Vaccination contre l’hépatite B : succès et perspectives

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hepatitis B vaccines

- HBV and the disease
- recombinant preventive vaccines
- chronic hepatitis B: toward an HBV cure
Global Burden of HBV

- > 2 billion individuals with markers of current or past infections
- 4 million acute cases of hepatitis B per year
- 200-300 million with chronic HBV disease
- Around one-third of persons with chronic HBV disease die from decompensated cirrhosis or hepatocellular carcinoma (HCC)
- 1 million deaths per year
- HBV causes 60% to 80% of all primary liver cancer
- HBV is second most important carcinogen behind tobacco

hepatitis B virus: HBV

- **Hepadnavirus**
  - partially double stranded DNA genome
  - 4 ORF

- **Viral proteins**
  - 3 envelope proteins (S, M, L)
  - viral polymerase (P)
  - HBx protein (X)
  - Capsid protein © and HBeAg (preC-C)

- **Viral cycle**
  - Host range: humans, chimpanzees
  - hepatocyte
  - Non cytopathic

- **Routes of transmission**
  - Perinatal (Mother-to-infant)
  - Infected blood (IVDU), sexual (30% in USA)
  - Horizontal (intra-familial)
  - Unknown (up to 30%)

Seeger C & Mason WS; Virology 2015
Structure of HBV genome and viral antigens

10 HBV genotypes

HBsAg: group “a”
Subtypes: “ayw, adr, adw”
Geographical distribution of hepatitis B virus genotypes and subgenotypes
Outcome of hepatitis B virus infection
the younger the age of infection, the higher the HBV carrier rate!

Horizontal transmission
(adult infection)
- 90% HBV clearance
- Full immune response (B+T +NK cells)
- asymptomatic or acute hepatitis B

Vertical transmission
(neonatal, childhood infection 2-4yo)
- 5-10% HBV clearance
- 15-40% activation of CD4/8+ T cells
- 15-40% liver injury: CAH, Cirrhosis, HCC
- T cell ignorance
- Exhaustion
- asymptomatic chronic hepatitis B
- HBV and HBsAg persistence

>90% (25%)
Plasma-derived vaccine: HBV envelope proteins from sera of HBV-carriers

<table>
<thead>
<tr>
<th>Proteins</th>
<th>HBV Particles</th>
<th>HBs Filaments</th>
<th>HBs Spheres</th>
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<tbody>
<tr>
<td>LHBs</td>
<td>GP42, P39</td>
<td></td>
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</tr>
<tr>
<td>MHBs</td>
<td>GP36, GP33</td>
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<tr>
<td>SHBs</td>
<td>GP27, P24</td>
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<tr>
<td>HBc</td>
<td>P22</td>
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</table>
From plasma-derived to recombinant hepatitis B vaccine

- **1964** B. Blumberg discovered the «Australia Ag». Nobel price 1976.
- **1968** F. Prince: Australia Ag = HBsAg on viral particles and on VLPs.
- The first hepatitis B vaccine derived from inactivated HBV or HBsAg particles purified from plasma of HBV chronic carriers
  - HBsAg stimulates the production of protective anti-HBs antibodies in vaccinated children (*S. Krugman, 1970*), in chimps and in adults (*P. Maupas, 1976*)
  
  - Lack of cell culture system susceptible to HBV infection in vitro (at that time...)


From the bench to recombinant hepatitis B vaccine

- HBV genome cloned and sequenced in 1976 (Galibert F. & al. Nature 1979)
- Localization on the viral genome of the gene coding for the major polypeptide of HBsAg (Charnay P. & al. NAR 1979)
- HBsAg expression toxic in E. coli & problems with purification (Charnay P. Nature 1980)
- Expression of HBV envelope proteins (HBsAg) in eucaryotic cells transfected with plasmids coding for HBV envelope proteins
  - Animal cells (Dubois MF & al. PNAS, 1980) mouse L cells, HBV endogenous promoter

(Chinese hamster ovary) CHO cells and gene amplification system
(HBsAg produced as secreted VLPs, glycosylation+)
Recombinant CHO cells expressing HBsAg
HBsAg particles produced from CHO cells

(Michel M-L & al. PNAS 1984)
Protein composition of HBsAg particles secreted by rec. CHO cells: Envelope proteins are glycosylated
Structure of the small (S) HBV envelope protein

Antigenic loop: HBsAg: group « a », sub-types « d, y, w, r »

AGL: interact with heparan sulfates (infectiosity)
Intra/intermolecular disulfide bounds (antigenicity)
Targeted by vaccine-induced NT Ab

Slide courtesy of R. Patient/ P. Roingeard/ A. Desrames /C. Sureau
Structure of the middle (M) HBV envelope protein

preS2-antigenic domain

Antigenic loop: HBsAg

Role? Not implicated in viral cycle
preS2 Ab are neutralizing
Contain Th cell epitopes

Slide courtesy of R. Patient/ P. Roingeard
Vaccin GenHevac B Pasteur (20µg)
HBsAg-producing CHO patented by I. Pasteur, INSERM & CNRS
Licenced to «Pasteur Mérieux sérums et vaccins»
now Sanofi Pasteur MSD

Yeast-derived recombinant vaccines
Engerix B (10 & 20µg), GSK
HBvaxPro (5&10µg), Merck)....
HBV vaccines contain only envelop proteins! No DNA

Hepatitis B small surface antigen particles are octahedral
Robert J. C. Gilbert et al.; PNAS, 102; 2005
Mechanisms of Action

1- Ag Capture

Vaccine = HBs Ag + Alum (i.m.)

2- Ag transport

Macrophage or dendritic cell

3- activation and proliferation of T lymphocytes

Proximal LN

HBsAg

peptides

4- effector functions

cytokine secretion

B cell activation

anti-HBs antibody

HBV

MHC class II
+peptide

B cell

Th2: IL-4, IL-5, IL-6

B7, CD28

2-CD4+
cell

T CD4+ cell

HIV

CD4+ cell

MHC class II + peptide

peptides

HBsAg
Vaccine protection against hepatitis B

1st mechanism: immediate viral neutralisation
- Neutralizing antibodies anti-HBs "a" (> 10 mUI/ml) prevent initial infection
- Efficient if antibodies persist > 10 mUI/ml (chimp.)

2nd mechanism: Induction of CD4+ T helper response (HBs = T-cell dependent Ag)
- Activation of B lymphocytes secreting anti-HBs antibodies
- Activation or recall of memory B cell response
Serology of acute hepatitis B

- HBsAg
- HBeAg
- Anti-HBs Ab
- Anti-HBc Ab
- Anti-HBe Ab
- HBV-T cells (CD8+, CD4+)

% of maximal level

Months post-infection

0 1 2 3 4 5 6 7 8 9

0 25 50 75 100

viral DNA sera

Serum transaminases

Incubation  Acute phase  recovery

preventive vaccines block infection at 2 steps
Factors Associated with Reduced Vaccine Responses

<table>
<thead>
<tr>
<th>Patient-Related</th>
<th>Vaccine-Related</th>
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<tbody>
<tr>
<td>Older age (&gt; 50 years)</td>
<td>Schedule (accelerated &lt; 0, 1, 2… 12 months)</td>
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<tr>
<td>HLA DRB1*0301, *0701</td>
<td>Double vs single dose</td>
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<tr>
<td>Male gender</td>
<td>Use of “adjuvants”</td>
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<tr>
<td>Smoking</td>
<td>MPL (TLR4), CpG ODN (TLR9), …</td>
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<tr>
<td>Obesity</td>
<td>IM &gt; ID</td>
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<td>Immune deficiency</td>
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<td>HIV</td>
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<td>Transplant recipients</td>
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<td>Dialysis</td>
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<tr>
<td>Compliance</td>
<td></td>
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Launay et al. JAMA 2011
Rey et al. Lancet Inf. Dis. 2015
Piroth et al. JID 2016
Vaccination VHB et infection par le VIH: intérêt du schéma vaccinal alternatif

- Essai multicentrique randomisé
  437 adultes VIH+, CD4 > 200/mm3, vaccination VHB
  - 3 injections (20µg) IM (M0, M1, M6),
  - 4 injections (40µg) IM (M0, M1, M2, M6),
  - 4 injections (4µg) ID (M0, M1, M2, M6).

- Critère d’ évaluation principal
  % de répondeurs 4 semaines après la dernière injection (S28)

- Résultats
  Supériorité des 2 schémas alternatifs par rapport au schéma standard :
  - répondeurs (Ac anti-HbS ≥ 10 mUI/ml) (65%, 82%, 77%),
  - forts répondeurs (Ac anti-HbS ≥ 100 mUI/ml) (41%, 74%, 53%),
  GMT: 55, 795 et 104 mIU/mL.

Pas d’ effet sur CD4 et CV VIH

Launay O et al, JAMA 2011;305(14):1432-1440
Vaccination contre l’hépatite B des populations immunodéprimés : chez les patients VIH

- persistance de la réponse avec primo vaccination par 4 injections double dose
- perte des anticorps anti HBS chez 15% des patients:
  - 33,1 mois dans le bras IM40 × 4
  - 8.7 mois dans le bras IM20 × 3
  - 6.8 mois dans le bras ID4 × 4

JAMA Intern Med. 2016 May 1;176(5):603-10
Vaccination contre l’hépatite B des populations immunodéprimées: intérêt de schémas intensifiés chez les patients vivant avec le VIH

- Non répondeurs à une vaccination antérieure: supériorité de la vaccination par 3 double doses en terme de réponse anticorps

- Ac anti-HBc isolés:
  - 46% de réponse après une dose de vaccin
  - En cas de non réponse : 89% sont répondeurs aux 3 double doses
Targets of anti-hepatitis B vaccine

- individuals at risk of infection
- babies born to HBV infected mothers

Since 1992 Hepatitis B vaccine is included in EPI

- as of 2012, 183 nations have this vaccine in their immunization program for infants (79% of children are protected worldwide)

One billion of vaccinated individuals worldwide

Weintraub K, Nature 2014
Impact of anti-hepatitis B vaccination

• Decrease in the number of acute and fulminant hepatitis
  • $5.4 \times 10^5 (1975-1984) >> 1.7 \times 10^5 (1985-1998) = 68\%$
decrease in fulminant hepatitis in Taiwan
• Decrease in mother-child transmission
• Decrease in HBsAg in serum and in HBV reservoir
• Decrease in hepatitis delta virus infections
• Decrease in the number of deaths related to cirrhosis and HCC
Efficacy of vaccination on the prevalence of HBsAg chronic carriers

Global eradication of hepatitis B: feasible or fallacy?: Thursz M. Nature 2012
# Active and passive hepatitis B vaccination: post-exposure prophylaxis in infants

<table>
<thead>
<tr>
<th>Maternal screening</th>
<th>Vaccine</th>
<th>HBIg</th>
<th>Efficacy</th>
<th>Cost</th>
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<td>Infants/</td>
<td>Higher</td>
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<td>0, 1, 6 mths</td>
<td>HBeAg+ mothers only</td>
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<td>HBeAg</td>
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<tr>
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<td>YES</td>
<td>Infants /</td>
<td>High</td>
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<td>0, 1, 6 mths</td>
<td>HBeAg+ mothers only</td>
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<tr>
<td>Yes</td>
<td>YES</td>
<td>recomman ded</td>
<td>high</td>
<td>highest</td>
<td>Thailand</td>
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<tr>
<td>HBsAg</td>
<td>1, 2, 4, 6 mths</td>
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</table>
hepatitis B vaccine: first anti-cancer vaccine

Prevalence of HBsAg and HCC children <12 yrs in Taiwan

Relationship Between HBV Vaccination and HCC Incidence

- 3,855,485 newborns vaccinated in Taiwan (1984-2000)
  - 43,134,217 person-years of follow-up
- 158 cases of newly diagnosed HCC during follow-up
  - Rates higher in boys vs girls
  - Receiving 4 vs 1-2 doses increased preventive effects against HCC

<table>
<thead>
<tr>
<th></th>
<th>All Participants, n</th>
<th>1-2 Vaccine Doses, n</th>
<th>3 Vaccine Doses, n</th>
<th>4 Vaccine Doses, n</th>
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<tbody>
<tr>
<td>Boys</td>
<td>2,009,182</td>
<td>217,768</td>
<td>1,061,759</td>
<td>729,655</td>
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<tr>
<td>Girls</td>
<td>1,846,303</td>
<td>197,921</td>
<td>979,213</td>
<td>669,169</td>
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</table>

Age- and Sex-Specific Mortality and Incidence Rates of Chronic Liver Disease and Hepatocellular Carcinoma for Birth Cohorts Born Before and After the Launch of the Hepatitis B Immunization Program in 1984 in Taiwan

Thirty-Year Outcomes of the National Hepatitis B Immunization Program in Taiwan
Reduction of HCC in childhood by vaccination against HBV for infants born to HBV-carrier mothers (Japan)

Tajiri H et al., 2011

- Start 1986: 494 babies born to HBV-infected mothers vaccinated
- 93.5% protection efficacy
- HBV carrier rate decreased from 0.8% (1985) to 0.005% (2005)

2 doses of HB Ig (1 at birth, 1 at 2mths) + 3 doses of vaccines (2, 3, 5mths)

<table>
<thead>
<tr>
<th>Period</th>
<th>HB cases</th>
<th>Total HCC</th>
<th>Ratio to HB</th>
<th>HBV+ HCC</th>
<th>Ratio to HB</th>
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<tbody>
<tr>
<td>1981-1985</td>
<td>124</td>
<td>20</td>
<td>0.161</td>
<td>11</td>
<td>0.089</td>
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<td>1986-1990</td>
<td>119</td>
<td>25 (0-4yr)</td>
<td>0.210</td>
<td>10</td>
<td>0.084</td>
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<td>1991-1995</td>
<td>147</td>
<td>22 (0-9yr)</td>
<td>0.150</td>
<td>9</td>
<td>0.061</td>
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<tr>
<td>1996-2000</td>
<td>133</td>
<td>15 (0-14yr)</td>
<td>0.113</td>
<td>7</td>
<td>0.053</td>
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<tr>
<td>2001-2005</td>
<td>133</td>
<td>8 (0-19yr)</td>
<td>0.060</td>
<td>1</td>
<td>0.008</td>
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<tr>
<td>2006-2008 (3years)</td>
<td>84</td>
<td>5 (0-22yr)</td>
<td>0.060</td>
<td>0</td>
<td>0.000 (p&lt;0.0001)</td>
</tr>
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</table>
hepatitis B vaccination: Unresolved issues

• Decline in anti-HBs titers: Is a booster dose required?
  – No (countries with low HBV endemicity, subjects with low infection risk)
  – Yes (immunocompromised subjects & subjects with high risk to HBV exposure)

• Anamnestic effect of booster dose on a-HBs Ab: stimulation of memory B cells
  – Few significant breakthrough infections (Ni YH et al. Gastroenterology 2007)
  – Unusual clinical courses of HBV infection in previously vaccinated subjects:
    transient viremia and no biochemical hepatitis after infection resulting from sexual
    contact or blood transfusion (Stramer SL et al. NEJM 2011; Liu et al. J Hepatol 2006)

• Eliminating HBV through neonatal vaccination?
  – Overall post-vaccination HBsAg carrier rate <1%
  – HBsAg carrier rate 7-17% or occult HBV found in infants from mothers with high
    titer viremia (HBeAg+)
  – Administration of anti-viral agents (Lam, Tenofovir, Telbivudine) to pregnant
    mothers before vaccination of neonates
Vaccination and global elimination of hepatitis B

- Vaccination of infants and neonates:
  - has already prevented 210 million of new chronic infections by 2015
  - will prevent 1.1 million deaths by 2030
- Scaling up the coverage of infant vaccination
  to 90% of infants, 80% of neonates (birth dose)
  combined with the use of peripartum antivirals

  would prevent 7.3 million deaths between 2015-2030 and 63 million new chronic infections

Nayagam S et al. The Lancet Sept 2016
hepatitis B vaccines

- HBV and the disease
- recombinant preventive vaccines
- immuno-modulatory and anti-viral approaches to treat CHB
HBV INFECTIONS: STRONG NEED FOR DEVELOPMENT OF NEW THERAPEUTIC INTERVENTIONS

Worldwide HBV chronic carriers
300 Millions

Inactive carriers of HBsAg
HBV DNA < 2000 IU/ml
not treated

IFN-alpha
successful in 30%
Side effects

Antiviral treatments

“e” Ag mutants increase

Lamivudine/Adefovir therapy
emergence of resistant virus
Annual resistance rate 15-20%

Entecavir/tenofovir therapy
Low rate of anti-HBe+, cccDNA+
long term treatment

Existing vaccine
Stages of Chronic Hepatitis B (CHB) Infection

<table>
<thead>
<tr>
<th>Immune tolerance</th>
<th>Immune clearance</th>
<th>Low replicative phase</th>
<th>Reactivation phase</th>
</tr>
</thead>
<tbody>
<tr>
<td>HBeAg positive</td>
<td>HBeAg negative/ anti-HBe positive</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

**HBV-DNA**
- $10^9 - 10^{10}$ cp/mL
- $10^7 - 10^8$ cp/mL
- $< 10^4$ cp/mL
- $> 10^5$ cp/mL

**ALT**
- Normal/mild CH
- Moderate/severe CH
- Normal/mild CH
- Moderate/severe CH

- Cirrhosis
- Inactive cirrhosis
- Cirrhosis

**HBeAg positive CHB**
**Inactive-carrier state**
**HBeAg negative CHB**

$< 10^4$ cp/ml = 2 000 IU/ml

Trepo C, Chan HL, Lok A. Hepatitis B virus infection. Lancet. 2014
Medical needs in chronic HBV infection

- Inhibition of viral replication
- Normalization of ALT
- Improvement in liver necroinflammation
- Improvement in fibrosis

Fulfilled by nucleos/tide analogs (NUC) treatments

- HBeAg negativation and a-HBe seroconversion
- Elimination of cccDNA and HBV-infected hepatocytes
- HBsAg loss and seroconversion to anti-HBs Ab

HBV cure: an achievable goal by using immune stimulation (IFN-α, vaccine therapy, cytokines, TLR agonists...
Acute self-limited infection

- *hepatitis B virus*
- *HBV DNA*
- *HBV RNA*
- *HBsAg*
- *HBcAg*
- *ISG: IFN-α/β??*
- *Viral capsides*
- *HBeAg*
- *HBV antigens*
- *Macrophages or dendritic cells*
- *MHC class I*
- *MHC class II*
- *Infected hepatocytes*
- *peptides*
- *peptides*
- *CD4+ T cell*
- *CD8+ T cell*
- *Lysis of infected hepatocytes & control of viral replication*
- *TNFα & IFNγ*
- *IL-2 & IFNγ*
- *B cell*
- *anti-HBs Ab*
- *anti-HBe Ab*
- *adaptive response (days)*
- *Innate response (hours)*
Acute self-limited HBV infection: Co-ordinated immune responses

- IR delayed by 4-6 wks post infection
- HBV Infection = high viral replication (>10^8 copies /ml) all hepatocytes are infected
- IFN-γ production by NK, NK T & MAIT cells
- non-cytolytic control of viral replication (IFN-γ / TNF-α; LTβ)
  - Strong multi-specific CD8 T cells
  - Strong proliferation of CD4+ T cells
  - HBV-specific CD8+ T recruited in liver
  - Hepatic lysis = >ALT


Weeks after infection
Chronic HBV infection:
uncontrolled viral replication and ongoing liver damage
or persistent episomal form of HBV cccDNA, resistant to antivirals

Low frequency HBV-specific CD8 T-cell responses
- with exhausted phenotype (PD-1, CTLA-4, CD244, Tim3…)
Impaired IL-2 production /proliferation of T cells
Impaired production of anti-viral cytokines (IFN-γ, TNF-α)
- increase in Tregs and IL-10-secreting T cells
Impaired NK cell responses

(Bertoletti & Maini, Antiviral. Ther., 2010)
**Therapeutic options: towards an HBV cure…**

**Stimulation of innate immunity**  
TLR agonists

**Stimulation of HBV-specific T cells:**  
Therapeutic vaccines

**Restoration of functional T cells:**  
Combined therapy with NUCs

Targeting the virus: **Direct Acting Agents**  
Inhibitors of cccDNA  
inhibitors of transcription (RNAi)  
Inhibitors of capsid assembly

Blocking inhibitory mechanisms in liver:  
**Host Targeting Agents**  
Therapeutic antibodies  
Blocking HBV entry

*(Michel M-L Virologie, 2014, vaccine 2017)*
hepatitis B vaccination combined with anti-viral treatments would avert 1.5 million of cancer deaths (2015-2030)

- **Preventive vaccine**
  - Neonates/early childhood: > 95%
  - Adulthood: < 5%
- **Immune Tolerance**
- **Inactive Carrier**
- **HCC**
- **HBeAg- Chronic Hepatitis B**
- **HBeAg+ Chronic Hepatitis B**
- **Immuno-modulatory drugs + NUCs**

**Questions:**
- Preventive vaccine effectiveness in adulthood?
- Impact of preventive vaccine on HCC prevention?
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