Do we need better HBV vaccines?

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National Reference Centre (NRC) for Hepatitis B and D Viruses,
Justus Liebig University Giessen, Germany
The burden of Hepatitis B

THE BURDEN OF HEPATITIS B
More than 250 million people live with the virus; few of them are diagnosed and not enough children are vaccinated against it.

Rising death toll
Hepatitis infections are now associated with more deaths globally than are tuberculosis, HIV or malaria.

Graber-Stiehl, Nature 2018
FORGOTTEN NO MORE
A long-overlooked scourge of millions, hepatitis B is in the crosshairs at last

“Hepatitis B is completely overlooked and the funding is totally out of proportion to the problem and the need.”

Timothy Block, Hepatitis B Foundation

AFRICA’S SILENT EPIDEMIC
Hepatitis now kills more people worldwide than HIV, tuberculosis or malaria. Tackling the hepatitis B virus in Africa is key to fighting back.

Ian Graber-Stiehl, Nature 2018
How does HBV remain in a population?

Currently, more than 250 million people are chronically infected with HBV

- 25 HBV-DNA positive from 304 examined skeletons
- from ancient Europe to Asia,
- from 5,500 to 800 years before present (BP)


HBV user manual

1) Don’t kill your host
2) Healthy female chronic carriers
3) Infect offspring soonest possible

Ancient hepatitis B viruses from the Bronze Age to the Medieval period

- 25 HBV-DNA positive from 304 examined skeletons
- from ancient Europe to Asia,
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Neolithic and medieval virus genomes reveal complex evolution of hepatitis B

- 3 HBV-DNA positive from 53 examined skeletons
- from ancient Western Europe,
- from 7,000 to 1,100 years before present (BP)
How does HBV remain in a population?

Currently, more than 250 million people are chronically infected with HBV


**HBV user manual**

1) Don’t kill your host
   - Vaccinate possible hosts
2) Healthy female chronic carriers
   - Female healthy vaccinees
3) Infect offspring soonerest possible
   - Vaccinate at birth (active/passive)

**Ancient hepatitis B viruses from the Bronze Age to the Medieval period**

- 25 HBV-DNA positive from 304 examined skeletons
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**Neolithic and medieval virus genomes reveal complex evolution of hepatitis B**

- 3 HBV-DNA positive from 53 examined skeletons
- from ancient Western Europe,
- from 7,000 to 1,100 years before present (BP)
Successes of vaccination against HBV

During the 20-year follow-up, no subject acquired new chronic HBV infection or clinical hepatitis B disease

Thailand

Evidence of protection against clinical and chronic hepatitis B infection 20 years after infant vaccination in a high endemicity region

Y. Poovorawan,1 V. Chongsrisawat,1 A. Theamboonlers,1 G. Leroux-Roels,2 S. Kuriyakose,3 M. Leyssen1 and J.-M. Jacquet1 1Department of Pediatrics, Faculty of Medicine, Center of Excellence in Clinical Virology, Chulalongkorn University, Bangkok, Thailand; 2Center for Vaccinology, Ghent University and Hospital, De Pintelaan, Ghent, Belgium; and 3GlaxoSmithKline Biologicals, Rixensart, Belgium

“During the 20-year follow-up, no subject acquired new chronic HBV infection or clinical hepatitis B disease“

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**Vertical HBV mother-to-child Transmission (MTCT)**

MTCT despite active/passive immunization of newborns

<table>
<thead>
<tr>
<th>MTCT despite active/passive immunization of newborns</th>
<th>Maternal serum HBV-DNA, viral load (VL)</th>
<th>Study country</th>
</tr>
</thead>
<tbody>
<tr>
<td>0 %</td>
<td>&lt; 2 x 10^5</td>
<td>&lt; 10^6</td>
</tr>
<tr>
<td>3.2 %</td>
<td>2 x 10^5-6</td>
<td>10^6-7</td>
</tr>
<tr>
<td>6.7 %</td>
<td>2 x 10^6-7</td>
<td>10^7-8</td>
</tr>
<tr>
<td>7.6 %</td>
<td>&gt; 2 x 10^7</td>
<td>&gt; 10^8</td>
</tr>
<tr>
<td>9 to 10 %</td>
<td>&gt; 2 x 10^7</td>
<td>&gt; 10^8</td>
</tr>
</tbody>
</table>

- **Up to 10 % of newborns** of HBV-pos. mothers with high VL are not protected despite vaccination
- **Over 90% risk of chronic infection in newborns**
  - **Antiviral therapy of HBV-infected pregnant women reduces MTCT**
    - TDF superior to Telbivudine or Lamivudine
    - Should be started early during pregnancy
    - Appears to be safe during pregnancy

**HBV user manual**

1) **Don’t kill your host**
   - Vaccinate possible hosts
2) **Healthy female chronic carriers**
   - Female healthy vaccinees
3) **Infect offspring soonest possible**
   - Vaccinate at birth (active/passive)

Terrault et al., Hepatology, 2016; 63:261-283
Problems with the HBV vaccine
Non/low-responders (below 10 IU/L anti-HBs)

“A primary 3-dose series induces protective antibody concentrations in > 95% of healthy infants, children and young adults” (WHO, 2017)

### Table 1  Factors determining the immune response to HB vaccine

<table>
<thead>
<tr>
<th>Reduced response is correlated with</th>
<th>References</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Subject characteristics</strong></td>
<td></td>
</tr>
<tr>
<td>Male gender</td>
<td>[12, 54]</td>
</tr>
<tr>
<td>Older age</td>
<td>[20, 21]</td>
</tr>
<tr>
<td>Obesity (BMI ≥ 30)</td>
<td>[12, 55]</td>
</tr>
<tr>
<td>Malnutrition</td>
<td>[56]</td>
</tr>
<tr>
<td><strong>Lifestyle</strong></td>
<td></td>
</tr>
<tr>
<td>Smoking</td>
<td>[12, 54]</td>
</tr>
<tr>
<td>Drug abuse</td>
<td>[57]</td>
</tr>
<tr>
<td><strong>Genetic non-response</strong></td>
<td></td>
</tr>
<tr>
<td>HLA haplotype (DPB1<em>02 or 1101, DRB1</em>03, 1302, 14, DQA1<em>0301, DQB1</em>02**, 0401, 0604)</td>
<td>Reviewed in [58]</td>
</tr>
<tr>
<td><strong>Health/disease status</strong></td>
<td></td>
</tr>
<tr>
<td>Chronic kidney disease</td>
<td>[59, 60]</td>
</tr>
<tr>
<td>Haemodialysis</td>
<td>[61, 62]</td>
</tr>
<tr>
<td>Diabetes</td>
<td>[63]</td>
</tr>
<tr>
<td>HIV</td>
<td>[64, 65]</td>
</tr>
<tr>
<td>Hematopoietic stem cell recipients</td>
<td>[66]</td>
</tr>
<tr>
<td>Pre-existing hepatitis C infection</td>
<td>[67, 68]</td>
</tr>
</tbody>
</table>

- Response decreases with age to 60-75% at the age of 60.
- With combination of negative factors up to 70% non/low-response (Wolters et al., 2003)

- Erika Garner-Spitzer: primary vaccine failure
- Pieter Meysmann: Transcriptome profiling

adapted from: Leroux-Roels, Med.Microbiol Immunol, 2015; 204:69-78
What is a low/non-responder?

Question: Which anti-HBs titre is protective against infection with HBV after vaccination?

“An anti-HBs antibody concentration of $\geq 10$ IU/L measured 1–2 months after administration of the last dose of the primary vaccination series is considered a reliable serological marker of long-term protection against HBV infection.” (WHO 2017)

<table>
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<th>Response</th>
<th>Full</th>
<th>Low</th>
<th>Non</th>
</tr>
</thead>
<tbody>
<tr>
<td>Anti-HBs (IU/L)</td>
<td>$\geq 10$</td>
<td>1-9</td>
<td>0</td>
</tr>
<tr>
<td></td>
<td>$\geq 100$</td>
<td>10-99</td>
<td>0-10</td>
</tr>
<tr>
<td>Protective</td>
<td>Yes?</td>
<td>No ?</td>
<td>No ?</td>
</tr>
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WHO, UK, Ireland, Switzerland, Germany
Does the HBV vaccine protects against infection?

Question: Which anti-HBs titre is protective against infection with HBV after vaccination?

“An anti-HBs antibody concentration of ≥ 10 IU/L measured 1–2 months after administration of the last dose of the primary vaccination series is considered a reliable serological marker of long-term protection against HBV infection.” (WHO 2017)

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“During the 20-year follow-up, no subject acquired new chronic HBV infection or clinical hepatitis B disease”

22.8% asymptomatic HBV infections in the 2nd decade
Increase in occult HBV infection (OBI) caused by partial protection after vaccination?

- 5.4% seronegative OBI in vaccinated children (birth dose) in Taiwan
  - Lai et al., Medicine (2016) 95:49(e5625)
Occult HBV infection (OBI) and transfusion medicine

**Transient occult HBV-infection in vaccinated blood donors from US**
- Of 2.1 million donations, 28 showed markers of a recent HBV infection
- Nine donors with transient OBI (up to four months duration)
- Titres up to 10,000 IU/mL HBV-DNA

<table>
<thead>
<tr>
<th>Anti-HBs titre (IU/L) of donors with transient OBI</th>
<th>HBV DNA positive</th>
</tr>
</thead>
<tbody>
<tr>
<td>Not vaccinated</td>
<td>3</td>
</tr>
<tr>
<td>&lt; 10, vaccinated</td>
<td>2</td>
</tr>
<tr>
<td>10 - 100, vaccinated</td>
<td>4</td>
</tr>
<tr>
<td>&gt; 100, vaccinated</td>
<td>0</td>
</tr>
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</table>

- Only an anti-HBs > 100 IU/L protects also against occult infection

**Immunity of blood donors against HBV from US**
- 62 % vaccinated
- 41 % anti-HBs below 100 IU/L
  - Partially protected
  - Occult infection after exposition
- 21 % anti-HBs above 100 IU/L

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UK, Ireland, Switzerland, Germany

Are our current anti-HBs tests reliable?

“Anti-HBs […] is considered a reliable serological marker…(WHO)”

- Many anti-HBs tests are not suitable to generate reliable quantitative anti-HBs results in the range 5 to 20 IU/L (individual sera; Huzly et al., 2008)
- “Different anti-HBs assays were associated with statistically significant ($P < 0.05$) differences in anti-HBs titres in all dilutions.” (pooled sera; Raven et al., 2016)
Neutralizing epitopes of HBV surface proteins

Liver sinusoid

(1) Three surface proteins
(2) PreS1 and S domain relevant for infection
(3) Both carry neutralizing epitopes

HBV surface proteins

(1) Three surface proteins
(2) PreS1 and S domain relevant for infection
(3) Both carry neutralizing epitopes

Anti-preS1 alone can neutralize HBV infection

Pre-S1 Antigen-Dependent Infection of *Tupaia* Hepatocyte Cultures with Human Hepatitis B Virus

Dieter Glebe,¹* Mehriar Aliakbari,¹ Peter Krass,¹ Eva V. Knoop,¹ Klaus P. Valerius,² and Wolfram H. Gerlich¹

Institute of Medical Virology¹ and Institute of Anatomy and Cell Biology,² Justus Liebig University Giessen, 35392 Giessen, Germany

Received 13 February 2003/Accepted 3 June 2003

In vivo neutralization of hepatitis B virus infection by an anti-preS1 humanized antibody in chimpanzees

Hyo Jeong Hong,⁠¹* Chun Jeih Ryu,⁠¹ Hyang Suk Hur,⁠¹ Seho Kim,⁠¹ Han Kyu Oh,⁠¹ Mee Sook Oh,⁠¹ and Song Yong Park⁠¹

⁠¹Antibody Engineering Research Unit, Korea Research Institute of Bioscience and Biotechnology, Taejon 305-600, South Korea
⁠²Central Research Center, Korea Green Cross Corp., Kyunggi-Do 449-903, South Korea
⁠³R&D Center, Aprogen, Inc., Taejon 305-600, South Korea

Received 1 August 2003; returned to author for revision 11 September 2003; accepted 11 September 2003
Neutralising antibodies generated by different HBV vaccines

1st generation
HBsAg, purified from plasma of HBV-infected patients

Neutralising antibodies generated by different HBV vaccines

Characterization of the 3rd International Standard for hepatitis B virus surface antigen (HBsAg)

Pia L. Seiz a, Christina Mohr a, Dianna E. Wilkinson b, John Ziebuhr a, Christian G. Schütter a, Wolfram H. Gerlich a, Dieter Glebe a, *

HBsAg from patient

<table>
<thead>
<tr>
<th>Fraction</th>
<th>1</th>
<th>2</th>
<th>3</th>
<th>4</th>
<th>5</th>
<th>6</th>
<th>7</th>
<th>8</th>
<th>9</th>
<th>10</th>
<th>11</th>
<th>12</th>
</tr>
</thead>
<tbody>
<tr>
<td>relative reactivity [%]</td>
<td>10</td>
<td>20</td>
<td>30</td>
<td>40</td>
<td>50</td>
<td>60</td>
<td>70</td>
<td>80</td>
<td>90</td>
<td>100</td>
<td></td>
<td></td>
</tr>
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HBsAg from vaccine

<table>
<thead>
<tr>
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<th>4</th>
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<th>6</th>
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<td>30</td>
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<td>60</td>
<td>70</td>
<td>80</td>
<td>90</td>
<td>100</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Prepared from vaccine bulk material from different donors in Vietnam
HBsAg Gt B4, ayw1/adw2

Neutralising antibodies generated by different HBV vaccines

1\textsuperscript{st} generation
HBsAg, purified from plasma of HBV-infected patients

2\textsuperscript{nd} generation
HBsAg from yeast
WHO vaccine: HBV Gt A2 Serotype adw2

WHO 2nd generation vaccine contains only SHBs of HBV genotype A2

99 % of all chronic carriers have different HBV genotypes

Breakthrough of distantly related HBV genotypes?
Genotype-effect during vertical transmission

- Subtype-specificity of the 2nd generation vaccine
  - **Vaccine: subgenotype A2, serotype adw2**
  - Vaccine protects against all genotypes at high anti-HBs (> 100 IU/L)
  - Genotype-effect during vertical transmission (Taiwan)

HBV-infected children

<table>
<thead>
<tr>
<th>Genotype</th>
<th>Serotype</th>
</tr>
</thead>
<tbody>
<tr>
<td>B</td>
<td>adw2</td>
</tr>
<tr>
<td>C</td>
<td>adr</td>
</tr>
<tr>
<td>A2</td>
<td>adw2</td>
</tr>
<tr>
<td></td>
<td>vaccine</td>
</tr>
</tbody>
</table>

Wen et al., Hepatology 2011
Vaccine breakthrough of distant HBV genotypes

- WHO 2nd generation vaccine contains only SHBs of HBV genotype A2
- 99% of all chronic carriers have different HBV genotypes
- Breakthrough of distantly related HBV genotypes?

1. Acute breakthrough with genotype F (Tacke et al., 2007)
   - Complete vaccination with Twinrix (HepA/B)
   - 10 months later acute HepB with icterus
   - Anti-HBs 82 IU/L at start of disease, no escape mutations

2. Chronic breakthrough with genotype F (O'Halloran et al., 2011)
   - Vaccinated with Engerix B (5x): 161 IU/L anti-HBs
   - After two years HBV-infection, no icterus
   - Establishes chronic HepB, no escape mutations

<table>
<thead>
<tr>
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<th>Serotype</th>
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<tbody>
<tr>
<td>B</td>
<td>adw2</td>
</tr>
<tr>
<td>C</td>
<td>adr</td>
</tr>
<tr>
<td>F</td>
<td>ayw4</td>
</tr>
<tr>
<td>A2</td>
<td>adw2</td>
</tr>
</tbody>
</table>

- HBV genotype F is phylogenetically most distant to the WHO 2nd generation vaccine of HBV genotype A2

A bat hepadnavirus with zoonotic potential

- TMBHBV isolated from New World bat (2013)
- The only “zoonotic animal hepadnavirus” (besides primate HBV)
- Infects primary human hepatocytes via NTCP
- Anti-HBs does not neutralize TMBHBV infection in vitro

An improved HBV vaccine for eradication of HBV?
Summary

(1) The hepatitis B virus (HBV) is a human pandemic virus that phylogenetically dates back at least to the Neolithic Age (7,000 years BP).

(2) HBV is usually not pathogenic and persists even in small human communities (e.g. hunter-gatherers) mainly through healthy chronic carriers and mother-to-child transmission (MTCT).

(3) Common HBV-Vaccine (2nd generation, genotype A2, SHBs only) protects against clinical and chronic infection, but asymptomatic infections are common.

(4) Problems are low/non-response, MTCT with high viral load of the mother; genotype-dependency of the vaccine, causing asymptomatic occult or rarely acute/chronic infections.

(5) Anti-HBs titre of 100 IU/L protects most likely against all forms of HBV infection.

(6) 1st and 2nd generation vaccines lack epitopes interfering with high affinity binding of HBV to its liver-specific receptor NTCP.

(7) Vaccination with third-generation vaccines that include preS1 induce additional antibodies targeting the preS1 receptor interaction with NTCP and could lead to improved protection of risk groups.