Hepatitis B Virus infection: virology

167 Falk Symposium:
Liver under constant attack – from fat to viruses
Congress Centrum Mainz

Maura Dandri

Department of Medicine
Gastroenterology, Hepatology, and Infectious Diseases
University Hospital Hamburg - Eppendorf
Hepatitis B virus

Hepatitis B virus causes acute and chronic hepatitis in humans

- 400 million people chronically infected worldwide
- Ca. 1 million deaths / year (despite vaccine) **WHO 2004**

- Major risk factor of developing cirrhosis and hepatocellular carcinoma (HCC)

**HBV particles in serum**

1) Infectious virion (Dane particles)

Non-infectious subviral particles:
2) filaments; 3) 22 nm spheres

H.-W. Zentgraf, Heidelberg
**HBV: morphology & genome organization**

Family *Hepadnaviridae* (pararetroviruses)

- smallest DNA enveloped animal viruses

- **High species-specific:**
  (HBV → humans, chimpanzees, Tupaia)

- **High tissue-specific:** liver tropism

*Compact, open circular, partially double stranded DNA genome (3.2 Kbp)*

4 major overlapping ORFs:

- **preC/C gene**: capsid protein, precore (HBeAg);
- **P gene**: viral polymerase (RT)
- **S gene**: 3 envelope proteins
  (three in frame start codons)
- **X gene**: non-structural regulatory protein;
Consequences of mutations on overlapping ORFs

Error prone HBV polymerase permits occurrence of nucleotide mutations;

Stronger conservatory constraints on protein sequences;

Polymerase mutations selected by antiviral therapy with nucleoside analogues may affect sequence and function of the overlapping HBsAg;

Amino acid substitutions induced in the major surface protein can reduce anti-HBs antibodies binding, leading to antibody escape;

Harrison, Semin.Liv.Dis.2006
HBV replication-cycle

Modified from W. Gerlich
HBV replication-cycle
Viral entry

- Unknown binding receptor(s)
- Host range is determined at an early step: (entry, attachment, fusion)
- State of hepatocyte differentiation is crucial for susceptibility of infection:
  - PHH shortly after plating
  - HepaRG after in vitro differentiation

The preS1 domain is needed for HBV infectivity

N-termini acylation with myristic acid is required for in vitro infection

Glebe, Urban W J Gastroenterol 2007
Viral entry

Chemically synthesized lipopeptides derived from the envelope of HBV blocks infection in cell culture (HepaRG & PTH, PHH) …

Grieco et al., PNAS, 99 (24) 2002
Urban et al., J. Virol, 79 (3), 2005
Gödde et al., Gastroenterology, 129, 2005
Engelke et al., Hepatology, 43, 2005
Schulze et al., Hepatology, 46, 2007
Acylated HBV preS1-derived peptides block HBV infection in vivo

Prevention of HBV infection after subcutaneous application of acylated preS1 derived peptides in uPA/RAG-2 mice repopulated with primary human hepatocytes

Petersen, Dandri, Urban et al. Nature Biotech. 2008
Acylated HBV preS1-derived peptides inhibit HBV infection in vivo

Mechanisms unclear - Binding to cellular targets

Acylated preS-peptides are promising candidates for future clinical applications:

- prevention of HBV/HDV reinfection after liver transplantation
- post exposure prophylaxis
- horizontal transfer from mother to child
- in combination with other antiviral agents to improve therapeutic outcome in CH-B patients (?)
HBV infection: the problem of cccDNA eradication
cccDNA: key molecule in infection and persistence

- It is formed in cell nucleus after de novo infection or upon nuclear re-import of mature nucleocapsids
- cccDNA formation is the first marker of HBV productive infection
- Stable non-integrated minichromosome
- Chronic HBV infection is due to maintenance of the cccDNA in the liver

Nassal, Virus Research 2008
cccDNA: key molecule in infection and persistence

- cccDNA serves as template of viral transcription; it does not replicate!
- cccDNA is not directly affected by polymerase inhibitors
- Long-term antiviral therapy can reduce the pool of cccDNA

There are presently no specific antivirals that directly target cccDNA!

HBV nucleotide mutations can be archived in the cccDNA (for ever?)

Open issues: cccDNA stability in liver inflammation

Immune mediated hepatocyte injury and compensatory cell division may favor cccDNA loss by acting through different mechanisms

1) Cytokine-mediated cccDNA destabilization (curing of cells) 
   Wieland, PNAS 2004

2) Due to cell division & inability to segregate with host chromosomes

3) Due to unequal cell distribution in dividing hepatocytes

4) cccDNA loss by dilution (killing for curing)

Sensitive molecular assays and infection models are needed to determine cccDNA stability in hepatocytes undergoing cell division
Open issues: cccDNA transcriptional activity

Little is known about mechanisms regulating cccDNA transcriptional activity

- cccDNA levels do not correlate with viremia levels in HBeAg(-) individuals
- Virion productivity (rcDNA/cccDNA) is significantly lower in the majority of treatment naïve HBeAg-negative individuals

Open issues: Factors affecting cccDNA activity

Virus and host-mediated mechanisms may control HBV replicative activity

• Expression of the regulatory HBx protein

• Immune-mediated (TNF$\alpha$, IFN $\gamma$) mechanisms may destabilize the cccDNA pool

• Epigenetic mechanisms may alter cccDNA transcriptional activity

Correlation between viremia levels and acetylation status of cccDNA-bound-histones

Antiviral treatments currently available do not permit eradication of the cccDNA minichromosome in the liver of CH-B patients. Strong down regulation and destabilization of the cccDNA, as well as enhancement of host immune responses may permit clearance of HBV infection.

Understanding of the factors affecting cccDNA stability and activity in vivo may assist in the design of novel therapeutic strategies aimed at silencing and eventually depleting the cccDNA reservoir in chronically infected patients.
Thank you!