BRIEF HISTORY OF HEPATITIS MILESTONES

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Stephen Hawking – Brief History of time
MENTORS AND FRIENDS

HEPATOLOGY

MASTER'S PERSPECTIVE

The Road Not Taken or How I Learned to Love the Liver: A Personal Perspective on Hepatitis History

Harvey J. Alter

EASL MONOTHEMATIC LYON 2013

WHAT HAVE WE LEARNED FROM THE HISTORY OF VIRAL HEPATITIS RESEARCH?

Hubert Blum
History of hepatitis – From jaundice to HCV

Hepatoscopy
I. Epidemic hepatitis (-3000 to 1900)

- 3000 Sumerians (Jaundice…)
- 420 Greek Hippocrates → « icterus »
+ 750 Middle Ages
   Pope Zacharie St Boniface → isolation
- 1800 Jaundice of camps

Sieges

St Jean d’Acre (1799)
Paris (1870)

→ prevention
I. Serum hepatitis (1880-1945)

« Syringe » hepatitis
injections for syphilis

Post-vaccine hepatitis
1882: anti-small pox: Lurman (Breme)
1937: anti-yellow fever: Findlay
1942: 28000 cases US navy

Serum and post transfusion hepatitis (1945-1975)
Duality of hepatitis

1947 Mc Callum

- Hepatitis A   epidemic
- Hepatitis B   serum

1964 Krugman (Willow Brook School)

- Hepatitis A → oral (30-45d)    « MS1 »
- Hepatitis B → parenteral (60-90d) « MS2 »
The discovery by Blumberg in 1964 of a new antigen in the serum of an Australian aborigine initiates a new era in the history of hepatitis. Blumberg, an ethnologist, demonstrates that this “Australian antigen” is a marker of hepatitis.

Nobel Prize in 1976 for the discovery of new mechanisms for the dissemination of infectious diseases
1968

• Described the SH Ag in patients who developed a post-transfusion hepatitis

• Confirmed
  – The specificity of this Ag for hepatitis B
  – The identity with Australia Ag
The virus

DANE

1970
### HBV Serologic Markers

<table>
<thead>
<tr>
<th>Antigens</th>
<th>Antibodies</th>
</tr>
</thead>
<tbody>
<tr>
<td>• HBsAg (1965)</td>
<td>• Anti-HBc total (1971)</td>
</tr>
<tr>
<td>• HBeAg (1972)</td>
<td>• Anti-HBc-IgM</td>
</tr>
<tr>
<td></td>
<td>• Anti-HBe</td>
</tr>
<tr>
<td></td>
<td>• Anti-HBs</td>
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<tr>
<td></td>
<td>• ADN POL (1972)</td>
</tr>
<tr>
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<td>• ADN (1974)</td>
</tr>
</tbody>
</table>
Tests / techniques

- 1965 Immunodiffusion (HBs/e Ag and Ab)
- 1970 Counter-electrophoresis (HBs/e Ag and Ab)
- 1970 RIA Ag/Ab
  - HBV DNA polymerase
- 1975 ELISA (Ag/Ab)
- 1980 DNA hybridization

PCR Real time

- $10^{10}$
- $10^9$
- $10^5$
- $10^2$
- 10❓
The Vaccine

Plasmatic vaccines (1975-76)
- Ph. Maupas / institut Pasteur (F)
- M. Hilleman / MSD (USA)

Recombinant vaccines (1981)
- P. Tiollais / institut Pasteur
- W. Rutter/ Chiron/MSD
HBV PARTICLES

**Serum**

**Serum-derived vaccine:** Szmuness W et al. N Engl J Med 1980; 303: 833-841

Pr Ph. Maupas

- Discovered the first HBV vaccine in humans (1976)
- Confirmed the association between HBV and primitive liver cancer
IMPACT OF HBV VACCINATION IN TAIWAN

• Incidence of HBs Ag:
  - 1985: 9%
  - 1995: 1%

• Réduction of HCC incidence and almost disappearance in children
HEPADNAVIRUSES

Orthohepadnavirus

- HBV - Humans
- WHV - Woodchucks
- GSHV - ground squirrel
- TSHV - Tree squirrel
- ASHBV - Artic squirrel

Old world monkeys:
- Gibbon (GiHBV)
- Gorilla (GoHBV)
- Orang-outang (OuHV)
- Chimpanzee (ChHBV)

New world monkeys:
- Woolly Monkey (WMHBV)

Avihepadnavirus

- DHBV - Duck
- HHBV - Heron
- SGHBV - Goose
- STHBV - Stork

Missing small available monkey
Discovery of Naturally Occurring Transmissible Chronic Hepatitis B Virus Infection Among Macaca fascicularis From Mauritius Island

Tatiana Dupinay,1,2,3,4* Tarik Gheit,5* Pierre Roques,6,7 Lucyna Cova,1,2,3 Philippe Chevallier-Queyron,1,2,8 Shin-i Tasahsu,9 Roger Le Grand,6,7 François Simon,10 Geneviève Cordier,11 Lahcen Wakrim,12 Soumaya Benjelloun,12 Christian Trépo,1,2,3,8 and Isabelle Chemin1,2,3

• Editorial – Hepatology (November 2013)
Persistent Human Hepatitis B Virus Infection in Cynomolgus Monkeys: A Novel Animal Model in The Search for a Cure?
J Bukh, R.E. Lanford, and R.H. Purcell

• Comment – Nature/middleeast (May 15, 2013)
Macaques-new animal models to test anti-HBV drugs and vaccines. B. Das
HBV protein expression in Macaques liver sections

Negative Control

Macaque HBV +
HBV transmission from cynomolgus to sylvanus Macaques

ALT peak
3/3 animals

HBsAg+ week 4/7 PI
HBsAg+/HBcAg
+ by IF on liver sections

PCR HBV DNA (+) X 12 weeks
About $10^3$ copies HBV DNA/ ml
at week 9 pi
HEPATITIS VIRUSES DISCOVERY

1963: HBV (Blumberg et al)
1973: HAV (Feinstein et al)
1977: HDV (Rizzetto et al)
1983: HEV (Balayan et al)
1989: HCV (Houghton et al)

- Serology
- IEM (stool)
- Serology, IF (liver)
- Serology, IEM (stool)
- Cloning (liver)
HDV 37th Birthday

Immunofluorescence detection of new antigen-antibody system (δ/anti-δ) associated to hepatitis B virus in liver and in serum of HBsAg carriers

M. Rizzetto, M. G. Canese, S. Aricò, O. Crivelli, C. Trepo, F. Bonino, and G. Verme

From the Department of Gastroenterology, Ospedale Mauriziano Umberto I, Turin, Italy, the Electron Microscope Centre of the Faculty of Medicine, University of Turin, Italy, and INSERM U41, and Laboratory of Hygiene, University Claude Bernard, Lyon, France

SUMMARY A new antigen-antibody system associated with the hepatitis B virus and immunologically distinct from the HB surface, core, and e systems is reported. The new antigen, termed δ, was detected by direct immunofluorescence only in the liver cell nuclei of patients with HBsAg positive chronic liver disease. At present, the intrahepatic expression of HbcAg and δ antigen appears to be mutually exclusive. No ultrastructural aspect corresponding to the δ antigen could be identified under the electron microscope, δ antibody was found in the serum of chronic HBsAg carriers, with a higher prevalence in patients with liver damage. The nuclear fluorescence patterns of HbcAg and δ antigen were similar; it is only possible to discriminate between the two antigens by using the respective specific antisera.

While studying liver biopsies from patients who were seropositive for the hepatitis B surface antigen (HBsAg) in direct immunofluorescence, it was noted that an antiserum against the hepatitis B core antigen (HbcAg), as well as staining specimens which core particles could be demonstrated by the electron microscope (EM), also reacted with additional biopsies which did not contain core particles (at electron microscopy) and were negative with other reference antisera against HBsAg.

When the EM core positive and core negative specimens were tested with several HBsAg positive sera, it soon became apparent that some sera reacted with either one or the other liver substrate; this suggested that there were two distinct nuclear antigenic specificities.

The identification of this new antigen and of its antibody as an immunological system independent of other known reactions associated with the HB virus is reported in this communication. Provisionally, we propose that it should be called δ.

*Address for correspondence: Dr. M. Rizzetto, Department of Gastroenterology, Ospedale Mauriziano Umberto I, Cis. Torali 44, 10128 Turin, Italy.

Received for publication 30 May 1977
Hepatitis D (Delta) Virus

δ antigen
HBsAg
RNA

CDC
MAJOR CHARACTERISTICS

- Unique agent
- Defective virus
- Highly pathogenic
- Reemerging
- Most challenging therapy
HDV INHIBITS HBV REPLICATION

- Anti-transcriptional effect
- Competition envelope
- Cytokines (MxA ?)
1980s: Global Anti-HDV Prevalence in HBsAg Carriers (15,000,000 Positive)

Anti-HD(HBsAg (+)) □ ?  0-5%  6-20%  21-60%  >60%
Epidemiology of HDV in Europe

1980s → 2010

- **Endemic**
- **In immigrants**
HEPATITIS VIRUSES DISCOVERY


HBV Blumberg et al
HAV Feinstone et al
HDV Rizzetto et al
HEV Balayan et al
HCV Houghton et al

Serology
IEM (stool)
Serology, IF (liver)
Serology, IEM (stool)
Cloning (liver)
The approach

1. **1979** Tubule Forming Agent → Shimizu (NIH)
2. **1985** D. Bradley (CDC)
   - Togavirus
   - Flavivirus
   - HDV
3. **1987** <50 nm filter
4. Post-transfusion NANB hepatitis 1980-
   incidence 7% in France → 10% USA
Dead End

- 1983  HDV like → Kamimura/Purcell
- 1984  Retrovirus → Seto/Gerety
- 1984  Spumavirus → Prince
- 1985-9 Non-A, non-B Ag/Ab systems Shimizu & many others
The ascent

• 1st viral isolation
  - without culture
  - without electronic microscopy
  - without serology

Direct molecular approach

Since then:
  - HEV, HHV8
  - TTV
  - HGV
Discovery of the hepatitis C virus

Isolation of a cDNA Clone Derived from a Blood-Borne Non-A, Non-B Viral Hepatitis Genome

QUI-LIM CHOO, GEORGE KUO, AMY J. WEINER, LACY R. OVERBY, DANIEL W. BRADLEY, MICHAEL HOUGHTON

The HCV discovery team
(from left to right; M. Houghton, Q-L Choo, G. Kuo and D. Bradley)
Timeline | Milestones in hepatitis C virus (HCV) research

- Description of non-A, non-B hepatitis
- Delineation of HCV genome organization and polyprotein processing
- First infectious clone of HCV constructed
- Replicon system established
- Production of recombinant infectious HCV in tissue culture

- Identification of HCV
- First three-dimensional structure of an HCV protein (NS3 serine protease)
- Interferon-α and ribavirin combination therapy
- Proof-of-concept clinical studies of an HCV protease inhibitor
- Functional HCV pseudoparticles described

Temptative model of HCV 
“Lipo-Viro-Particle”

Francois PENIN & Patrice ANDRE

Phospholipid monolayer

E1 and E2 
enzyme 
glycoproteins

Phospholipid bilayer

Nucleocapsid 
(Core protein and ss(+)RNA)
HISTORY OF HEPATITIS THERAPY
DELETERIOUS EFFECT OF PREDNISOLONE IN HBsAg-POSITIVE CHRONIC ACTIVE HEPATITIS


Figure 5. Cumulative Rate of Complications in 51 Patients with HBsAg-Positive Chronic Active Hepatitis Who Were Receiving Prednisolone (Solid Line) or Placebo (Dotted Line). Figures above each line denote the numbers of patients remaining in the study. Prednisolone increased the rate of complications (z = 1.6709, P < 0.0001).

Figure 6. Actuarial Survival Rate in 51 Patients with HBsAg-Positive Chronic Active Hepatitis Who Were Receiving Prednisolone (Solid Line) or Placebo (Dotted Line). Figures above each line represent numbers of patients remaining in the study. The survival rate was decreased by prednisolone (z = 0.5171, P < 0.01).
Chronic Hepatitis

- 1955 - From steroids to abstention
  - Aciclovir and herpes
  - 1981 - Interferons
    - Alpha
    - Beta
- 2004 - PEG IFNs
Treatment with Oral Nucleos(t)ides

- 1978  Vidarabine
- 1979  Foscarnet
- 1985  Ganciclovir
- 1997  Lamivudine
- 2003  Adefovir
- 2006  Entecavir/Telbivudine
- 2008  Tenofovir

12 years
Evolution of Anti-HBV Agents

Raymond SCHINAZI
HBV Treatment Landscape in 2012

Timeline:
- 1990: Interferon alfa-2b
- 1998: Lamivudine
- 2002: Peginterferon alfa-2a, Entecavir
- 2005: Adefovir
- 2006: Telbivudine
- 2008: Tenofovir
**SUSTAINED BENEFIT**

Entecavir in HBeAg negative patients: Proportion of Patients with HBV DNA <300 copies/mL

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**ETV-027**

- **EOD†**: 94%
- **Baseline**: 4%
- **Wk 12**: 59%
- **Wk 24**: 83%
- **Wk 48**: 93%
- **Wk 72**: 94%
- **Wk 96**: 91%
- **Wk 144**: 95%

**ETV-901**

- **EOD†**: 93/99
- **Baseline**: 4/99
- **Wk 12**: 56/95
- **Wk 24**: 79/95
- **Wk 48**: 84/90
- **Wk 72**: 72/77
- **Wk 96**: 67/74
- **Wk 144**: 54/57†

† EOD= end-of-dosing
‡ 10 patients who remained on treatment at the Week 144 of ETV-901 visit had missing PCR samples

VIRAL LOAD PREDICTS OUTCOME

Entire Cohort (N = 3653)

Cumulative incidence of HCC

Year of Follow-up

Baseline HBV DNA Level, copies/mL

- > 1 million
- 100,000-999,999
- 10,000-99,999
- 300-9,999
- < 300
Cumulative Risk for HCC

Cumulative risk of HCC (%)

Follow-up (month)

HBsAg seroclearance at age ≥ 50

HBsAg seroclearance at age < 50

Age of HBsAg seroclearance

<table>
<thead>
<tr>
<th>No. of patients at risk</th>
<th>&lt; 50</th>
<th>151</th>
<th>124</th>
<th>102</th>
<th>87</th>
<th>71</th>
<th>56</th>
<th>47</th>
<th>37</th>
<th>21</th>
<th>15</th>
<th>10</th>
</tr>
</thead>
</table>

≥ 50

147 | 120 | 86 | 63 | 51 | 46 | 38 | 31 | 24 | 18 | 12 |

Paradigm shift

1) Break of tolerance
   - Disease
     - Therapy

2) Replication is the driving force of complications
   - Anticipate
   - Prevention >> cure
BEYOND VIROSUPPRESSION:

Can we achieve HBsAg clearance, thereby preventing disease progression and resistance?
PAN – Proof of Concept for Cure

Stopping viral replication

Take over by the immune system

Controls the disease and the infection
HBV-RELATED POLYARTERITIS NODOSA

Mr Gu.,
19 ans

transaminases

1 jan 15 jan 30 jan 15 fev

Anti Hbe

Anti Hbs

50 140

60 kg s

15 nov
# Results from the PAN Treatment According to Antiviral Protocol

<table>
<thead>
<tr>
<th>Protocol</th>
<th>Cure</th>
<th>Anti HBe</th>
<th>Anti HBs</th>
</tr>
</thead>
<tbody>
<tr>
<td>Vidarabine - 1978</td>
<td>75%</td>
<td>45%</td>
<td>19%</td>
</tr>
<tr>
<td>(3 weeks)</td>
<td>25/3</td>
<td></td>
<td></td>
</tr>
<tr>
<td>+PE-cortc/sev</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Interferon - 1976</td>
<td>80%</td>
<td>64%</td>
<td>50%</td>
</tr>
<tr>
<td>(6 months)</td>
<td>11/14</td>
<td></td>
<td></td>
</tr>
<tr>
<td>+PE+cortic/sev</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Lamivudine - 1997</td>
<td>90%</td>
<td>80%</td>
<td>60%</td>
</tr>
<tr>
<td>Tenofovir - 2010</td>
<td>9/10</td>
<td>90%</td>
<td>80%</td>
</tr>
</tbody>
</table>
CHRONIC HEPATITIS C THERAPY
HCV infection and global mortality risk

The REVEAL HCV Cohort Study

- 23,820 adults, Taiwan
- 1,095 anti-HCV positive; 69.4% HCV-RNA detectable

**Hepatic causes**

- p<0.001 for intergroup comparison
- p<0.001 for HCV-RNA detectable vs undetectable

**Extrahepatic causes**

- p<0.001 for intergroup comparison
- p<0.001 for HCV-RNA detectable vs undetectable

SVR improves global survival


SVR : Sustained virological response
What is the Goal?

- Interferon-free combination therapy
- High barrier to antiviral resistance
- Once daily oral therapy
- Pan-genotypic antiviral activity
- Reasonable safety and minimal drug-drug interactions
- Short duration (12 weeks)
- SVR rates > 90%
- ... and affordable

Inspired by Jordan Feld, Donald Jensen and Hubert E. Blum
The Hurricane of HCV Drug Development

Status 11/2013 (Selection)
Potential Evolution of HCV Therapy for GT 1
Small Molecules will be Added in an Effort to Improve SVR Rates
One of the most beautiful histories of medicine since small pox, polio and... tuberculosis
CONCLUSIONS AND PERSPECTIVES
HISTORY OF CHRONIC VIRAL HEPATITIS
Major Advances in 50 Years: 1960s - 2013

- **Analytical tools**
- **Clinical aspects**
  - Epidemiology
  - Natural Course
  - Pathogenesis
  - Diagnosis
  - Therapy
  - Prevention
- **Virological aspects**
  - Structure and genetic organization
  - Life cycle, incl. receptors
- **Individualized hepatology**
« I Have a dream »

HCV

1. Predictive genetic tests
   - fibrosis
   - IFN response
   - efficacy/tolerance of DAAs

2. Specific anti-HCV Ig

3. Prevention of HCC
   - primary
   - secondary
« I have a dream »
HBV

1) Chronic hepatitis B must become priority research
2) We should aim at HBV CURE
3) Since most HBV infected people live in resource limited countries new immunotherapic appraoches including therapeutic vaccine are needed
4) Availability of a small primate macaque model may be most helpful.

Yes we can!
We should have a dream

Worldwide famous wizard

They paved the way
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The Global Viral Hepatitis Summit
15th International Symposium on Viral Hepatitis and Liver Disease

Berlin, Germany
June 26–28, 2015