Hepatitis B Virus Serum Biomarkers
Virtual Workshop

What’s Hepatitis B Core-Related Antigen (HBcrAg)

Jianming Hu
Penn State College of Medicine
Peripheral HBV Markers of Intrahepatic Viral Activity

- Complete Virion (HBs+HBc+DNA): 10^6/ml
- RNA Virion: 10^5/ml
- Empty Virion (HBs+HBc): 10^11/ml
- HBeAg: 10^{14}/ml
- HBsAg: 10^{14}/ml

- HBeAg
- p22
- PreC protein
- Incomplete processing
- PreCore

- 3.5 kb PreCore RNA
- 3.5 kb pgRNA
- cccDNA
- Integrated HBV DNA
- rcDNA
- Pol
- HBc
- L-HBs M-HBs S-HBs
- HBsAg

- 2.4 kb RNA
- 2.1 kb RNA
- 10^{10}/ml
- 10^{9}/ml
- 10^{10}/ml
HBV Precore/Core Gene – Differential Biogenesis

- **Complete Virion**
- **Empty Virion**

**HBc (p21)**

**pgRNA**

**PreCore/Core ORF**

**PreCore mRNA**

**precore (p25)**

**p22**

**HBeAg (p17)**

**PreC (“p22cr”)**

**HBeAg**

**Empty Virion ?**
Current HBcrAg CLEIA Assay (Fujirebio)

- **Multiple NTD mAbs**: target common sequences in HBc, HBeAg, PreC (p22cr)
- **SDS-heating denaturation**: release HBc from virions, HBeAg/PreC from Ag-Ab complexes
Characterization of Secreted Precore/Core Products

- mAbs targeting different regions of Precore/core (-10, NTD, CTD)
- Identification of HBcrAg components by SDS-PAGE and western blots
  - species, concentrations, physical states, correlations to cccDNA, etc.
Multiple of HBeAg and PreC (p22cr) Species Due to Differential CTD Processing

-10 (HBeAg & PreC)
NTD (All HBcrAg components-HBc, HBeAg, PreC)

PreC3 (A) → PreC2 (A) → PreC1
HBc → e3 (A) → e2 (A) → e1 (A)

CTD (HBc & long HBe/PreC in gtA only)
-10 (HBeAg & PreC)
NTD (All HBcrAg components-HBc, HBeAg, PreC)
Genotype-Dependent CTD Processing of Both HBeAg and PreC (p22cr)

PreC (p22cr) is processed similarly to HBeAg at CTD but retains N-terminal signal peptide.
HBeAg Is the Predominant Component of HBcrAg

**HBeAg**: ca. 1 ug/ml

**HBc**: ca. 0.1 ug/ml

**PreC**: ca. 0.1 ug/ml
PreC (p22cr) Is Not Major Component of Virions In HBV (gt D) Infected PHH

- HBeAg & PreC have higher density (expected of proteins) than virions on CsCl gradient.
- Migrate faster on agarose gel than virions.
Some Human Serum HBeAg and PreC in Low- and High-Density Fractions

1A11

PreC1

e1

e2

T2221

PreC1 & HBc

e2

e1

366-2

HBc

(CsCl) (Low)

V

Bottom (high)
HBcrAg Components - HBc/HBeAg/PreC (p22cr) in Human And Chimpanzee Serum

- Complete Virion (HBs+HBc+DNA) - HBeAg/PreC
  - HBeAg: $10^{14}$/ml
  - PreC: $10^{13}$/ml
- Empty Virion (HBs+HBc)
  - Complete Virion (HBs+HBc+DNA) - HBV PreCore
    - HBV PreCore
    - Low density serum factor (lipid, lipoproteins)?
  - Empty Virion (HBs+HBc) - HBV Core
    - HBsAg
      - $10^{14} \times (10^{16} \text{ molecules})$/ml
Differential Clearance Kinetics of Serum HBc (Empty Virion) vs. HBe/PreC in HBV Infected Chimpanzees
HBc, Not HBcrAg, in Chimpanzee Serum Correlates with Intrahepatic cccDNA

- For HBc: $R = 0.76$, $p < 0.05$, $n = 9$
- For HBcrAg: $R = 0.52$, $p = 0.15$, $n = 9$
Differential Clearance Kinetics of HBV DNA (Complete Virion)/HBc (Empty Virion)/HBsAg in HBV Infected Chimpanzees

1st phase (red) - viremia drop alone: block of pgRNA packaging (IFN)

2nd phase (purple) - antigenemia drop also: cccDNA suppression or loss
Sequence Conservation Between WHV and HBV Precorre/Core Gene

Signal Peptide

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<td>HBV gtD</td>
<td>MQLFHLC</td>
<td>7E9</td>
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<tr>
<td>WHV7</td>
<td>MYLFHLC</td>
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Precorre/Core Gene

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<td>LDTASALYREALESPHECSPHHTALRQAILCWGELMTLATWVGVNL</td>
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<tr>
<td>WHV7</td>
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Both Serum WHeAg and WPreC Are Glycosylated and Similarly Processed At CTD

A. HBV-Chimp (gt D) M1002

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<tr>
<td>4</td>
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B. F6005 (Chronic) M7392 (Acute)

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PNGase F - 12 KDa, 17 KDa, 24 KDa
Both Serum WHeAg and WPreC Are Glycosylated and Similarly Processed At CTD

WHeAg and WPreC are shown with their respective signal peptide (SP) and mature protein domains. The CTD (C-terminal domain) is indicated with a blue circle. The glycosylation sites are marked with a yellow box. The figure illustrates the processing and glycosylation of both WHeAg and WPreC proteins.
Low-Density WHeAg and WPreC in CsCl Gradient Fractions Correlate with Chronic, But Not Acute, WHV Infection
Differential Response of Serum WHV Markers During wIFN Treatment

1st phase - viremia drop alone: block of pgRNA packaging (in most animals)

2nd phase - antigenemia drop also: cccDNA suppression or loss (only in some animals, e.g., not in F1018)

Serum WHeAg loss, alone (F1013): antibody-mediated clearance?
Differential Response of Serum WHV Markers During TLR7 Agonist and NUC Treatment

1st phase - viremia drop alone:
block of pgRNA packaging (TLR7 agonist-IFN) or reverse transcription (NUC)

2nd phase - antigenemia drop also: cccDNA suppression or loss
Components of HBcrAg as Markers of Intrahepatic HBV Activity

- **HBeAg**: 10^{14}/ml
- **PreC**: 10^{13}/ml
- **Complete Virion (HBs+HBc+DNA)**: 10^9/ml
- **Empty Virion (HBs+HBc)**: 10^{11}/ml
- **HBsAg**: 10^{14}/ml

**Components of HBcrAg**

- **HBeAg**
- **PreC protein**
- **Incomplete processing**
- **PreCore protein**

**RNA and DNA Components**

- **3.5 kb PreCore RNA**
- **3.5 kb pgRNA**
- **2.4 kb RNA**
- **2.1 kb RNA**

**Silencing (secondary)?**

**IFN**

**NUC**

**HBc**

**Pol**

**rcDNA**

**cccDNA**

**Integrated HBV DNA**

**L-HBs**

**M-HBs**

**S-HBs**

**HBsAg**
Components of HBcrAg as Markers of Intrahepatic HBV Activity

Clearance Mechanisms

Non-cytolytic

1. HBe antibody clearance
2. Core promoter/PreCore ORF mutations

Transcriptional/post-transcriptional suppression

Cytolytic

* Also produced from integrated HBV
Conclusions

1. Serum HBeAg is the predominant component of HBcrAg.
   - Most factors affecting HBeAg will also affect HBcrAg, as a cccDNA marker.

2. Serum HBc (Empty virions) is normally a minor component of HBcrAg.
   - HBc levels does not always track those of HBeAg or PreC (p22cr)
   - HBcAg is a better marker for cccDNA – not affected by factors affecting HBeAg.
     - Need sensitive HBc assays!

3. HBc, not HBeAg or PreC (p22cr), is the major component of virions (complete or empty).

4. Measurement of individual HBcrAg components - HBc, HBeAg (PreC?), together with classical (viremia, HBsAg) and new (HBV RNA) serum markers provide better insights into intrahepatic HBV gene expression, replication, and cccDNA levels/activity during natural course of infection and antiviral treatment.

5. Serum HBeAg and PreC may be associated with host factors – role in HBeAg/PreC function and viral persistence?
   - Function of PreC (p22cr) vs. HBeAg – distinct?
Acknowledgements

Penn State College of Medicine
Xupeng Hong
Laurie Luckenbaugh
Megan Mendenhall

University of Basel, Basel, Switzerland
Stefan Wieland, PhD

Royal Melbourne Hospital, Melbourne, Australia
Renae Walsh
Liza Cabuang
Sally Soppe
Peter Revill, PhD

Georgetown University Medical Center
Stephan Menne, PhD

Gilead Sciences
Dara Burdette, PhD
Becket Feierbach, PhD
William Delaney, PhD

Funding - NIAID/NIH