clinical pharmacological and toxicological studies with NASVAC confirmed its safety and efficacy and these have also been documented in HBV transgenic mice which expressed Dane particle, HBV DNA, and HBsAg/HBeAg.
A phase I clinical trial carried out in healthy volunteers confirmed the safety and immunogenicity of NASVAC in humans.

Subsequently, a phase I/II clinical trial was conducted in 18 CHB patients with further encouraging results in terms of safety and antiviral effect.

In the present paper, the results of a phase III clinical trial are reported.
Methods

- Conducted at Bangabandhu Sheikh Mujib Medical University, and Farabi Hospital, Dhaka, Bangladesh
- All patients gave written informed consent for the study and diagnosed as CHB
- Treatment naive and none has received any antiviral or immune stimulatory drug for HBV infection
- Both genders, aged 18-65
- HBsAg and HBV DNA in blood for more than 6 months
- Levels of alanine aminotransferase (ALT) at enrolment had to be above the upper limits of normal (ULN) values
- HBV levels were more than $10^3$ copies/mL for hepatitis B e antigen (HBeAg(-))
- $10^4$ for HBeAg(+) patients
Study interventions

- Randomly divided into two groups (1:1)
- Eighty patients were assigned to receive 180 μg of Peg-IFN once weekly for 48 consecutive weeks
- Other 80 patients received NASVAC
- Formulation comprises of equal amounts of 100μg of HBsAg and 100μg of HBcAg
- Therapeutic vaccination was conducted in two cycles
- In the first cycle, NASVAC was administered in a volume of 1.0 ml via the intranasal route using a nasal spray on five occasions at 2-weekly intervals
The second cycle started at week 12, the same vaccine formulation was administered simultaneously via the nasal and subcutaneous routes.

Five occasions at 2-weekly intervals.

Patients were observed for 2 h after each vaccination.

Serum was collected from each patient before the study started, before each vaccination, after 5 nasal vaccinations (end of first cycle) and after the end of second cycle (end of treatment).

The follow-up study was conducted 24 weeks after the EOT.
Occurrence of adverse events to the treatment was most carefully assessed in this clinical trial.

Adverse reactions were measured immediately and up to 2 hours after immunization.

Levels of HBV DNA, ALT, HBeAg, Anti-HBe, and levels of fibrosis were evaluated before treatment and at different points during and after treatment.

Assessments were made at tertiary level reference laboratories of Dhaka, Bangladesh.
Study outcomes

- Viral load reduction was taken as its primary outcome
- The success criterion was in relation with the proportion of patients showing reduction of the viral load
- The virological response was measured by the HBV serum DNA quantification of the patients by real time PCR systems
- Biochemical response as measured by the serum ALT transaminase level
- Serological response as measured by HBsAg detection and their specific antibodies
- Serum HBeAg detection and its conversion to anti-HBeAg antibodies
- Histological response as measured by Fibroscan
Sample size and statistical analysis

- To accomplish hypothesis 80 patients needed for group 1 and 80 patients for group 2
- Nominal and categorical parameters were expressed as absolute numbers and percentages
- Mean, standard deviation, median, interquartile range and/or ranges were calculated for all quantitative variables
- Statistical analysis was performed using SPSS version 15.0 for Windows
- For all analyses, a two-tailed significance testing and a significance level of 0.05 were used
Results

- Only 2 patients voluntarily dropped out of the trial before start of NASVAC treatment (none due to AE)
- 4 out of 80 patients in the Peg-IFN group withdrew from treatment (2 due to AE)
- NASVAC from June, 7th 2011 up to January 3rd, 2012
- Peg-IFN from June, 7th 2011 up to May 5th 2012
- Mean age of 29±7 years in both groups
- Only 15 patients (19.2%) in NASVAC group and 18 patients (22.5%) in the Peg-IFN group were HBeAg positive
<table>
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<tr>
<th>Variables</th>
<th>NASVAC</th>
<th>Peg-IFN</th>
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<tbody>
<tr>
<td></td>
<td>N</td>
<td>%</td>
</tr>
<tr>
<td>Total</td>
<td>78</td>
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<td>17.9</td>
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<tr>
<td>Male</td>
<td>64</td>
<td>82.1</td>
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<tr>
<td>Age (years)</td>
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<td></td>
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<tr>
<td>Median ± IQR (range)</td>
<td>28 ± 10 (18–50)</td>
<td>28 ± 11 (18–48)</td>
</tr>
<tr>
<td>Height (m)</td>
<td>1.52 ± 0.00 (1.22–1.83)</td>
<td>1.52 ± 0.00 (1.22–1.83)</td>
</tr>
<tr>
<td>Weight (kg)</td>
<td>59 ± 16 (36–81)</td>
<td>61 ± 15 (35–85)</td>
</tr>
<tr>
<td>Body mass index (kg/m²)</td>
<td>25.78 ± 6.83 (15.50–36.85)</td>
<td>26.36 ± 6.43 (17.22–40.26)</td>
</tr>
<tr>
<td>HBV DNA (log copies/mL)</td>
<td>4.7 ± 1.8 (3.2–13.0)</td>
<td>5.2 ± 2.6 (3.1–12.5)</td>
</tr>
<tr>
<td>ALT (IU/L)</td>
<td>30.0 ± 22.0 (10.0–262.0)</td>
<td>37.0 ± 19.8 (10.0–226.0)</td>
</tr>
<tr>
<td>HBeAg(+) at baseline</td>
<td>15 (19.2)</td>
<td>18 (22.5)</td>
</tr>
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</table>
At EOT, both NASVAC and Peg-IFN therapy induced similar proportion of HBV DNA reduction.

The statistical hypothesis of the present clinical trial was verified during treatment free follow up 24 weeks after EOT.

Higher proportion of patients with viral load below 250 copies/ml was found in NASVAC recipients (57.7%) as compared to the Peg-IFN treated group (35.0%).

Levels of HBV DNA<1000 copies were detected in 71.8% and 45% patients receiving NASVAC and Peg-IFN.
Peg-IFN treated group evidenced a more pronounced post-treatment (EOT) viral rebound

The mean levels of HBV DNA in the sera of these patients were also compared at baseline and different times after therapy

At baseline, the levels of HBV DNA were comparable between NASVAC recipients and Peg-IFN treated CHB patients (5.4 ± 2.1 versus 5.8 ± 2.3 log copies/ml)

At EOT, the levels of HBV DNA were reduced in both groups

24 weeks after EOT NASVAC maintained HBV DNA suppression almost at EOT level

Peg-IFN experienced rebound of HBV DNA from 3.0 ± 1.5 log copies/ml to 4.3 ± 2.2 log copies/ml
Elevation of ALT by nasal administration of NASVAC in CHB patients

- ALT increase was recorded in 85% of NASVAC-treated patients after 5 nasal vaccinations.
- Flares occurred independently of patients HBe serology, sex, age or initial viral load.
- Nature of the ALT increases in NASVAC group was transient, homogeneously related to week 12 and reached five to ten times baseline levels.
- Peg-IFN had increases occurred in only 30% patients and was not homogeneously related to the week 12.
HBeAg & anti-HBeAg determinations

- 5 out of 14 NASVAC treated patients (35.7%) seroconverted from HBeAg to anti HBe within 24 weeks of treatment
- 3 out of 16 patients seroconverted to anti HBeAg (18.7%) in the Peg-IFN group
- HBe/anti-HBeAg seroconversions were associated to strong reduction of HBV DNA
- Patients who cleared HBeAg had a significantly deeper reduction in mean viral load value
Assessment of liver fibrosis

- None of the NASVAC-recipient patients had stiffness values over 18.3 kPa at 24 week after EOT

- 7 patients receiving Peg-IFN treated had liver stiffness of more than 18.3 kPa cut off at 24 week after EOT

- 18.3 kPa is level corresponding to cirrhosis on the Metavir score
Harms

- No serious adverse event in any of the groups
- The NASVAC treatment was not stopped due to any severe event
- Severe adverse events were reported during Peg-IFN treatment.
Among drugs which are used for treatment of CHB, Peg-IFN is endowed with both antiviral and immunomodulatory activities.

A phase III, open label randomized controlled clinical trial was designed to directly compare the therapeutic efficacy and tolerance of a therapeutic vaccine candidate, NASVAC versus Peg-IFN in treatment-naïve CHB patients.

NASVAC was significantly safer compared to Peg-IFN.

The overall data indicate that NASVAC is a safe immune therapeutic agent for CHB patients in both short-term and long-term perspectives.
NASVAC therapeutic vaccine combines both HBsAg and HBcAg

Evidence from early 2000 did show that HBcAg-specific immunity is essential to control HBV replication and liver damage.

Studies in HBV transgenic mice revealed that HBcAg acts like an adjuvant to induce and sustain HBV-specific immunity which is safe and exhibits antiviral activity.

NASVAC has been administered by both nasal and parental routes allowing stimulation of both mucosal and systemic immune compartments of CHB patients.

NASVAC triggered increases of ALT, an indirect marker of restoration of host immunity, after nasal administration.

This finding mimics the natural history of immune restoration in CHB patients evolving from an immune tolerance state to an immune clearance phase.
Conclusion

- We present here a phase III study which used a therapeutic vaccine comprising both HBsAg and HBcAg, at high doses (up to 100–200 μg of each antigen), administered 10 times by the nasal and five by subcutaneous routes, in treatment naïve patients with CHB.

- The therapeutic benefit of NASVAC was obtained after 15 nasal/subcutaneous immunizations within only 6 months as compared to 48 doses of Peg-IFN over a year.

- This study indicates that the impact of therapeutic vaccination in the treatment of chronic infectious diseases will depend on the capacity of designing the adequate antigens and adjuvant strategies as well as the selection of the most suitable immunization route(s) and candidate recipients.
Thank you for your attention!

- Azra Karić
- Esma Kurtanović
- Ademir Abdukić