VHPB Technical meeting
MULTI TOPIC meeting
Vilnius, Lithuania. April 25-26, 2019

Do we need better HBV vaccines ?

Dieter Glebe

Institute of Medical Virology,
National Reference Centre (NRC) for Hepatitis B and D Viruses,
Justus Liebig University Giessen, Germany

The burden of Hepatitis B

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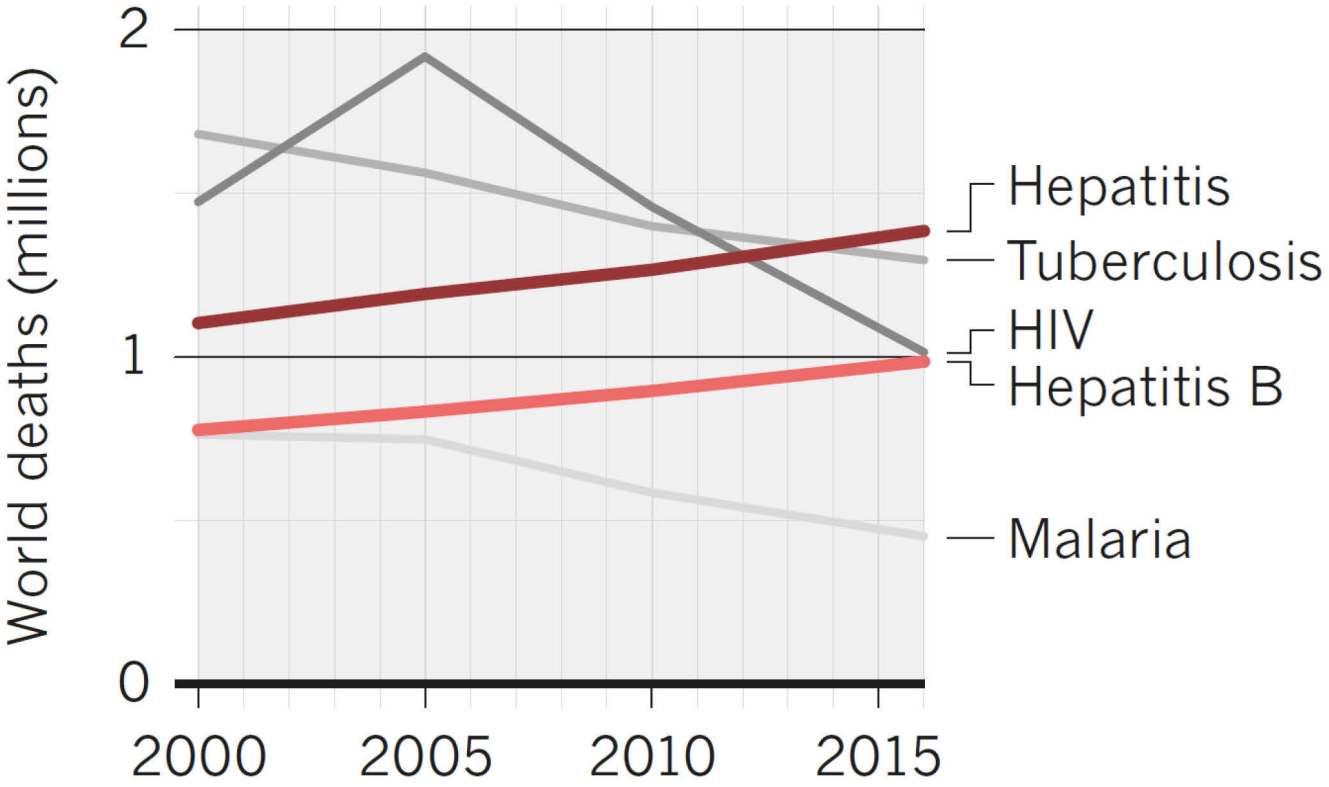
THE BURDEN OF HEPATITIS B

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

HepB is back

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Science
Nov 30, 2018

FORGOTTEN NO MORE

A long-overlooked scourge of millions, hepatitis B is in the crosshairs at last

Jon Cohen, Science 2018

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

Timothy Block, Hepatitis B Foundation

Nature
Dec 6, 2018

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?

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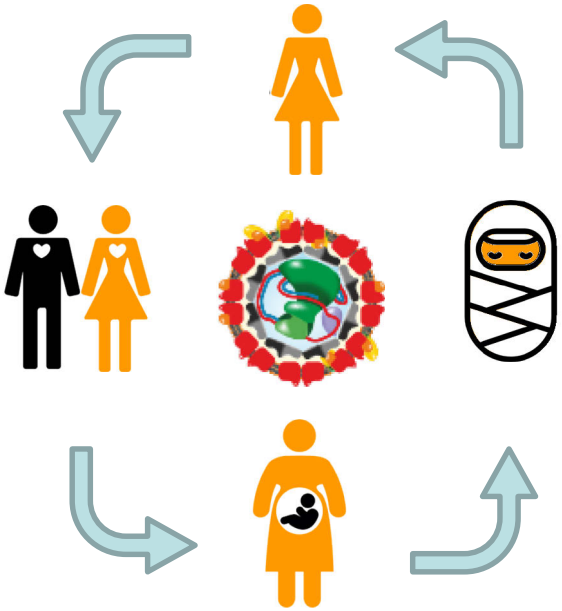
Currently, more than 250 million people are chronically infected with HBV



HBV user manual

1) **Don't kill your host**

2) **Healthy female chronic carriers**

3) **Infect offspring soonest possible**

Mühlemann et al., 2018. **Nature** Vol.557(7705): 418-423

LETTER

https://doi.org/10.1038/s41586-018-0097-z

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,
- from 5,500 to 800 years before present (BP)

Krause-Kyora et al., 2018. **Elife**. pii:e36666.

Neolithic and medieval virus genomes reveal complex evolution of hepatitis B

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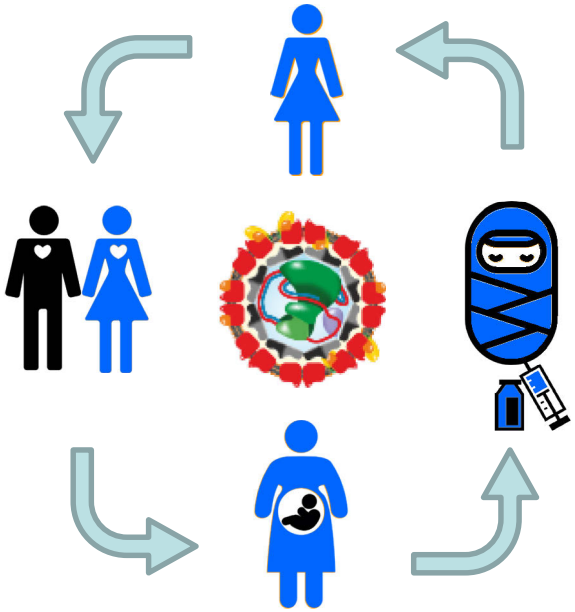

HBV user manual

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)**

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Successes of vaccination against HBV

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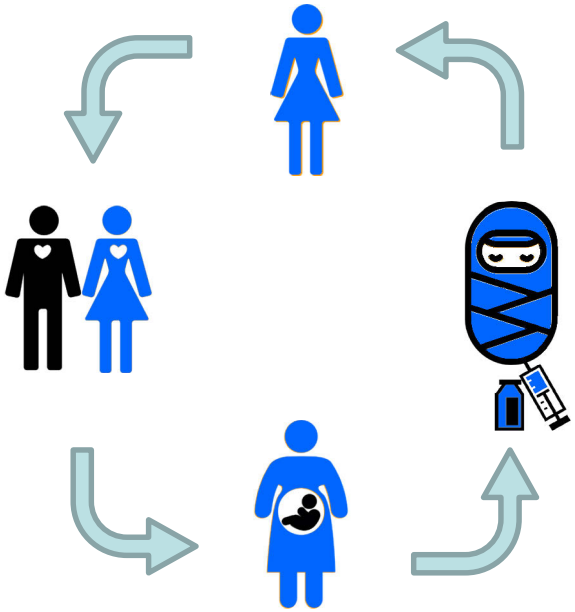

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Journal of Viral Hepatitis, 2011, **18**, 369–375

Thailand

doi:10.1111/j.1365-2893.2010.01312.x

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. Leyssen[3] and J.-M. Jacquet[3] [1]*Department of Pediatrics, Faculty of Medicine, Center of Excellence in Clinical Virology, Chulalongkorn University, Bangkok, Thailand;* [2]*Center for Vaccinology, Ghent University and Hospital, De Pintelaan, Ghent, Belgium; and* [3]*GlaxoSmithKline Biologicals, Rixensart, Belgium*

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

Vertical HBV mother-to-child Transmission (MTCT)

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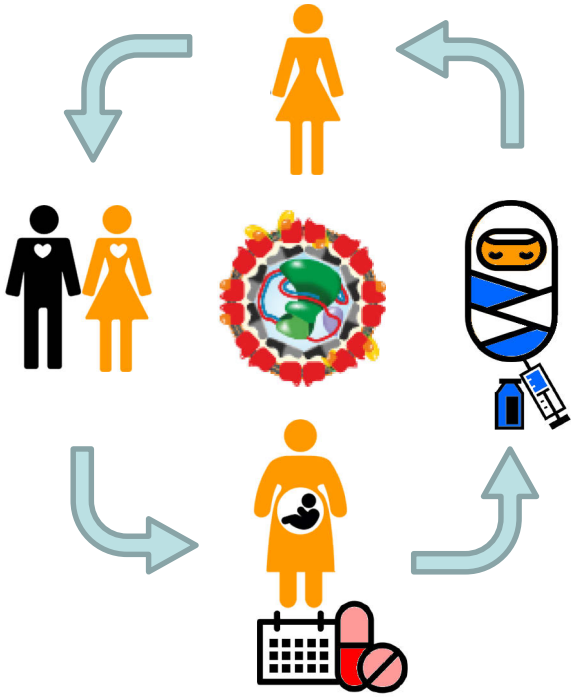
MTCT despite active/passive immunization of newborns



HBV user manual

1) Don't kill your host
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   - ➢ Female healthy vaccinees
3) Infect offspring soonest possible
   - ➢ Vaccinate at birth (active/passive)

MTCT despite active/passive immunization of newborns	Maternal serum HBV-DNA, viral load (VL)		
	IU/mL	Copies/mL	Study country
0 %	< 2 x 10^5	< 10^6	China, Australien
3.2 %	2 x 10^{5-6}	10^{6-7}	China
6.7 %	2 x 10^{6-7}	10^{7-8}	China
7.6 %	> 2 x 10^7	> 10^8	China
9 to 10 %	> 2 x 10^7	> 10^8	Australia

- **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

Terrault et al., Hepatology, 2016; 63:261-283

Problems (low/non-responder)

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

- **Problems with the HBV vaccine**
- Non/low-responders (below 10 IU/L anti-HBs)

Table 1 Factors determining the immune response to HB vaccine

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

- **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 ?

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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)

Response	Full	Low	Non	
Anti-HBs (IU/L)	≥ 10	1-9	0	WHO
	≥ 100	10-99	0-10	UK, Ireland, Switzerland, Germany
Protective	Yes?	No ?	No ?	

Does the HBV vaccine protects against infection ?

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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*

Occult HBV infection (OBI) and vaccination

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Universal Infant Immunization and Occult Hepatitis B Virus Infection in Children and Adolescents: A Population-Based Study

Hsu et al., Hepatology, 2015, Vol 61(4): 1183-1191

Taiwan

Vaccinated	Infected (HBV DNA positive)	
	Anti-HBc neg.	Anti-HBc pos.
No	4/218 (1.8%)	3/181 (1.7 %)
Yes	0/392 (0 %)	**16/334 (4.8 %)**

- 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

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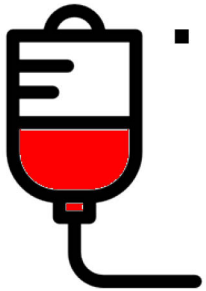

- **Transient occult HBV-infection in vaccinated blood donors from US**
  - Of 2.1 million donations, 28 showed markers of a recent HBV infection
  - Nine donors with transient OBI (up to four months duration)
  - Titres up to 10,000 IU/mL HBV-DNA

Anti-HBs titre (IU/L) of donors with transient OBI	HBV DNA positive
Not vaccinated	3
< 10, vaccinated	**2**
10 - 100, vaccinated	**4**
> 100, vaccinated	**0**

➢ 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

Stramer et al., New Engl. Med. 2011; 364.3:236-247

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	≥ 100	10-99	0-10	UK, Ireland, Switzerland, Germany
Protective	Yes?	No ?	No ?	

- **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

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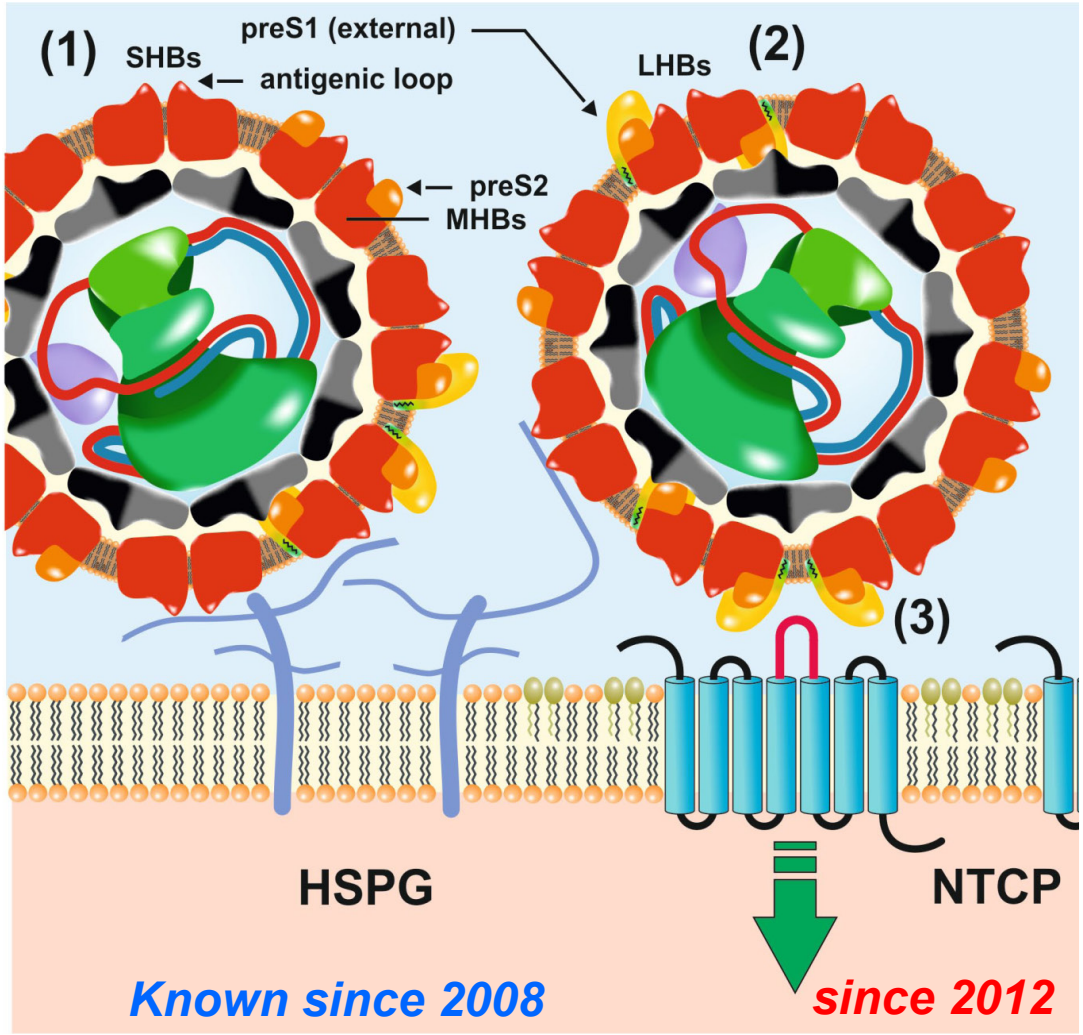
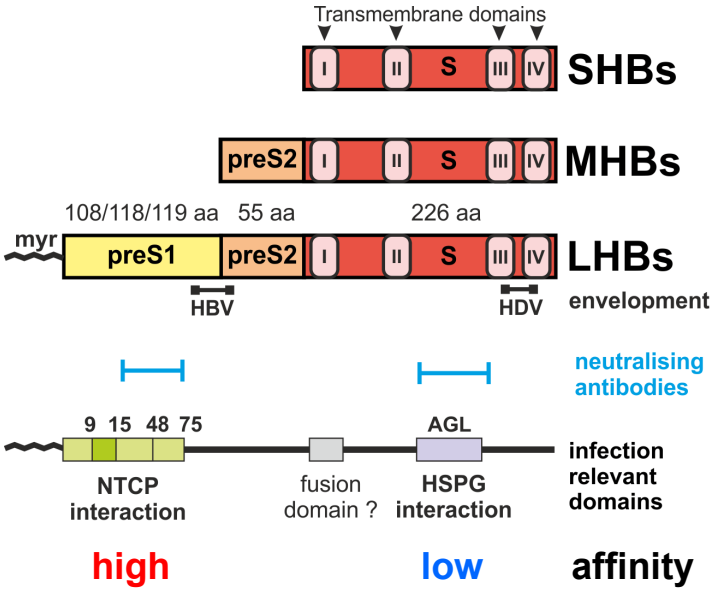
Liver sinusoid



Hepatocyte

HBV surface proteins



(1) Three surface proteins

(2) PreS1 and S domain relevant for infection

(3) Both carry neutralizing epitopes

Glebe D, Bremer CM. Semin Liver Dis. 2013 May;33(2):103-12.

Anti-preS1 alone can neutralize HBV infection

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JOURNAL OF VIROLOGY, Sept. 2003, p. 9511–9521 Vol. 77, No. 17
0022-538X/03/$08.00+0 DOI: 10.1128/JVI.77.17.9511–9521.2003

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. Gerlich[1]

Institute of Medical Virology[1] 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,[a,*] Chun Jeih Ryu,[a] Hyangsuk Hur,[a] Seho Kim,[b] Han Kyu Oh,[b] Mee Sook Oh,[c] and Song Yong Park[b]

[a] *Antibody Engineering Research Unit, Korea Research Institute of Bioscience and Biotechnology, Taejon 305-600, South Korea*
[b] *Central Research Center, Korea Green Cross Corp., Kyunggi-Do 449-903, South Korea*
[c] *R&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

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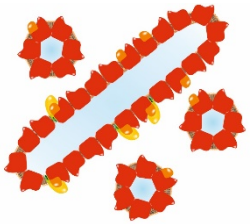
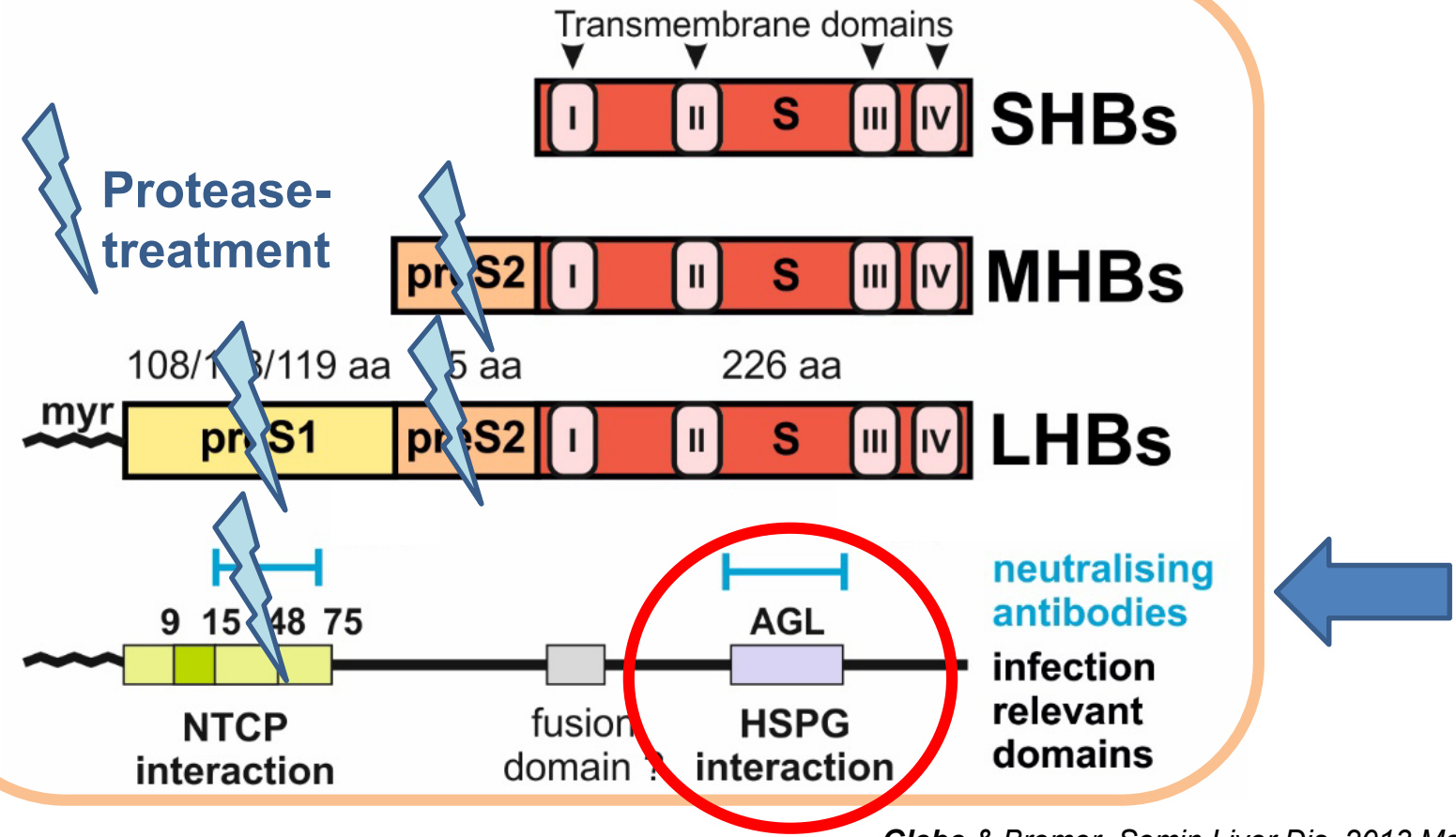
Virion

Subvirale Partikel



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



Glebe & Bremer, Semin Liver Dis. 2013 May;33(2):103-12.

Neutralising antibodies generated by different HBV vaccines

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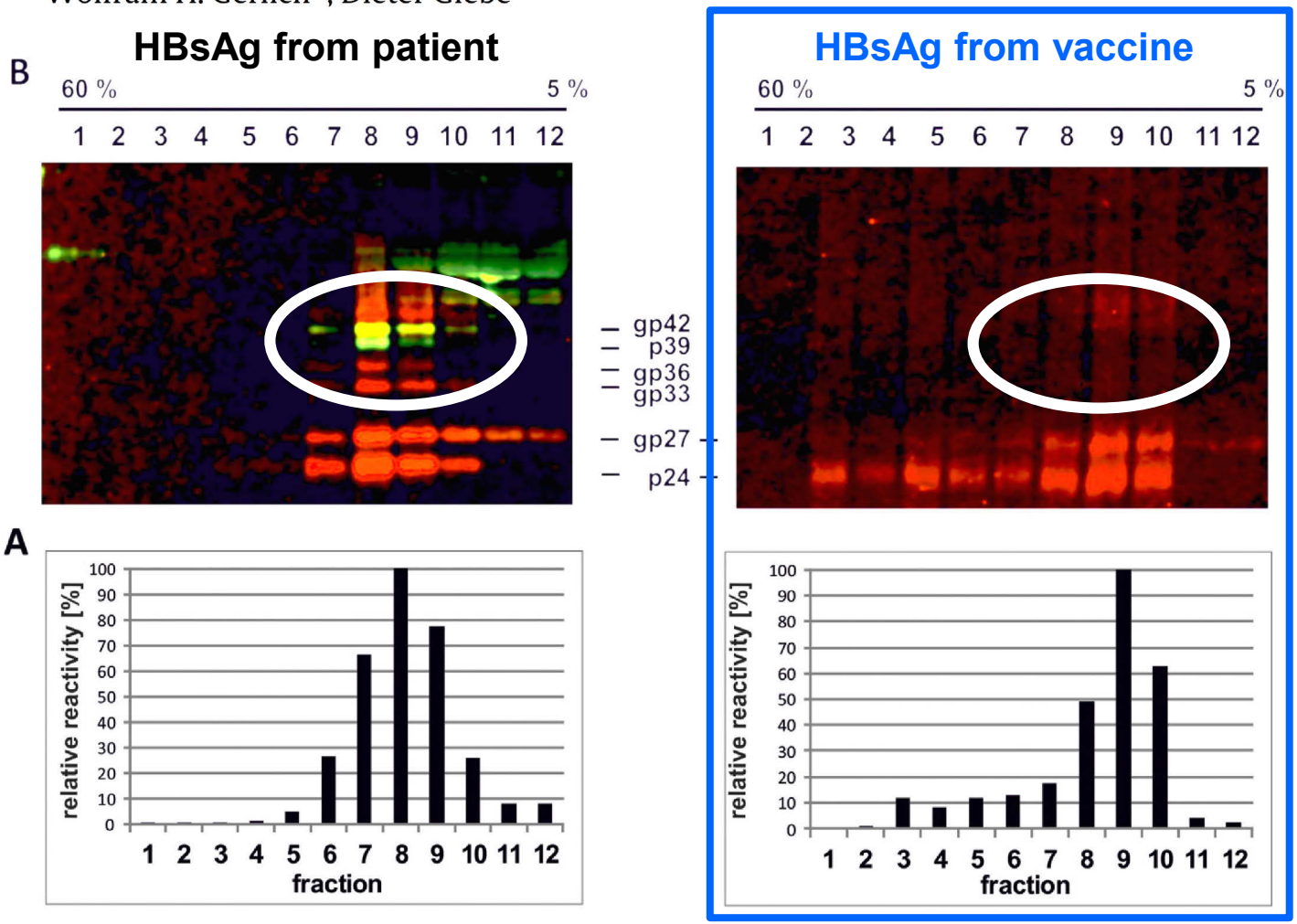
Characterization of the 3rd International Standard for hepatitis B virus surface antigen (HBsAg)

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



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

preS1

HBsAg – Western Blot

HBsAg - ELISA

Seiz et al., . J Clin Virol 2016 82:166-172.

Neutralising antibodies generated by different HBV vaccines

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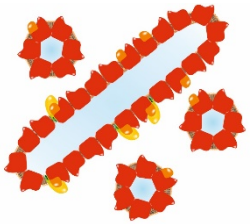
Virion

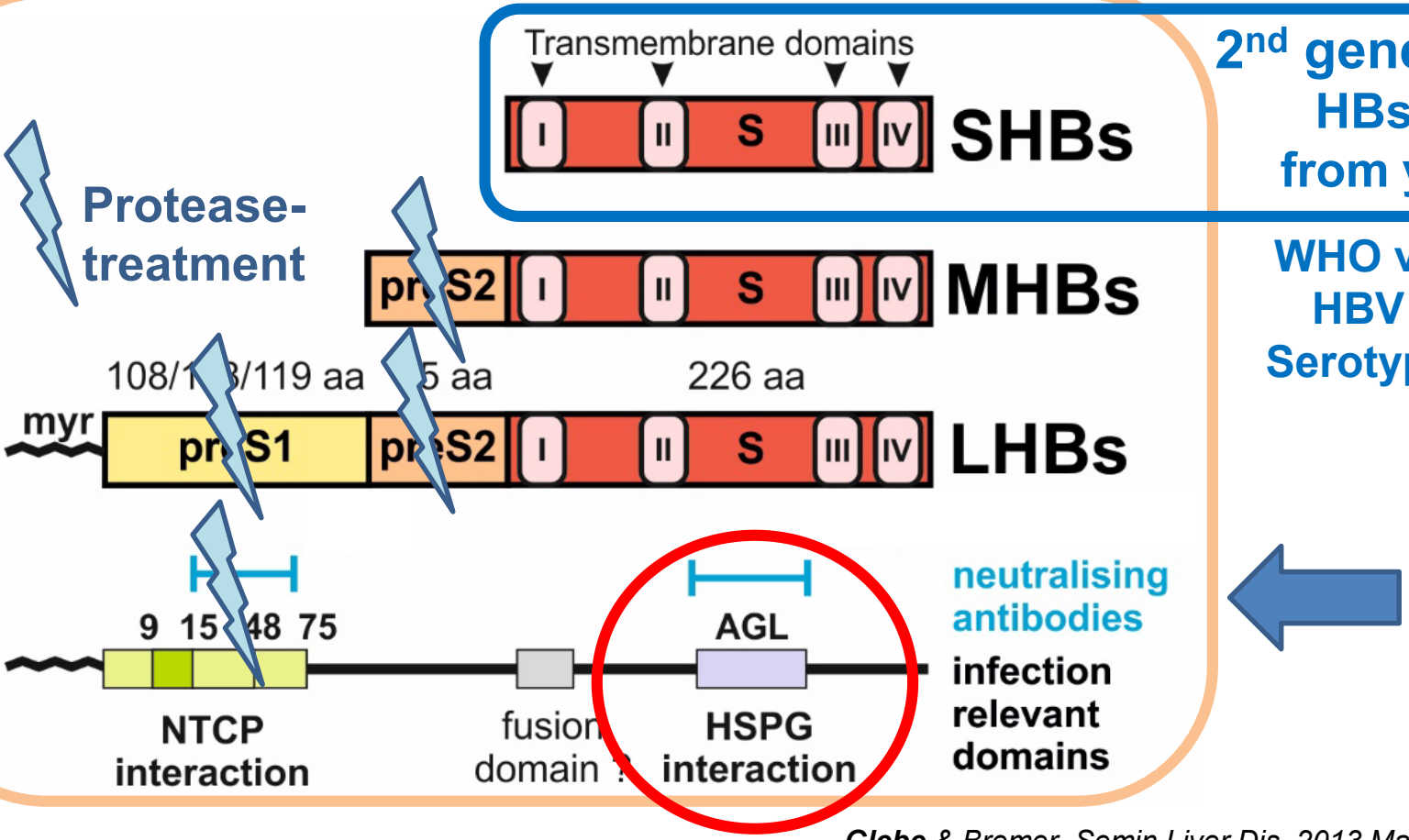
Subvirale Partikel



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

2nd generation
HBsAg from yeast

WHO vaccine:
HBV Gt A2
Serotype adw2



Glebe & Bremer, Semin Liver Dis. 2013 May;33(2):103-12.

Global prevalence of HBV genotypes

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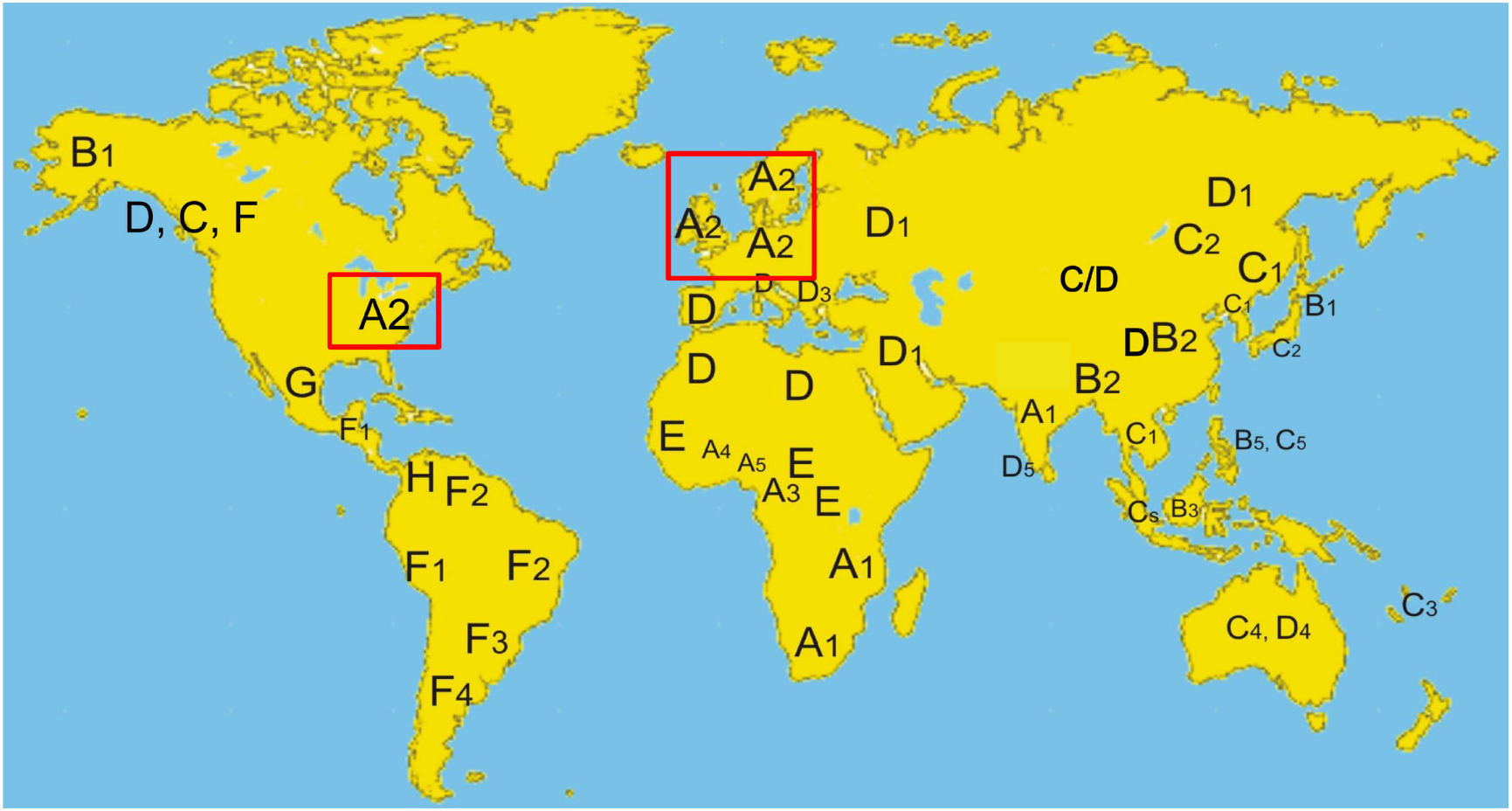
- WHO 2nd generation vaccine contains only SHBs of HBV genotype A2
- 99 % of all chronic carriers have different HBV genotypes
- Breakthrough of distantly related HBV genotypes ?



Adapted from: Schaefer & Gerlich, Textbook of Hepatology 2007, 825.

Genotype-effect during vertical transmission

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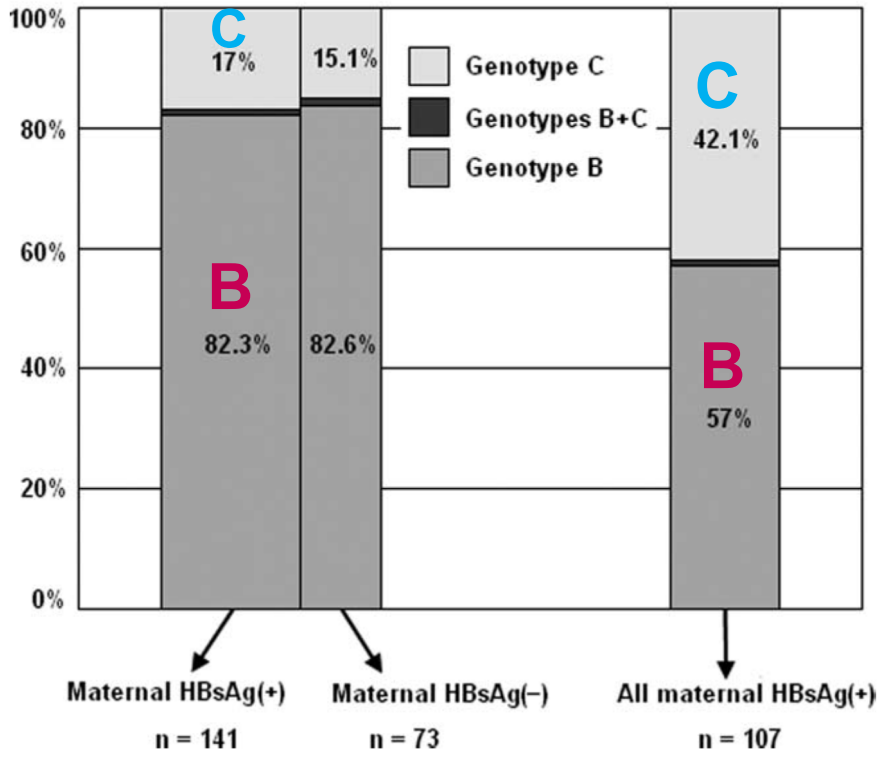
- Subtype-specificity of the 2nd generation vaccine
  - **Vaccine: subgenotype A2, serotype adw2**
  - Vaccine protects against all genotypes at high anti-HBs (> 100 IU/L)
  - Genotype-effect during vertical transmission (Taiwan)

HBV-infected children

not vaccinated vaccinated



Genotype	Serotype	
B	adw2	
C	adr	
A2	adw2	vaccine

Wen et al., Hepatology 2011

Vaccine breakthrough of distant HBV genotypes

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- 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**

Genotype	Serotype	
B	adw2	
C	adr	
F	**ayw4**	
A2	adw2	vaccine

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

Adapted from: Schaefer & Gerlich, Textbook of Hepatology 2007, 825.

A bat hepadnavirus with zoonotic potential

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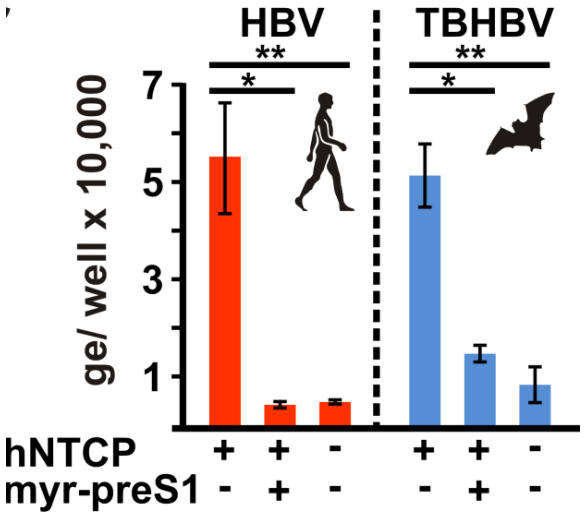
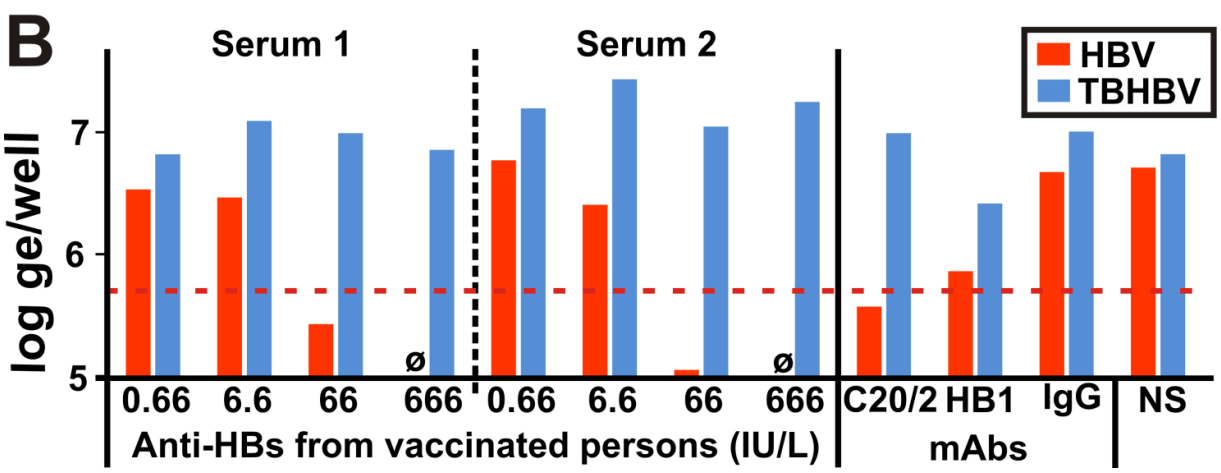
Tent-making bat

TMBHBV

- 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 ?**

Drexler/Geipel et al. PNAS 2013, Oct 1; 110(40):16151-16156.

Summary

JUSTUS-LIEBIG-UNIVERSITÄT GIESSEN

(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.