Hepatitis B virus genotypes: Clinical significance and biological characterization

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Hepatitis B virus: the small virus causing a big medical problem

- enveloped;
- 3.2-kb genome;
- double stranded DNA;
- 4 genes: overlapping, unidirectional
- multiple proteins from core & envelope genes
The envelope gene encodes three envelope proteins

env gene

preS1  preS2  S

L protein
M protein
S protein

L protein: mediates virion formation.
M protein: enhances virion formation.
S protein: drives release of both virions and subviral particles.
Core gene encodes two related proteins with distinct functions

<table>
<thead>
<tr>
<th></th>
<th>core protein</th>
<th>HBeAg</th>
</tr>
</thead>
<tbody>
<tr>
<td>translated from</td>
<td>pg RNA</td>
<td>pc RNA</td>
</tr>
<tr>
<td>particle formation</td>
<td>yes</td>
<td>no</td>
</tr>
<tr>
<td>for DNA replication</td>
<td>required</td>
<td>not required</td>
</tr>
<tr>
<td>induces</td>
<td>immune clearance</td>
<td>immune tolerance</td>
</tr>
</tbody>
</table>
Genotype A produces multiple size forms of HBeAg

Ito et al., 2009, J. Virol. 83: 3507-3517.
Core protein expression is coupled with HBV DNA replication

- only pg RNA is required for HBV DNA replication;
- It serves as mRNA for core protein and DNA polymerase, and also as the pregenome for conversion to DNA;
- Therefore, amount of core protein translated correlates with level of genome replication;
- In the immune clearance phase, increased genome replication will trigger stronger immune attack.
- That may explain the association of core promoter mutations with both fulminant hepatitis and HCC.
Functional consequences of core promoter mutations

- The 3.5-kb pc RNA specifies HBeAg, whereas the 3.5-kb pg RNA is devoted to genome replication;

- Transcription of the two 3.5-kb RNAs is under the transcriptional regulation of core promoter;

- Core promoter mutations reduce HBeAg expression but enhance genome replication through transcriptional regulation of pc RNA and pg RNA;

- The 1762/1764/1766 triple core promoter mutation could increase genome replication by > 10 fold!

The two aspects of HBV genetic variability: genotypes and mutants
HBV and host immune clearance reaction: a cat-and-mouse game

- **Immune tolerance phase**
  - WT virus

- **Immune clearance phase (HBeAg+)**
  - Core promoter mutations
    - High HBeAg expression
    - High HBsAg expression
    - Low DNA replication
    - Reduce HBeAg expression
    - Enhance DNA replication

- **Immune clearance phase (HBeAg-)**
  - Precore mutation
    - Prevent HBeAg expression

- **Immune clearance phase (low HBsAg)**
  - PreS deletion immune escape mutation
    - Delete antigenic epitope
    - Reduce HBsAg secretion
    - Reduce HBsAg recognition
Geographic distribution of HBV genotypes

- A: India, Europe, Africa, North America
- B: China, Japan, Southeast Asia
- C: China, Korea, Japan, Southeast Asia
- D: India, Europe, Africa, North America
- E: Africa
- F: South America
- **G: Homosexual men**
- H: South America
Genotypes with highly overlapping geographic distributions

• A, D: India, Europe, Africa, North America

• B, C: China, Japan, Southeast Asia

• F, H: South America
Contrasting clinical manifestations between genotypes A and D

<table>
<thead>
<tr>
<th></th>
<th>A: sexual contact</th>
<th>D: transfusion &amp; transplantation</th>
</tr>
</thead>
<tbody>
<tr>
<td>route of transmission</td>
<td></td>
<td></td>
</tr>
<tr>
<td>chronicity rate of acute infection</td>
<td>A&gt;D</td>
<td></td>
</tr>
<tr>
<td>fulminant hepatitis</td>
<td></td>
<td>D&gt;A</td>
</tr>
<tr>
<td>HBeAg+ phase</td>
<td></td>
<td>A&gt;D</td>
</tr>
<tr>
<td>G1896A precore mutation</td>
<td></td>
<td>D&gt;&gt;A</td>
</tr>
<tr>
<td>core promoter mutations</td>
<td></td>
<td>A&gt;D</td>
</tr>
<tr>
<td>efficacy of IFN therapy</td>
<td></td>
<td>D&gt;A</td>
</tr>
</tbody>
</table>
Different properties between A and D genotypes

- Genotype D isolates often possess higher replication capacity;
- Genotype D isolates display reduced S protein expression and HBsAg secretion;
- Genotype A secretes several size forms of HBeAg;
- Genotype A cannot shut off HBeAg expression simply by G1896A mutation.
Genotype A is unable to shut off HBeAg expression via the G1896A mutation alone.

Contrasting clinical features between B and C genotypes

<table>
<thead>
<tr>
<th>Feature</th>
<th>Comparison</th>
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</thead>
<tbody>
<tr>
<td>Perinatal transmission</td>
<td>C &gt; B, more breakthrough infections</td>
</tr>
<tr>
<td>Transmission among adults</td>
<td>B2: sexual</td>
</tr>
<tr>
<td></td>
<td>C2: intrafamilial</td>
</tr>
<tr>
<td>Chronicity rate of adult infection</td>
<td>C2 &gt; B1 &amp; B2</td>
</tr>
<tr>
<td>Fulminant hepatitis</td>
<td>B &gt; C</td>
</tr>
<tr>
<td>HBeAg+ phase of chronic infection</td>
<td>C &gt; B (by 10 years)</td>
</tr>
<tr>
<td>Core promoter mutations</td>
<td>C &gt; B</td>
</tr>
<tr>
<td>Response to IFN therapy</td>
<td>B &gt; C</td>
</tr>
<tr>
<td>Age of HCC detection</td>
<td>B2 earlier than C2(?)</td>
</tr>
<tr>
<td>Lifelong HCC risk</td>
<td>C2 &gt; B2</td>
</tr>
</tbody>
</table>
Contrasting biological properties between B and C genotypes

- Wild-type genotype B isolates transcribe more 3.5-kb RNAs than corresponding genotype C isolates, leading to higher genome replication;

- Core promoter mutations could enhance genome replication of genotype C isolates;

- Wild-type genotype C isolates display more efficient virion secretion than corresponding genotype B isolates.

Our hypothesis

- Low replication and core protein expression prolongs the immune tolerance phase of genotype C infection, extending the HBeAg+ phase of infection;
- Low replication and core protein expression render genotype C less likely to trigger fulminant hepatitis, but more likely to cause chronic infection in adults;
- Efficient virion secretion compensates for low replication, thus enabling genotype C to spread efficiently inside the liver as required for persistent infection;
- At the immune clearance phase, genotype C can enhance its replication capacity by core promoter mutations, which offsets viral destruction and prolongs virus replication.
Immune tolerance vs fulminant hepatitis: opposing outcomes of HBV infection

<table>
<thead>
<tr>
<th></th>
<th>immune tolerance</th>
<th>fulminant hepatitis</th>
</tr>
</thead>
<tbody>
<tr>
<td>mostly occur in</td>
<td>infants</td>
<td>adults</td>
</tr>
<tr>
<td>duration</td>
<td>chronic</td>
<td>acute</td>
</tr>
<tr>
<td>replication capacity</td>
<td>low</td>
<td>high</td>
</tr>
<tr>
<td>HBeAg expression</td>
<td>high</td>
<td>low or absent</td>
</tr>
<tr>
<td>HBsAg expression</td>
<td>high</td>
<td>low?</td>
</tr>
<tr>
<td>dominant viral species</td>
<td>genotypes A, D wild-type</td>
<td>genotypes B, D core promoter mutant precore mutant</td>
</tr>
</tbody>
</table>
Dissecting the contribution of age of transmission vs. viral genotype towards chronic infection

• age of transmission:
  90% of perinatal transmission will end up as chronic infection;
  nearly all the perinatal transmission is caused by genotype B or C

• HBV genotype:
  the chronicity rate of acute adulthood infection is far greater for genotype A than genotype B or C
Prolonged HBV replication is a major risk factor for HCC development

• perinatal transmission (genotypes B & C);
• HBeAg positivity;
• High HBV DNA titer;
• core promoter mutations;
• preS deletions.
Conclusions

• The biological properties are different between genotypes A & D, as well as between genotypes B and C;

• HBV genotypes differ in terms of their prevalence of precore and core promoter mutations, as well as preS deletions;

• The interplay between viral genotype and mutations most likely underlies contrasting clinical features between genotypes A and D, as well as between genotypes B and C.
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