Basic Concepts of Hepatitis B

Professor Stephen Locarnini
Victorian Infectious Diseases Reference Laboratory, North Melbourne, Victoria 3051, AUSTRALIA
Liver Disease Progression

HBV ACUTE / CHRONIC HEPATITIS B
CIRRHOSIS CIRRHOSIS

PRIMARY PREVENTION
Vaccination

CHEMO PREVENTION
Cytokines
IFN-α, Peg-IFN-α
Antivirals
LMV, ADV, ETV, LdT, TFV

CIRRHOSIS ESLD

CANCER SCREENING
Tumour Marker Ultrasound Transplantation

HEPATOCELLULAR CARCINOMA

Time 20-30 years

Normal Cirrhosis Cirrhosis HCC
Outline of Presentation

1. Molecular Virology

2. Natural History of Chronic Hepatitis B

3. Molecular Pathogenesis

4. Treatment and Associated Challenges

5. Future Directions
HBV Genome Map

Circular ss/ds DNA virion, supra-genomic RNA

Four major overlapping ORFs

Alternative start sites

Many regulatory elements
HBV Replication: Early Events

- Attachment and Penetration
- uncoating
- transport to cell nucleus
- CCC DNA
- DNA repair
- MINICHROMOSOME
- Nucleus
- ER/Golgi
- Cytosol
HBV Replication: Packaging & Reverse Transcription

Attachment and Penetration

HBV RNA transcripts

DNA repair

MINICHROMOSOME

CCC DNA

HBV RNA transcripts

HBV polymerase protein

transport to cell nucleus

HBV DNA SYNTHESIS

core proteins (HBcAg)

translocation

new (-) strand DNA synthesis

pgRNA

attachment

uncoating

pregenomic RNA

Nucleus

LA PROTEIN
HBV Replication: Assembly & Release

- CCC DNA
- HBV RNA transcripts
- HBV polymerase protein
- HBV RNA thetrancripts
- HBV polymerase protein
- HBV DNA SYNTHESIS
- HBsAg
- HBeAg
- Precore protein
- secretory pathway
- LA PROTEIN
- attachment and penetration
- uncoating
- envelope proteins S, M, L
- Golgi complex
- Re-entry
- DNA repair
- transport to cell nucleus
- core proteins (HBcAg)
- Re-entry
- new (-) strand DNA synthesis
- pgRNA
- (A)n (A)n
- S' Cap
- Translocation
Persistence and Variability

- HBV circular DNA genome comprises four major overlapping ORF
- No origin of DNA replication
- Regulatory elements contained with coding sequences

**Genetic stability**
- Genomic DNA/cccDNA

**Genetic variability**
- Replicates via reverse transcription
- Spliced pregenomic RNA
Natural History of Chronic Hepatitis B
Phases of CHB infection: Serum and Liver Compartment

<table>
<thead>
<tr>
<th></th>
<th>Immune Tolerant</th>
<th>HBeAg-Positive CHB [Immune Clearance]</th>
<th>Immune Control [Low or Non-Replicative]</th>
<th>HBeAg-negative CHB [Immune Escape]</th>
</tr>
</thead>
<tbody>
<tr>
<td>HBeAg</td>
<td>Positive (2,000-5,000 PEIU/ml)</td>
<td>Positive (100-1,000 PEIU/ml)</td>
<td>Negative</td>
<td>Negative</td>
</tr>
<tr>
<td>Anti-HBe</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>HBsAg (log IU/ml)</td>
<td>4.5-5</td>
<td>4.0-4.5</td>
<td>2.9-3.0</td>
<td>3.3-3.9</td>
</tr>
<tr>
<td>Anti-HBs</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>HBV DNA (IU/ml)</td>
<td>&gt; 20,000</td>
<td>&gt; 20,000</td>
<td>&lt; 2,000</td>
<td>&gt; 2,000</td>
</tr>
<tr>
<td>Viral Diversity (PC/C ORF)</td>
<td>Persistently normal</td>
<td>Elevated (1-2X) and fluctuating</td>
<td>Normal</td>
<td>Elevated and fluctuating</td>
</tr>
<tr>
<td>Serum ALT Level (U/L)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Liver Histology</td>
<td>Normal or mild hepatitis</td>
<td>Moderate to severe hepatitis</td>
<td>Normal to mild hepatitis. May have cirrhosis</td>
<td>Moderate to severe hepatitis. May have cirrhosis</td>
</tr>
<tr>
<td>Intra-Hepatic HBV Replicative, Intermediates</td>
<td>rcDNA/cccDNA (100-1,000) &gt;1 cccDNA/cell</td>
<td>rcDNA/cccDNA (10-100) 1 cccDNA/cell (0.1-10/cell)</td>
<td>rcDNA/cccDNA (10-100) 0.1 cccDNA/cell (0.001-1/cell)</td>
<td>rcDNA/cccDNA (100-1000) cccDNA/cell (0.1-10/cell)</td>
</tr>
</tbody>
</table>

Dandri, M & Locarnini, S. 2012. GUT;61(Suppl 1):i6-i17
# Phases Of Chronic Hepatitis B: A Regional Perspective

<table>
<thead>
<tr>
<th>Clinical Phases of CHB</th>
<th>Western Europe</th>
<th>Africa</th>
<th>Asia</th>
</tr>
</thead>
<tbody>
<tr>
<td>“Immune tolerant”</td>
<td>Duration</td>
<td>Often absent</td>
<td>Short</td>
</tr>
<tr>
<td>“Immune active” (clearance)</td>
<td>HBeAg seroconversion</td>
<td>Early</td>
<td>Very Early</td>
</tr>
<tr>
<td></td>
<td>Early HBeAg seroconversion</td>
<td>A &gt; D</td>
<td>A</td>
</tr>
<tr>
<td>“Inactive/Latent” (Non-replicative)</td>
<td>Durability of HBeAg seroconversion</td>
<td>Intermediate</td>
<td>High</td>
</tr>
<tr>
<td>“Reactivation”</td>
<td>HBeAg negative CHB</td>
<td>Frequent in the Mediterranean region</td>
<td>Unknown</td>
</tr>
</tbody>
</table>

### Clinical / Epidemiological Correlations: A Regional Perspective (I)

<table>
<thead>
<tr>
<th></th>
<th>Western Europe</th>
<th>Africa</th>
<th>Asia</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Endemicity</strong></td>
<td>Low</td>
<td>High</td>
<td>High</td>
</tr>
<tr>
<td><strong>Mode of transmission</strong></td>
<td>Sexual or Percutaneous</td>
<td>Horizontal</td>
<td>Perinatal (Vertical)</td>
</tr>
<tr>
<td><strong>Age of infection</strong></td>
<td>Adolescent or Adult</td>
<td>Childhood (&lt;5yrs)</td>
<td>At birth or Infant</td>
</tr>
<tr>
<td><strong>Family History</strong></td>
<td>Often none</td>
<td>HBeAg- mother or HBeAg+ relatives</td>
<td>HBeAg+ mother or relatives</td>
</tr>
<tr>
<td><strong>Chronicity</strong></td>
<td>Low (1-5%)</td>
<td>High (30-50%)</td>
<td>Very High (&gt;90%)</td>
</tr>
</tbody>
</table>

Clinical / Epidemiological Correlations: A Regional Perspective (ii)

<table>
<thead>
<tr>
<th></th>
<th>Western Europe</th>
<th>Africa</th>
<th>Asia</th>
</tr>
</thead>
<tbody>
<tr>
<td>Risk of End-Stage Liver Disease</td>
<td>Low</td>
<td>Low</td>
<td>High</td>
</tr>
<tr>
<td>Risk of Hepatocellular Carcinoma</td>
<td>Low</td>
<td>High</td>
<td>High</td>
</tr>
<tr>
<td>Age at risk of HCC</td>
<td>Older persons (M&gt;F)</td>
<td>Young males often without cirrhosis</td>
<td>Older persons (M&gt;F)</td>
</tr>
<tr>
<td>Mortality from HBV</td>
<td>Low</td>
<td>Low</td>
<td>High</td>
</tr>
</tbody>
</table>

**Clinical / Epidemiological Correlations: A Regional Perspective (iii)**

<table>
<thead>
<tr>
<th></th>
<th>Western Europe</th>
<th>Africa</th>
<th>Asia</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>HBV genotype</strong></td>
<td>A2, D</td>
<td>A1, E, D</td>
<td>B1, B2; C1, C2</td>
</tr>
<tr>
<td><strong>Naturally Occurring</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>HBV Mutations</strong></td>
<td>A1896 in HBV/D</td>
<td></td>
<td>T1762/A1764</td>
</tr>
<tr>
<td></td>
<td>No A1896 in HBV/A</td>
<td>T1809-T1812</td>
<td>A1896</td>
</tr>
<tr>
<td><strong>Response to</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>Interferon-based</strong></td>
<td>Good (A&gt;&gt;D)</td>
<td>Unknown</td>
<td>Poor</td>
</tr>
<tr>
<td><strong>Therapy</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Geographic Distribution of HBV Genotypes and Sub-Genotypes

> 350 million people chronically infected worldwide
HCC Risk Associated with Baseline HBV DNA Level and Genotype: Case-Control Study

Log$_{10}$(HBV DNA), HBV genotype

- >5.90, C: 26.49 (10.41-67.42)
- 4.23-5.90, C: 13 (4.65-19.27)
- <4.23, C: 6.55 (2.23-19.27)
- >5.90, mixed/non-C: 6.99 (2.97-16.49)
- 4.23-5.90, mixed/non-C: 2.95 (1.29-7.75)
- <4.23, mixed/non-C: 1 (referent)

Adjusted odds ratio (95% confidence interval)

Yu et al. JNCI 2005.
Molecular Pathogenesis
Hepatitis B Pathogenesis

- Hepatocellular injury caused by HBV infection is predominantly immune mediated
- Key HBV epitopes: include HBcAg
- Key mediators: HLA-class I Restricted CD8+ cells
- Key role of anti-HBs and anti-HBe
- The breadth and strength of the specific adaptive immune response determines the outcome of acute or chronic HBV infection

**ACUTE:** CD8+ and CD4+ T cell responses are strong, polyclonal and multispecific

**CHRONIC:** these responses are weak and narrowly focused

**CD8+ T cell has a direct cytolytic effect**

Hepatitis B Pathogenesis #2

- CD8+ T cells also have a non-cytolytic effect through production of IFN-γ and TNF-α (also produced by NK and NKT cells)
- Role of alpha/beta interferons to non-cytopathically suppress HBV replication via suppression of viral gene expression

Current Concepts

- Both non-cytolytic and cytolytic mechanisms are required for successful HBV control and eventual clearance

Kinetic Models of Self-Limited HBV and HCV Infection

Adaptive immune response

Undetectable

Detectable

HBV/HCV viral kinetics

Weeks after infection

HCV

HBV

HCV/Liver damage

ALT

HBV/Liver Damage

ALT

HBV

The Innate Immune System

- Evolutionarily ancient
- Universal - all multicellular organisms
- Constitutive - germ-line
- Immediate pathogen response (minutes to hours)
- Components: Pattern Recognition Central
  - Pattern recognition receptors (PRR): pathogen-associated molecular patterns (PAMP)
  - At least three families of PRRs relevant to viruses:
    1. Toll-like Receptors (TLRs) [all microbes]
    2. RIG-I-like RNA helicases (RLH/RLRs) [viruses]
    3. Nod-like Receptors (NLRs) [bacteria and viruses]

TRINITY OF CO-OPERATIVE SIGNALLING

NO MEMORY

Overview of Pathogen Sensing

[Pattern Recognition Central: PAMP]
TLR’s: What Are They?

- Type I integral membrane glycoproteins
- Members of larger superfamily that includes IL-1 receptors (considerable homology in cytoplasmic regions)
- Highly conserved
- Tolls are activated by molecules common to pathogens but absent from self.
- Toll activation leads to activation of ‘danger signals’

TLR Signaling: MyD88 vs IRF dep

TLR2 Expression in HBeAg-POS and HBeAg-NEG CH-B Patients

A

B

C

Visvanathan, K. et al
2007. Hepatology;45:102
HBV Life Cycle and Innate Immunity

Potential intracellular PAMPS:
- nucleocapsid
- viral DNA
- viral RNA (ss and ds)
- viral proteins

LA PROTEIN

Potential PAMPS for virus and sub-viral particles:
- glycoproteins (HBsAg)
- nucleocapsid (HBcAg)
- rcDNA

HBV Virion

HBsAg

Potential intracellular PAMPS:

LA PROTEIN

Other potential PAMPS:
- secreted HBsAg
- secreted HBeAg
- secreted non enveloped nucleocapsids
- free viral nucleic acids

TLRs & Specific Immunity

Innate immune response

- Secreted receptors (complement, MBL)
- Recognition and signaling (TLRs)
- Phagocytosis

Adaptive immune response

- Co-stimulatory molecule
- Antigen presentation
- Antigen presenting cell

- TH1
- TH2

IL-1, IL-6, TNF-α
IL-12
Stimulation of the interleukin-1-receptor and toll-like receptor-2 inhibits hepatitis B virus replication in hepatoma cell-lines *in vitro*


TLR2 Stimulation Inhibits HBV Replication: WT (HBeAg-Positive) HBV \textit{(in vitro)}
Treatment and Associated Challenges
Current Treatment Limitations

- Currently Two types of drugs currently approved:
  - **Antivirals** (e.g. LMV, ADV, ETV, LdT, TFV)
    - target the virus directly
    - limited potential treatment pool
    - High incidence of drug resistance
  - **Immunomodulators** (e.g. IFN)
    - stimulate the body’s immune system to attack the virus
    - T-Cell and B-Cell modulators
    - limited potential treatment pool
    - High incidence of serious adverse side effects
    - Low efficacy in patients with high levels of HBeAg titre
Entecavir, Telbivudine and Tenofovir Achieves Potent Viral Suppression in Nucleos(t)ide Naïve Patients at One Year

Data not from head-to-head studies therefore cross comparisons cannot be made

<table>
<thead>
<tr>
<th></th>
<th>ADV 10 mg/day</th>
<th>LdT 600 mg/day</th>
<th>ETV 0.5 mg/day</th>
<th>TDF 300 mg/day</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>LVD 100 mg/day</strong></td>
<td>(+)¹ (-)¹</td>
<td>(+)² (-)³</td>
<td>(+)¹ (-)¹</td>
<td>(+)³ (-)³</td>
</tr>
<tr>
<td><strong>HBeAg (+) (+)</strong></td>
<td>5.5</td>
<td>6.5</td>
<td>6.9</td>
<td>8.6⁻⁷ 6.9⁻⁷</td>
</tr>
<tr>
<td><strong>HBeAg (-) (-)</strong></td>
<td>4.4</td>
<td>3.6</td>
<td>5.2</td>
<td>6.2</td>
</tr>
</tbody>
</table>

**Mean baseline viral load (log₁₀ copies/mL)**

Does NUC Therapy Prevent HCC?

YES!

Review

Incidence of hepatocellular carcinoma in chronic hepatitis B patients receiving nucleos(t)ide therapy: A systematic review

George V. Papatheodoridis¹,*; Pietro Lampertico²; Spilos Manolakopoulos¹; Anna Lok³

¹2nd Department of Internal Medicine, Athens University Medical School, Athens, Greece; ²1st Division of Gastroenterology, Fondazione Policlinico IRCCS Ospedale Maggiore, Mangiagalli e Regina Elena, Università degli Studi di Milano Milan, Italy; ³Division of Gastroenterology, University of Michigan, Ann Arbor, MI, USA

2010 Journal of Hepatology vol. 53; 348-356
Nucleos(t)ide Analogue (NA) Therapy and Incidence of Hepatocellular Carcinoma

- Patients with CHB receiving effective oral antiviral therapy for at least 46 months reduced HCC risk by >56% (6.4% vs 2.8%; p=0.003)

- Rate of HCC significantly higher in patients with lamivudine resistance than in NA-naïve patients (7.1% vs 3.8%; p=0.001)

- Incidence of HCC significantly higher in patients with lamivudine resistance with cirrhosis than in those who were NA-naïve cirrhosis (18% vs 11%; p=0.015)

- It is unethical not to offer treatment to patients with CHB who meet treatment criteria

- The maintenance of viral suppression over a long period of time is needed to reduce the risk of HCC

The paradigm of antiviral therapy is the suppression and maintenance of viraemia below the limit of detection.

No Replication = No Resistance

NR = NR

Rates of Development of Resistance (HBeAg+)

Data not from head-to-head studies. Design and inclusion criteria may differ.
Recommend serum HBV DNA levels measured at week 12 and 24

- Week 12: compliance
- Week 24: risk of resistance if using low genetic barrier NA
  : predict likelihood of a sustained virological response
Indications of Emergence of Drug-Resistant Virus

1. Increasing viral load (≥ 1.0 log IU/ml)
2. Identification of known genotypic markers of drug resistance within viral polymerase:
   * primary resistance mutations (rtM204I)
   * secondary resistance mutations (rtL180M with rtM204V)
   * compensatory mutations (rtV173L)
3. Increasing serum ALT levels
4. Clinical deterioration

Rates of Development of Resistance (LAM-RES)

Data not from head-to-head studies. Design and inclusion criteria may differ.
Switch to or Add on Rescue Therapy?

- **Lamivudine-R or Telbivudine-R**
  - Adefovir: add-on (**not optimal**)
  - Tenofovir: switch to seems sufficient
  - Entecavir: switch to (**not optimal rescue**)

- **Adefovir-R**
  - Lamivudine: add-on if no prior LAM-R (**not optimal**)
  - Tenofovir: switch to (**not optimal rescue**)
  - Entecavir: switch to seems sufficient

- **Entecavir-R**
  - Tenofovir: switch to seems sufficient

- **MDR (rtA181T containing)**
  - Tenofovir plus Entecavir: seems sufficient

Zoulim F & Locarnini S. 2012. J Hepatol;56(Suppl.1):S112-S122
Summary

KEEP IT SIMPLE

• EASL recommends the use of high genetic barrier drugs (Entecavir or Tenofovir) as First Line Agents
• Reduces laboratory-based monitoring (DNA/Pol Sequencing)
• Reduces/eliminates the burden of managing NA-resistant Patients in the clinic
• By treating appropriately……………

NO Replication = No Resistance

NR = NR
Future Directions
Main Factors that Influence HBV Persistence and Pathogenesis

1. **Immune-mediated factors:**
   - Lack of robust type-1 IFN production
   - Weak CD8+T- and CD4+T-cell responses
   - NK-, NKT cell-mediated liver damage

2. **Virological factors:**
   - cccDNA stability
   - Interference with the innate immune system of the hepatocytes:
     a) HBeAg-mediated inhibition of TLR-2 signalling
     b) HBx-mediated inhibition of RIG-1 pathways
     c) SVPs-mediated inhibition of TLR-9 signalling
     d) Impairment of STAT translocation/methylation
   - Alteration of cellular pathways
     a) Cell-signalling pathways
     b) Mitochondria functions
     c) Lipid metabolism
     d) Epigenetic status of host genome

3. **Emergence of HBV variants:**
   - Induced by immune pressure (PC/BCP)
   - Induced by antiviral therapy with polymerase inhibitors

4. **Co-infection with other viruses:**
   - HBV/HCV
   - HBV/HDV
   - HBV/HIV

Dandri, M & Locarnini, S. 2012. GUT;61(Suppl 1):i6-i17
# Potential Viral Biomarkers

Multiple Motifs in the HBV genome (Carmen W 1999)

<table>
<thead>
<tr>
<th>Region</th>
<th>Genotype</th>
<th>Phenotype</th>
</tr>
</thead>
<tbody>
<tr>
<td>Precore</td>
<td>G1896A</td>
<td>Loss of HBeAg</td>
</tr>
<tr>
<td></td>
<td>G1899A</td>
<td>Replication ↑</td>
</tr>
<tr>
<td>BCP</td>
<td>A1762T/G1764A T1753V</td>
<td>↓ HBeAg Replication ↑</td>
</tr>
<tr>
<td>EnhII (Box α)</td>
<td>C1653T</td>
<td>Replication ↑</td>
</tr>
<tr>
<td>HBx</td>
<td>xK130M and xV131I (1762/1764) xI127T/N (1753)</td>
<td>Affects transactivation of HBx</td>
</tr>
<tr>
<td>Pre-S2 C trunc</td>
<td>Up to aa175 of HBsAg</td>
<td>Intracellular retention/ER stress</td>
</tr>
<tr>
<td>Pol-Env</td>
<td>rtM204I rtA181T</td>
<td>Drug resistance/vaccine escape</td>
</tr>
</tbody>
</table>

**OTHER:** Viral load

Viral genotype
Liver cirrhosis
Host immune response markers
Conclusions

- Infection with HBV results in a diverse clinical spectrum and patient outcome
- Virus-hepatocyte-immune response relationship drives emergence of most HBV mutants
- Control measures (selection pressures) can result in emergence of escape variants:
  - Vaccines/HBIg
  - Antiviral drugs
- Require improvements in quantitative diagnostic virology
  - Quantitative measures of HBsAg and HBeAg in serum
  - Quantitative enumeration of quasispecies 
    eg: BCP, 1762, 1764
    rtA181T/sW172*
- Linking these data into individualized patient outcomes based on personalized intervention strategies

RESPONSE GUIDED THERAPY HAS ARRIVED FOR HEPATITIS B!!