Chronic viral hepatitis:
Human Disease and Animal Models

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BE.450  April 20, 2005
Hepatitis viruses

- **HAV**: Acute gastroenteritis and/or hepatitis
- **HBV**: Acute or chronic hepatitis; significantly increases risk of hepatocellular carcinoma (HCC)
- **HCV**: Chronic hepatitis, cirrhosis and HCC
- **HDV**: Delta agent; requires HBV for packaging
- **HEV**: Usually acute and self-limiting, but 20% mortality in pregnant women; HEV > HAV in India
- **HFV**: Single reported outbreak; agent unidentified
- **HGV**: Part of GB virus group; lymphotropic
# Hepatotropic Hepatitis Viruses of Humans

<table>
<thead>
<tr>
<th>Virus</th>
<th>Type/Old name</th>
<th>Disease</th>
</tr>
</thead>
<tbody>
<tr>
<td>Hepatitis A (HAV)</td>
<td>RNA; hepatovirus/infectious hepatitis agent</td>
<td>Sporadic or epidemic; acute only. Faecal-oral spread</td>
</tr>
<tr>
<td>Hepatitis B (HBV)</td>
<td>DNA; hepadnavirus/serum hepatitis agent; Australia antigen</td>
<td>Acute or chronic, including hepatocellular carcinoma (HCC). Parenteral spread</td>
</tr>
<tr>
<td>Hepatitis C (HCV)</td>
<td>RNA; flavi- and pestivirus-like/transfusion-associated NANB hepatitis virus</td>
<td>Acute, often chronic, including HCC. Spread typically parenteral, but also sporadic</td>
</tr>
<tr>
<td>Hepatitis D (HDV)</td>
<td>RNA, defective virus/delta agent</td>
<td>HBV needed for pathogenicity; increases severity of type B hepatitis</td>
</tr>
<tr>
<td>Hepatitis E (HEV)</td>
<td>RNA virus/enteric NANB hepatitis virus</td>
<td>Sporadic or epidemic; probably acute disease only. Faecal-oral spread</td>
</tr>
<tr>
<td>Others</td>
<td>RNA; <em>Flaviviridae</em>, also known as GBV-C</td>
<td>Perhaps causes mild disease, but may not; often associated with HCV or HBV</td>
</tr>
<tr>
<td></td>
<td>Paramyxovirus/syncytial giant-cell hepatitis</td>
<td>Reported association with aggressive hepatitis may be in doubt</td>
</tr>
<tr>
<td></td>
<td>Toga-virus</td>
<td>May be implicated in a fulminant type of hepatitis</td>
</tr>
<tr>
<td></td>
<td>TT-virus</td>
<td>Implicated in fulminant and post-transfusion hepatitis</td>
</tr>
<tr>
<td></td>
<td>Parvovirus B19</td>
<td>Implicated in fulminant hepatitis associated with aplastic anaemia in children</td>
</tr>
</tbody>
</table>

Figure by MIT OCW.
## Clinicopathological Syndromes of Viral Hepatitis

<table>
<thead>
<tr>
<th>Acute</th>
<th>Chronic</th>
</tr>
</thead>
<tbody>
<tr>
<td>Classical (icteric) acute type</td>
<td>Carrier state</td>
</tr>
<tr>
<td>Subclinical (anicteric)</td>
<td>Typical forms (formerly known as chronic active and chronic persistent hepatitis)</td>
</tr>
<tr>
<td>Cholestatic</td>
<td>Atypical variants in immunocompromised patients#</td>
</tr>
<tr>
<td>Fulminant</td>
<td></td>
</tr>
<tr>
<td>Neonatal</td>
<td></td>
</tr>
<tr>
<td>Atypical variants in immunocompromised patients#</td>
<td></td>
</tr>
</tbody>
</table>

# Fibrosing cholestatic or cholestatic forms with more aggressive clinical presentations
Acute viral hepatitis

- Flu-like symptoms
- Anorexia & nausea
- ± Icterus (jaundice)
  - Yellow mucous membranes
  - More common in adult form
- ↑ hepatocyte enzymes
  - ALT, AST
- ± Biliary obstruction (cholestasis)
  - Itching
  - ↑ ALP, GGT, bilirubins

Figure removed for copyright reasons. Comparing normal and jaundiced faces.
Fulminant hepatic necrosis (rare)

- Very serious, often fatal complication
- Indistinguishable from toxic and idiosyncratic hepatic necrosis
- Occurs in ~0.1% of HAV infections (also sometimes HBV)
- Almost never in HCV

Source: Figure 7.1 in [MacSween].
Chronic viral hepatitis

- Persistent/intermittent fatigue
- Upper R quadrant pain
- Jaundice
- Weakness
- Muscle & joint pain
- Often asymptomatic
  - Detected during routine bloodwork

Figure removed for copyright reasons.
Source: Figure 7.25 in [MacSween].
Chronic hepatitis viruses

HBsAg = Hepatitis B Surface Antigen
HBcAg = Hepatitis B Core Antigen
HDAg = Hepatitis Delta Antigen

HEPATITIS B VIRUS
HBsAg → DNA
HBcAg

HEPATITIS C VIRUS
Protein C → AAA → RNA → Protein E

HEPATITIS D VIRUS
HDAg → HBsAg → RNA

Figure by MIT OCW.
Hepatitis B

• >350 million people persistently infected (6% of world population)
• 1 in 3 humans presumed exposed during lifetime
• Major cause of liver failure and cancer in sub-Saharan Africa and Far East
  – especially in combination with aflatoxin B1
• Vaccine has reduced incidence, but vertical transmission in developing countries remains a major hurdle
Hepatitis B virus (HBV)

• Time of infection critical to outcomes
  – Vertical transmission or infancy
    • Persistence
    • Liver failure and/or HCC in early adulthood
    • Most common form in Africa and Asia
  – Adult infection usually cleared or persistently subclinical
    • but can be progressive
HBV genome (Hepadnavirus)

- Incomplete dsDNA virus
- Genomic replication requires reverse transcription (like HIV)
- Integration into host chromosomes not required
  - but increases risk of HCC
- Major genes:
  - Surface/envelope (HBsAg)
  - Core (HBcAg) and pre-core (HBeAg)
  - X gene (HBx): transactivator

Figure removed for copyright reasons.
Source: Figure 7.30 in [MacSween].
Circulating HBV capsids

- 22 nm diameter
- Spheres and tubules
- Found in serum
- Empty self-assembled surface antigen proteins
  - = Australia antigen
    - Don’t confuse with Dane particle (full virus)

Photo removed for copyright reasons.
HBV serologic course: clearance (adult-acquired)

Figure removed for copyright reasons.
Source: Figure 7.31 in [MacSween].
HBV serologic course: persistent (infant-acquired)

Figure removed for copyright reasons.  
Source: Figure 7.32 in [MacSween].
Hepatocellular carcinoma

Photo removed for copyright reasons.
Hepatitis C

- Flaviviral etiology discovered in 1989
  - formerly “non-A non-B hepatitis”: NANBH
- Unlike HBV, persistence and chronic progressive disease is usual outcome in adult infection
- >170 million people persistently infected (3% pop.)
- #1 cause of liver failure and transplants in U.S.
- Most common chronic bloodborne infection
- Peak HCV incidence in 1970’s and 80’s--now progressing to liver failure, cirrhosis and cancer
HCV endemic in Africa and Far East

Figure removed for copyright reasons.
Source: Figure 7.25 in [MacSween].
HCV genome (Hepacivirus)

Structure of the Hepatitis C Viral Genome and Encoded Proteins

- 5’ internal ribosomal entry site (IRES)
- Single polyprotein cleaved by protease
- 3 structural proteins: core, E1, E2 (envelope)
- 6 major nonstructural genes: NS2, 3, 4A, 4B, 5A, 5B
- Other regulatory elements and genes of unknown function

Figure by MIT OCW.
HCV clinical course

• Acute infection usually inapparent or unrecognized
• >50% will be persistently infected
• Chronic relapsing bouts of clinical hepatitis with increases in serum transaminases (hepatocyte damage marker)
• 5-10% progress to cirrhosis and/or HCC
Pathology of HCV
(compare murine *H. hepaticus*)

Sequence of ten photos removed for copyright reasons.
Source: [MacSween].
Cirrhosis

- Criteria
  - Hepatocyte necrosis
  - Fibrosis
  - Nodular regeneration

- Occurs in 90% of HCV patients with progressive infection

Figure removed for copyright reasons. Source: Figure 7.19 in [MacSween].
Hepatocytes in HBV and HCV

HBV: “Ground-glass”
HCV: “Oncocytic” (nonspecific)

Figure removed for copyright reasons.
Source: Figure 7.33 in [MacSween].

Figure removed for copyright reasons.
Source: Figure 7.35 in [MacSween].
Animal models of HBV and HCV
Animal models: shortcomings

• Except for chimpanzee and a few other primates, no animal can be infected with HBV or HCV
• Equivalent animal viruses do not generally cause chronic hepatitis or HCC (except woodchucks and other sciurid species)
• Most animal models are useful for studying acute infection & immune clearance, or viral persistence without inflammation (e.g. transgenic mice), but not both
• Absence of good models has hindered research
## Animal hepadnaviruses

### Hepatitis B Viruses (Hepadnaviruses) of Animals

<table>
<thead>
<tr>
<th>Virus Scientific Name</th>
<th>Host</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Genus: Orthohepadnavirus</strong></td>
<td></td>
</tr>
<tr>
<td>Hepatitis B virus (HBV)#</td>
<td>Human</td>
</tr>
<tr>
<td>Woodchuck hepatitis virus (WHV)</td>
<td>Woodchuck, groundhog</td>
</tr>
<tr>
<td>California ground squirrel hepatitis virus (GSHV)</td>
<td>California ground squirrel</td>
</tr>
<tr>
<td>Arctic ground squirrel hepatitis virus (AGSHV)</td>
<td>Arctic ground squirrel</td>
</tr>
<tr>
<td>Woolly monkey hepatitis B virus (WMHBV)</td>
<td>Woolly monkey</td>
</tr>
<tr>
<td><strong>Genus: Avihepadnavirus</strong></td>
<td></td>
</tr>
<tr>
<td>Duck hepatitis B virus (DHBV)</td>
<td>Domestic duck, Pekin duck</td>
</tr>
<tr>
<td>Heron hepatitis B virus (HHBV)</td>
<td>Grey heron</td>
</tr>
<tr>
<td>Snow goose hepatitis B virus (SGHBV)</td>
<td>Snow goose</td>
</tr>
</tbody>
</table>

# Naturally acquired HBV infection also has been demonstrated in the chimpanzee, gorilla, gibbon, and orangutan.

Woodchuck hepatitis virus (WHV)

- Advantages
  - Closely related to HBV
  - High incidence of HCC
  - Patterns of neonatal and adult infection outcome mirror HBV

- Disadvantages
  - Few reagents available for woodchucks
  - Laboratory-reared animals expensive
  - Must be infected very young for persistence
  - HCC equally expressed between sexes (human HBV-associated HCC is male-predominant)

If Punxsutawney Phil sees his shadow, he has woodchuck hepatitis virus.
Duck hepatitis B virus (DHBV)

- **Advantages**
  - Pekin ducks readily available
  - Virus easily propagated in primary liver cell culture
    - useful to study virus lifecycle & in vitro interruption
- **Disadvantages**
  - Poorly characterized lab species
  - Few reagents available
  - No X gene in avihepadnaviruses
  - No HCC
HBV: transgenic mouse models

• First created in mid-1980’s
• Express one or more viral gene products
• Expression of Pre-S gene in commercially available mice causes cytoplasmic retention of surface protein
  – results in cell toxicity and HCC, but may not mimic natural HBV infection
HBV-transgenic mouse models

- **Advantages**
  - Well characterized lab animal w/many reagents
  - Can study specific viral gene expression
  - Can perform adoptive transfer of specific cells or cytokines
  - Some develop HCC in male-predominant fashion like humans (even in absence of inflammation)

- **Disadvantages**
  - Not naturally infected; cannot evaluate viral entry etc.
  - Tolerant to transgenes; no immune response (adoptive transfer or induced expression used to circumvent)
  - Because no complete virus life cycle, hard to do chemotherapeutic evaluations
Non-human primate models of HBV

- Chimpanzee can be infected and supports complete viral life cycle
  - but subclinical or mild hepatitis with viral clearance
  - expensive, endangered species;
- Other apes also infectable, but same caveats
- Wooley monkey HBV poorly characterized
- Tree shrews (*Tupalaia* spp.)
  - can be infected with human HBV
  - co-carcinogenesis with aflatoxin B1
  - poorly characterized experimental species
HBV animal model summary

- Woodchuck hepatitis virus most reliably mimics human disease
  - but few reagents and species poorly characterized
- Other sciurid models (squirrel, prairie dog, etc.)
- Avian hepadnaviruses useful for viral kinetics
- Transgenic mouse models best for studying specific molecular pathways
- Non-human primates have advantages and disadvantages, but expensive and many poorly characterized
Animal models of HCV

- Chimpanzee
- Tree shrew
- GBV-B in tamarins and marmosets
- Transgenic mice
- Chimeric rodents with human hepatocytes
HCV in chimpanzees

• Advantages
  – Support complete viral life cycle
  – Acute hepatitis common (at least upregulation of serum transaminases)
  – Were critical in identifying the causative agent of “non-A, non-B hepatitis”

• Disadvantages
  – Endangered species
  – Cannot do terminal experiments
  – Do not develop chronic hepatitis of HCC
  – Impractical for large-scale study
HCV in tree shrews

• Advantages
  – Can be infected with HCV, and sequentially passaged through multiple generations
  – Causes acute mild hepatitis with immune clearance

• Disadvantages
  – Very poorly characterized species
  – Difficult to acquire and maintain in laboratory setting
  – Poor model for chronic infection
  – Hard to tame
GBV-B virus in tamarins

**Advantages**
- Naturally infective for tamarin species
  - although whether original isolate of human or tamarin origin uncertain
- Genome similar to HCV
  - protease can cleave HCV polyprotein
- Causes acute hepatitis

**Disadvantages**
- Difficult to establish persistence
- Origin of virus unclear
- Expensive to use nonhuman primates
- HCC extremely rare
HCV transgenic mice

• Advantages
  – As for HBV
  – Some develop steatosis and/or male-predominant HCC
  – Adoptive transfer models have shed light on immune mechanisms

• Disadvantages
  – As for HBV
  – Highly variable phenotypes depending on gene expressed, mouse strain and environment (difficult to compare studies)
Rodent/human liver chimeras

- Seeding of rodent liver or extrahepatic site with human liver cells
- Must use immunodeficient recipients
  - SCID, Rag-/- etc.
  - Sublethal whole body irradiation
- Various strategies to deplete endogenous liver to allow for greater human cell engraftment
  - toxic necrosis (e.g. acetaminophen)
  - uPA transgenic mice
- Rats tolerized to human liver by neonatal exposure followed by implantation on day 17
- Human hepatocytes support viral replication, but difficult to evaluate immune responses
A bacterial model of chronic hepatitis and HCC: *H. hepaticus*

- History: Early 1990’s—high prevalence of HCC in control male A/JCr mice in 2-yr National Toxicology Program (NTP) carcinogenesis study at NCI
- NCI & MIT DCM collaborated to identify causative organism as *H. hepaticus*
- Prototype enterohepatic (non-gastric) Helicobacter species (EHS)
- EHS are only murine infectious agents known to cause chronic active hepatitis and HCC
**H. hepaticus** model of chronic hepatitis and HCC

**Advantages**
- Natural murine pathogen
- Except for cirrhosis, histologic presentation similar to human chronic viral hepatitis (especially hepatitis C)
- Invokes male-predominant disease and cancer like humans
- Resistant and susceptible mice allows study of factors protecting against disease

**Disadvantages**
- Not viral; hard to make direct comparisons to viral hepatitis (and to sell to M.D. reviewers)
- C57BL/6 mice not susceptible to clinical disease
- Long timecourse (>18 months for tumors)
HCV animal model summary

- Chimpanzees can be infected, but same caveats as HBV
- Tree shrew model may be useful for acute disease event investigation
- GBV-B tamarin model useful for therapeutic evaluations (e.g. protease inhibitors)
- Transgenic mice: same advantages and disadvantages as for HBV
- Rodent/human liver chimeras: useful to study viral replication in vivo, but not immune response
- *H. hepaticus* model useful to study chronic inflammation and HCC, but not viral gene function
Overall summary

• HBV and HCV are major worldwide human pathogens
• Treatments for viral hepatitis are palliative and lifelong; no cure
• Vaccine exists for HBV but not HCV
• Animal models helpful to investigate pathogenesis but all have limitations
  – Usually able to study early disease events with inflammation, or chronic gene expression without normal immune responses, but not both
We recommend the avian models
Further reading

- ILAR Journal, 2001, 42(2)
  - Animal models of hepatitis (topic dedicated issue).
  - http://dels.nas.edu/ilar_n/ilarhome/index.shtml