Chronic viral hepatitis:
Human Disease and Animal Models

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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

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

Figure by MIT OCW.

Clinicopathological Syndromes of Viral Hepatitis

Acute	Chronic
Classical (icteric) acute type	Carrier state
Subclinical (anicteric)	Typical forms (formerly known as chronic active and chronic persistent hepatitis)
Cholestatic	Atypical variants in immunocompromised patients[#]
Fulminant	
Neonatal	
Atypical variants in immunocompromised patients[#]	

[#]Fibrosing cholestatic or cholestatic forms with more aggressive clinical presentations

Figure by MIT OCW.

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

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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

Figure removed for copyright reasons.
Source: Figure 7.1 in [MacSween].
MacSween, R., et al. Pathology of the Liver, 4th ed. Philadelphia, PA: Elsevier, 2002.

Chronic viral hepatitis

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

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Source: Figure 7.25 in [MacSween].

Chronic hepatitis viruses

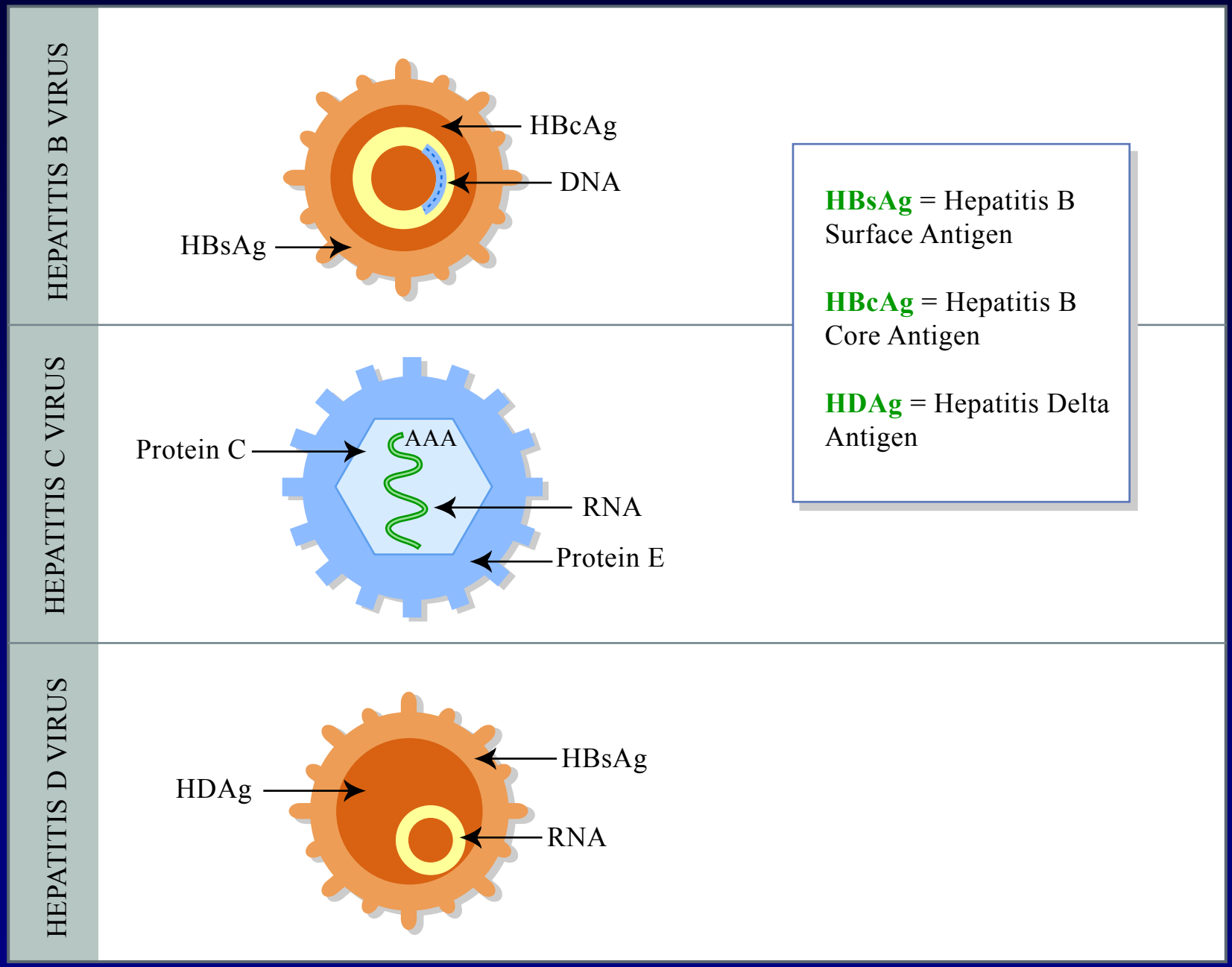

Figure by MIT OCW.

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

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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)

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HBV serologic course: clearance (adult-acquired)

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Source: Figure 7.31 in [MacSween].

HBV serologic course: persistent (infant-acquired)

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Source: Figure 7.32 in [MacSween].

Hepatocellular carcinoma

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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

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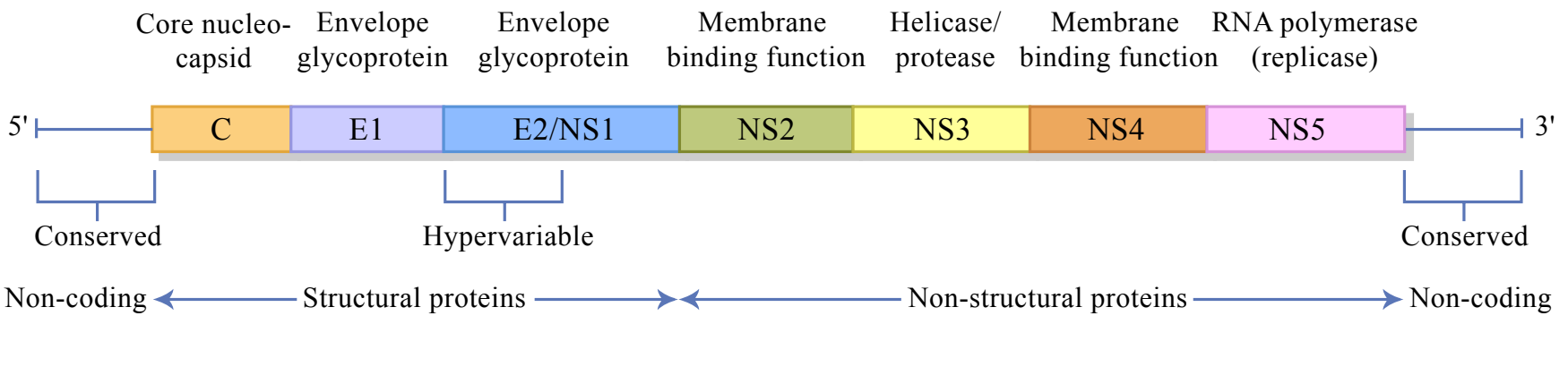
Source: Figure 7.25 in [MacSween].

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

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Source: Figure 7.19 in [MacSween].

- Criteria
  - Hepatocyte necrosis
  - Fibrosis
  - Nodular regeneration
- Occurs in 90% of HCV patients with progressive infection

Hepatocytes in HBV and HCV

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Source: Figure 7.33 in [MacSween].

HBV: “Ground-glass”

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Source: Figure 7.35 in [MacSween].

HCV: “Oncocytic” (nonspecific)

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

Virus Scientific Name	Host	
Genus: *Orthohepadnavirus*		
Hepatitis B virus (HBV)[#]	Human	*Homo sapiens*
Woodchuck hepatitis virus (WHV)	Woodchuck, groundhog	*Marmota monax*
California ground squirrel hepatitis virus (GSHV)	California ground squirrel	*Spermophilus beecheyi*
Arctic ground squirrel hepatitis virus (AGSHV)	Arctic ground squirrel	*Spermophilus parryii*
Woolly monkey hepatitis B virus (WMHBV)	Woolly monkey	*Lagothrix labotricha*
Genus: *Avihepadnavirus*		
Duck hepatitis B virus (DHBV)	Domestic duck, Pekin duck	*Anas domesticus*
Heron hepatitis B virus (HHBV)	Grey heron	*Ardea cineria*
Snow goose hepatitis B virus (SGHBV)	Snow goose	*Anser caerulescens*

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

See Tennant, B.C. and J. L. Guerin. "The woodchuck model of hepatitis B virus infection." ILAR J 42 no. 2 (2001):89-102.

Figure by MIT OCW.

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

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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

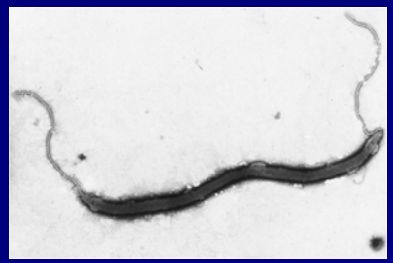
- Advantages
  - As for HBV
  - Some develop steatosis and/or male-predominant HCC
  - Adoptive transfer models have shed light on immune mechanisms
- Disadvantages
  - As for HBV
  - Highly variable phenotypes depending on gene expressed, mouse strain and environment (difficult to compare studies)

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*

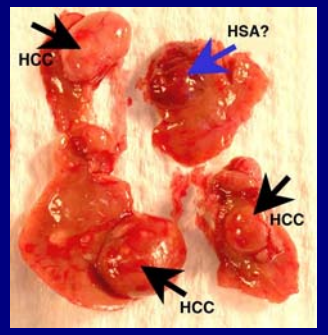
- History: Early 1990's--high prevalance of HCC in control male A/JCr mice in 2-yr National Toxicology Program (NTP) carcinogenesis study at NCI
- NCI & MIT DCM collaborated to identify causative organism as *H. hepaticus*
- Prototype enterohepatic (non-gastric) Helicobacter species (EHS)
- EHS are only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



MIT DCM photo

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)



MIT DCM photo

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
- Robbins and Cotran Pathologic Basis of Disease, 7th ed. 2005. Ch 18, pp. 890-902.
- Pathology of the Liver, 4th ed., 2002. Macsween RNM, ed. Ch. 7. Acute and chronic viral hepatitis.
- Guha C et al. Cell culture and animal models of viral hepatitis. Lab Anim (NY).
  - Part I. HBV. 2004 Jul-Aug;33(7):37-46.
  - Part II. HCV. 2005 Feb;34(2):39-47.