Viral Hepatitis: Biogeography and Pathogenesis

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Outline of Presentation

1. HBV and HDV Genotypes and Sub-Genotypes
   - Non-Indigenous vs Indigenous Distribution
2. HBV Recombinants: Impact on Vaccination and Pathogenesis
3. HDV Infection: Amazonian Basin

Viral Factors Associated with Outcome of Chronic Hepatitis B Virus Infection

- HBV DNA and HBsAg serum levels
  (↑ LC and HCC risk)
- Persistent HBeAg-positivity (precore/core)
  (↑ HCC risk)
- HBV Genotype & Sub-genotype
  (B1/B6 versus C2/F)
  [Liver Disease Progression]
- HBV Variants: [BCP mutants: (A1762T/G1764A)]
  (↑ LC and HCC risk)
- HBV Splice RNA/DNA
  (↑ HCC risk)
- Hepatitis Delta Virus Super-Infection
  [Fulminant hepatitis]

HBV Serotyping Versus Genotypes

<table>
<thead>
<tr>
<th>Genotype</th>
<th>AA122</th>
<th>AA160</th>
<th>Serotype</th>
</tr>
</thead>
<tbody>
<tr>
<td>A</td>
<td>K/K</td>
<td></td>
<td>adw, ayw</td>
</tr>
<tr>
<td>B</td>
<td>K/K</td>
<td></td>
<td>adw, ayw</td>
</tr>
<tr>
<td>C</td>
<td>R/K</td>
<td>R</td>
<td>adw, ayw</td>
</tr>
<tr>
<td>D</td>
<td>R/K</td>
<td>R</td>
<td>ayw</td>
</tr>
<tr>
<td>E</td>
<td>R/K</td>
<td></td>
<td>ayw</td>
</tr>
<tr>
<td>F</td>
<td>K/K</td>
<td></td>
<td>adw</td>
</tr>
<tr>
<td>G</td>
<td>K/K</td>
<td></td>
<td>adw</td>
</tr>
<tr>
<td>H</td>
<td>K/K</td>
<td></td>
<td>adw</td>
</tr>
<tr>
<td>I</td>
<td>K/K</td>
<td></td>
<td>adw</td>
</tr>
<tr>
<td>J</td>
<td>R/K</td>
<td></td>
<td>ayw</td>
</tr>
</tbody>
</table>

- 10 genotypes differ > 8% (entire genome)
- > 40 subgenotypes differ by 4% to 8% nt diversity

Maximum Clade Credibility Tree of the Hepatitis B Genotypes

Geographic Distribution of HBV Genotypes and Sub-Genotypes

> 350 million people chronically infected worldwide
> 2 billion people have been infected

CDC 2012
HBV Genotype and Natural History: Summary

• Genotype C causes more severe course of disease with cirrhosis and HCC than genotype B
• Same for genotype F versus H
• HCC risk may be higher in genotype D compared to genotype A-2
• Activity/severity of disease is comparable between genotype A and D

Therapeutic Significance of HBV Genotypes

(A) Interferon-alpha (IFN)
• HBV genotype C responds less to IFN treatment compared to B
• HBV genotype D responds less to IFN treatment compared to A

\[ A > B > C > D \]

(B) Nucleoside/nucleotide analogues
• Little evidence that genotypes respond differently

HBV Genotype Distribution in the North American Circumpolar Arctic

<table>
<thead>
<tr>
<th>Genotype</th>
<th>North American Circumpolar Arctic</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>A</td>
</tr>
<tr>
<td>Alaska</td>
<td>12%</td>
</tr>
<tr>
<td>Canada</td>
<td>1%</td>
</tr>
</tbody>
</table>
| Self-Identifying: Namaauut (B6) First Nation (D) B6 - Self Limiting Mild Disease F-1b - Progressive Liver Disease - High HCC Risk

Hepatitis B and Indigenous Populations #1

Geographical Distribution of the Ethnic Population Before 1519 and HBV Genotypes in the Mexican Population at Present

Results of Age- and Sex-Matched Case-Control Study of Clinical Differences Between HBV Subgenotypes B6, B1 and B2

<table>
<thead>
<tr>
<th>Characteristic</th>
<th>A (n=95)</th>
<th>B6 (n=82)</th>
<th>B1 (n=50)</th>
<th>B2 (n=50)</th>
<th>P-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age (years)</td>
<td>30 (11)</td>
<td>29 (11)</td>
<td>30 (12)</td>
<td>30 (11)</td>
<td>0.956</td>
</tr>
<tr>
<td>Gender (male/female)</td>
<td>49/46</td>
<td>44/38</td>
<td>27/23</td>
<td>27/23</td>
<td>0.567</td>
</tr>
<tr>
<td>Hepatitis B surface antigen</td>
<td>positive</td>
<td>26/95</td>
<td>24/82</td>
<td>13/50</td>
<td>17/50</td>
</tr>
<tr>
<td>Liver enzymes</td>
<td>normal</td>
<td>33/62</td>
<td>40/52</td>
<td>28/32</td>
<td>30/20</td>
</tr>
</tbody>
</table>

Geographical Distribution of the Ethnic Population Before 1519 and HBV Genotypes in the Mexican Population at Present

Osiowy, C et al 2013. Antiviral Ther;18:467-473

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Osiowy, C et al 2013. Antiviral Ther;18:467-473

Panduro, A et al 2013. Antiviral Ther;18:475-484
**Hepatitis B and Indigenous Populations #2**

**Central America / Mexico**

<table>
<thead>
<tr>
<th>Genotype</th>
<th>G</th>
<th>H</th>
<th>C</th>
<th>D</th>
<th>A</th>
<th>B</th>
</tr>
</thead>
<tbody>
<tr>
<td>Nahvas (Aztec)</td>
<td>-</td>
<td>62.5%</td>
<td>12.5%</td>
<td>12.5%</td>
<td>6.5%</td>
<td>6.5%</td>
</tr>
<tr>
<td>Huchol (Chichimicas) (west)</td>
<td>11.1%</td>
<td>11.1%</td>
<td>-</td>
<td>22.2%</td>
<td>55.6%</td>
<td>-</td>
</tr>
</tbody>
</table>

HBV-H: very low VL
Minimal disease
OBI
Aztec strain

Panduro, A et al 2013. Antiviral Ther; 18: 475-484

**Hepatitis B and Indigenous Populations #3**

**Latin America**

- Brazil: HBV-A and HBV-F predominate
- Rest of LA: HBV-F

Genotype F:
- Amerindian genotype
- Indigenous to the American Continent
- Wooly Monkey (WMV) – very close to HBV-F

F1-4
- F1a: Costa Rica, El Salvador
- F1b: Alaska, Argentina, Chile
- F2/F3: Northern Part of South America
- F2a: Brazil, Venezuela
- F2b: Venezuela
- F3: Colombia, Venezuela, Panama

Liver Cancer Risk: 8-10 fold increase

**Distribution of HBV Genotypes in Latin America**

**Hepatitis B Vaccination in Latin America**

- from 1990 to 2005 significant decrease in HBsAg prevalence in Tropical and Central Latin America
- Tropical LA changed from intermediate to low endemiocity region [HBsAg prevalence has halved]
- attributed to introduction of the universal HBV vaccination
- no serotype mismatch between genotype F (adw-4) and vaccine strain (genotype A-2, adw-2)


Liver Cancer Risk: 8-10 fold increase
Prevalence of HBsAg Positivity


HBV Replication: Sources of Heterogeneity

1. Minichromosome (cccDNA): RNA Pol II
2. Reverse Transcription: No Proof Reading
   - mutation rate: $5 \times 10^{-5}$ nt substitutions/sites/year
   - G $\rightarrow$ A hyper mutation
3. Recombination: genotype C
4. HBV Splice Pathway
5. Overlapping Reading Frames
   - stop codons

TreeOrder Scan of Nonrecombinant HBV and recB Sequences

Simmonds P and Midgley S 2005. JVirol;79:15467

HBV Recombination

C4: C-J Recombinant Reveals
[Indigenous Australians, Top End]

S-ORF (HBsAg) (ayw-3): J: POTENTIAL VACCINE ESCAPE MUTANTS

C – BACKBONE: INCREASED VIRULENCE
   (↑ LC progression)
   (↑ HCC risk)

- Serotype Mismatch with Vaccine (adw-2)
HBV Recombination: Mosaic Genome

B2 vs B1

Reveals: Okinawa Paradox (B1 minimal disease) versus high LC and HCC risk in Taiwan (B2)

Pre-C/C-ORF Virulence Marker

↑ LC progression
↑ HCC risk

Viral Factors Associated with Outcome of Chronic Hepatitis B Virus Infection

- HBV DNA and HBsAg serum levels
  (↑ LC and HCC risk)
- Persistent HBeAg-positivity (precore/core)
  (↑ HCC risk)
- HBV Genotype & Sub-genotype (B1/B6 versus C2/F)
  (Liver Disease Progression)
- HBV Variants: [BCP mutants: (A1762T/G1764A)]
  (Presence and proportion (> 45%))
  (↑ LC and HCC risk)
- HBV Splice RNA/DNA
  (↑ HCC risk)
- Hepatitis Delta Virus Super-Infection
  [Fulminant hepatitis]

HDV: Unique Features in Human Virology

- Smallest infectious agent in man
- Similar to viroid RNAs of plants
- Infectious at $10^{11}$ serum dilution
- Rolling circle mechanism of replication
- Self-cleaving ribozyme
- Transcription by host-RNA polymerases
Hepatitis Delta Virus Infection in the Amazon Basin

1. Labrea Hepatitis (Labrea Black Fever)
   - children and young adults
   - males more often than females
   - often clusters in families
   - fulminant form of viral hepatitis
   - high degree of mortality (within 1-2 weeks)
   - "morula-like" cells in the liver (microvesicular change)
   - described in the 1950s, Labrea Region on the Purus River
   - similar descriptions in Colombia and Peru as early as 1934

2. "Hepatitis of the Sierra Nevada de Santa Marta"
   - first described in 1930 in the Magdalena province of Northern Colombia

Labrea Hepatitis: Morula-Like Cells

Bartsch, G et al 1967. JAMA 199: 479-484

Delta Virus Infection and Severe Hepatitis

An Epidemic in the Yurupík Indians of Venezuela

Bensabath, G et al 1987. JAMA; 258: 479-488


HDV Genome

HDV Genome
Histologic Studies of Severe HDV Infection in Venezuelan Indians


Probability of Survival of Patients with Chronic Hepatitis D According to Histology of Clinical Staging at Enrolment

Global HDV Distribution

Genotype: > 30% nt diversity; subgenotype > 8% nt diversity

Pathogenesis of HDV Super-Infection in Amazon Basin

• Genotype III of HDV
  [versus Genotype I HDV Europe]

• Genotype F: HBsAg extra cysteine residues
  [versus Genotype H in Mexico]

• Waves of HDV Gen III Super-Infection in Amazon Basin (every 5-10 years).
• Associated with Very High Mortality Amongst Young Population

Therapy – Current State

• The current recommendation is pegylated interferon-alpha weekly for 12-18 months

• 20%-25% of the patients respond; HDV may relapse as long as HBsAg is present in blood

• Only reliable end-point of therapy is the clearance of HBsAg in blood
Possible Factors Influencing the Response to Therapy in Chronic Hepatitis D

- **HDV genotype**
  - Genotype non-1 (Hughes #138)

- **Baseline HDV RNA load**
  - Viral load lower than $<6 \log_{10}$ (Stern #186)

- **Early virological response**
  - PCR-negative within 6 months of therapy

- **Baseline HBsAg titre**
  - Lower in responders (Hughes #138)

- **Stage of fibrosis**
  - Absence of cirrhosis

HDV Therapy Challenges

- HBV required only to provide HBsAg for HDV entry and exit from cell

- Replication of HDV independent from HBV replication (i.e. from HBV-DNA levels)

- **NO KNOWN REPLICATION FUNCTION OF HDV** to be targeted by antivirals

Myrcludex B: An Entry Inhibitor

- ** PRIMARY INFECTION**
  - Myrcludex B

- **RE-INFECTION**
  - Myrcludex B

Myrcludex B: An Entry Inhibitor

- The GMP synthesis of 100 g Myrcludex B (API) is finished.
- A formulation for s.c. application has been developed.
- Vials for clinical studies have been filled.
- Myrcludex B has been characterized for purity, stability etc.

Status of Myrcludex B the First in Class Entry Inhibitor of HBV and Hepatitis Delta Virus (HDV).

Conclusions

1. Infection with HBV and/or HDV in Indigenous persons results in the full spectrum of clinical outcomes from asymptomatic infection to rapidly progression cirrhosis, fulminant hepatitis with death in weeks as well as early onset hepatocellular carcinoma.

2. In 2014, despite the successes of implementing effective vaccine prevention programs and the widespread availability of efficacious and cheap treatments for CHB, Indigenous peoples are still at high risk for developing the serious and severe complications of infections with HBV and/or HDV.

3. Important initiatives such as the Global Hepatitis Program (GHP) of the World Health Organisation (WHO) need to ensure that Indigenous populations who are currently NOT deriving benefit of these recent medical advancements, now do so.

Thank You