



HISTORY OF VIRAL HEPATITIS: WHAT COMES AFTER E ?

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VIRAL HEPATITIS

- Five established specific human hepatitis viruses (A to E)
 - Differ remarkably in physico-chemical composition and mechanisms by which they undergo replication within infected cells
 - Share in common only a particular tropism for the hepatocyte and the ability to induce liver injury, usually by an immune-mediated mechanism
- Other candidate liver-specific viruses have not unequivocally been linked to liver disease, in particular acute liver failure and chronic hepatitis



HISTORICAL PERSPECTIVE

- Epidemic jaundice described in ancient times by Hippocrates
 - Among the signs of an epidemic disease that plagued Thasos in 400 BC
 - Although often cited as evidence of epidemic viral hepatitis (possibly hepatitis A) among the ancient Greeks, the disease described seems more likely to have been Weil's disease (Leptospirosis) or possibly a viral haemorrhagic fever



HISTORICAL PERSPECTIVE (CONTINUED)

- Possibly contagious nature of jaundice observed in the middle of the 8th century AD by Pope Zacharias, who decreed that affected persons be isolated
- Other references to jaundice abound in writings from the Middle Ages, but it is not until the 19th century that epidemics of viral hepatitis begin to be described more clearly
- Prevailing view that of Virchow, published in 1865, who proposed that epidemic jaundice had a catarrhal origin, consequent to a mucous plug in the papilla of Vater



HISTORICAL PERSPECTIVE (CONTINUED)

- Several reports from the late 19th century and early 20th century first linked vaccination, blood drawing, blood transfusion and parenteral treatment of syphilitic patients with contaminated needles to the development of what was not initially differentiated from epidemic catarrhal jaundice, but what presumably was what later came to be known as “serum hepatitis”



HISTORICAL PERSPECTIVE (CONTINUED)

- The infectious nature of viral hepatitis was not widely appreciated until later in the 20th century
 - The disorder is notably unmentioned in the 3rd edition of Philip Moen Stimson's *Manual of the Common Contagious Diseases*, published in Philadelphia in 1940
 - This all changed with the outbreak of World War II, during which more than 200,000 cases of jaundice were recorded among American troops and more than 5 million cases were described among the military and civilian populations of Germany
 - Little attention seemingly having been paid to the fact that an estimated 70,000 Union troops had been disabled by the disorder during the American Civil War of the 1860's



HISTORICAL PERSPECTIVE (CONTINUED)

- Another pointer to the infectious nature of epidemic hepatitis was the outbreak of the disease in 1942 in nearly 30,000 US servicemen inoculated with yellow fever vaccine, 62 of whom died
 - Since vaccines are made from filtrates to eliminate bacteria, this occurrence provided substantial support for what, in retrospect, was the prophetic view of McDonald, who, writing on “acute yellow atrophy” in the Edinburgh Medical Journal in 1908, when the concept of pathogenic viruses was in its infancy, stated that epidemic jaundice was the result of viral infection



HISTORICAL PERSPECTIVE (CONTINUED)

- Clinical observations, originally reported by MacCallum in the late 1940's, suggested that both enteric (“infectious hepatitis”, “hepatitis A”) and parenteral/sexual (“serum hepatitis”, “hepatitis B”) transmission could occur
- Also appreciated by mid-20th century that these two forms of hepatitis could follow different clinical courses, suggesting different aetiologies



HISTORICAL PERSPECTIVE (CONTINUED)

- The landmark studies of Krugman and colleagues, from New York University School of Medicine, performed at the Willowbrook State School in New York and published in the 1950's and 1960's, were important in elucidating the infectious nature of aetiologic agents for hepatitis A and hepatitis B
- The first to prove the transmissibility of hepatitis from person to person



HISTORICAL PERSPECTIVE (CONTINUED)

- Willowbrook State School
 - Housed thousands of mentally-retarded children, mostly with trisomy 21
 - Frequent cases of jaundice
 - Serum levels of recently-identified liver transaminases determined and indicative of hepatitis



HISTORICAL PERSPECTIVE (CONTINUED)

- Newly admitted children exposed to infectious material, with parental consent
 - Much criticism followed
- Demonstrated that different filterable infectious agents caused the two clinical patterns of hepatitis
 - One inoculum of serum, termed MS1 (after the initials of an infected child) caused an illness with a short incubation period in keeping with epidemic hepatitis (hepatitis A)
 - Another inoculum of serum, termed MS2 (apparently obtained from the same child who later developed the second form of jaundice), caused an illness with a longer incubation period, corresponding to hepatitis B



HISTORICAL PERSPECTIVE (CONTINUED)

- MS1 preparation later used by Boggs and colleagues for the experimental transmission of the clinical illness of hepatitis A to:
 - Marmoset monkeys (reported in 1967)
 - Humans incarcerated at the Joliet State Penitentiary, under the auspices of the US Army (reported in 1970)



HISTORICAL PERSPECTIVE (CONTINUED)

- There followed frustrating and unsuccessful efforts to identify specific agents responsible for viral hepatitis continued
- No specific serologic markers identified until 1965
 - Description of Australia antigen by Blumberg and colleagues at the NIH
 - Would later prove to be the envelope of the Hepatitis B, virus, HBsAg



HISTORICAL PERSPECTIVE (CONTINUED)

- Description of the Australia antigen
 - Work driven by an interest in genetics and human anthropology, using agar double diffusion gels to detect immunological reactions between antibodies and antigens in human sera of different ethnic backgrounds
 - Multi-transfused patients, usually with leukaemia or haemophilia, selected for study as expected to display numerous antibodies
 - Initial breakthrough came with development of an immunoprecipitin line between the serum of a patient with haemophilia and that of an Australian aborigine
 - Association of the Australia antigen with the entity known as “serum hepatitis” (hepatitis B) was not recognised until 2 years later

HISTORICAL PERSPECTIVE (CONTINUED)

- This landmark discovery provided, for the first time, a serologic marker for one of the hepatitis viruses
- Epidemiologic and further clinical differentiation from other viruses could now be determined
- An explosion of new knowledge in the field of viral hepatitis would follow
- Hepatitis A, D, C and E viruses, in that order, would be identified



HISTORICAL PERSPECTIVE. HEPATITIS A VIRUS

- HAV was first identified in 1973 by scientists from the NIH, who discovered the virus in faecal samples collected from volunteers who had been inoculated with Krugman's MS1 serum obtained from the Willowbrook school
 - Immunoelectron microscopy revealed 27 nm spherical viral particles, which formed aggregates after incubation of faecal samples with convalescent serum
 - Two years later, Hollinger and colleagues reported immunological techniques to detect HAV antigen and antibodies in serum
 - At around the same time, Dienstag et al and Maynard et al separately demonstrated the transmission of hepatitis A to chimpanzees, which became the preferred animal model in which to study the disease
 - HAV was successfully adapted to growth in cell culture by the end of the 1970's
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HISTORICAL PERSPECTIVE: HEPATITIS D VIRUS

- In 1977, Rizzetto et al in Turin reported the identification of a new antigen in both liver and serum of patients who were carriers of HBsAg
- This led to the discovery of the HDV, a defective virus whose genome consists of a negative single-stranded circular RNA encoding a single nucleocapsid protein, delta antigen
- To form an infectious particle, the delta virus requires the envelope of the helper HBV containing HBsAg



HISTORICAL PERSPECTIVE: HEPATITIS C VIRUS

- With the advent of sensitive techniques to diagnose infections with HAV, HBV and HDV by the end of the 1970's came the realisation that most cases of post-transfusion hepatitis were not caused by these viruses
- The entity thus identified, which affected in the order of 20% of patients undergoing cardiac surgery who received a blood transfusion, came to be known as non-A, non-B hepatitis (NANBH)



HISTORICAL PERSPECTIVE: HEPATITIS C VIRUS

- A major breakthrough that stemmed from advances in molecular biologic techniques was the identification of HCV by Houghton and colleagues in 1989 at the Chiron Corporation
 - A cDNA was cloned from plasma that had a high titre of infectious virus collected from a chimpanzee
 - Use of serum from a patient with chronic NANBH to screen a cDNA phage expression library prepared from the chimpanzee's plasma RNA
 - Among millions of clones screened, one (designated 5-1-1) was recognised by the immunoglobulins in the patient's serum
 - Within 12 months of the discovery of the initial clone, the complete sequence of the genome of the new virus was determined



HISTORICAL PERSPECTIVE: HEPATITIS C VIRUS

- Retrospective testing of blood samples stored at the NIH and elsewhere has shown that up to 90% of cases of non-A, non-B hepatitis were caused by the hepatitis C virus



HISTORICAL PERSPECTIVE: HEPATITIS E VIRUS

- Hepatitis E is an epidemic waterborne type of hepatitis with many of the clinical features of hepatitis A
- Clinical entity first recognised in India in 1980
- Its possible existence raised after retrospective testing of sera from persons afflicted during a large waterborne epidemic of hepatitis during in 1956 in Delhi, along with another epidemic in Kashmir in 1978, lacked serologic markers of acute hepatitis A or B



HISTORICAL PERSPECTIVE: HEPATITIS E VIRUS

- Causative agent identified in Uzbekistan in 1983 by Bayalan and colleagues
 - Immunoelectron microscopy in stool samples of a volunteer immune to HAV, who had ingested fecal extracts from patients with epidemic NANBH
 - Virus identified as approximately 30 nm particles aggregated by antibodies in the patients' sera
- Genome cloned in 1990 and fully sequenced shortly after
- HEV has been propagated in a variety of animal models, most notably macaques



CLINICAL SPECTRUM OF VIRAL HEPATITIS

Asymptomatic Elevation
of Liver Enzymes (AST, ALT > ALP)



RUQ Pain, Jaundice
+/- Constitutional Symptoms



Acute Liver Failure

Self-Limited Syndrome  Progression to Cirrhosis



	<u>HAV</u>	<u>HBV</u>	<u>HCV</u>	<u>HDV</u>	<u>HEV</u>
Incubation (weeks)	2-6	4-12	2-24	4-24	2-6
Transmission	Faeco-oral	Parenteral Sexual Vertical	Parenteral Sexual Vertical	Parenteral Sexual Vertical	Faeco-oral
Carrier State/ Chronic Hepatitis	No	Carriers: 3-5% (adults) : 90% (neonates) CHB: 30% carriers	CHC: 70%	CHD: 70%	No
Risk of HCC	No	Yes (even carriers)	Yes	Yes	No
Diagnosis	IgM anti-HAV	HBsAg IgM-anti HBc	HCV RNA	IgM anti-HDV	IgM anti-HEV
Vaccine	Yes	Yes	No	No	No



Similarity in Incubation, Transmission, Potential for Chronicity and Risk of HCC

- Hepatitis viruses A, E
- Hepatitis viruses B, C, D



MAGNITUDE OF PUBLIC HEALTH PROBLEM

- Viral hepatitis A-E continues to be a major public health problem and the most common cause of liver disease worldwide
- Chronic viral hepatitis remains the most common cause of chronic liver disease worldwide (notwithstanding the rise of NAFLD in Western countries)



MAGNITUDE OF PUBLIC HEALTH PROBLEM (CONTINUED)

- Although the incidence of acute viral hepatitis is decreasing throughout the world as a result of more effective public health measures and the introduction of hepatitis A and B vaccination programmes, each new case of acute hepatitis C carries around a 70% chance of adding to the growing pool of people with chronic viral hepatitis and all its complications
- Annual cost is enormous, even when not taking into account the intangibles of impaired quality of life



Hepatitis with Other Viruses

- Epstein-Barr virus
- Cytomegalovirus
- Varicella zoster virus
- Herpes simplex virus
- Parvovirus B19
- Adenovirus
- Enteroviruses
- Papilloma virus
- Paramyxoviruses
- Toga-like viruses (“giant cell hepatitis”)
- Haemorrhagic fever viruses

No Progression to Chronicity



NEGATIVE TESTING FOR ALL ESTABLISHED HEPATITIS VIRUSES (USA DATA)

- **10% of transfusion-associated acute hepatitis**
 - [Alter & Bradley, 1995](#)
 - **20% of community-acquired acute hepatitis**
 - [Alter et al., 1992](#)
 - **30% of chronic hepatitis/cirrhosis (otherwise unexplained)**
 - [Kodali et al., 1994](#)
 - **Most cases of hepatitis-associated aplastic anaemia**
 - [Brown et al., 1997](#)
 - **20% of acute liver failure (otherwise unexplained)**
 - [Ferraz et al., 1996](#)
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**COULD “OCCULT” HBV ACCOUNT
FOR SOME OF THESE CASES ?**



OCCULT HBV INFECTION

- **Follow-up after apparent resolution of hepatitis B**

(HBsAg -ve, IgG anti-HBc +ve, anti-HBs +ve)

- **Up to 50% show persistence of HBV DNA in serum**
- **Persistence of HBV DNA in liver for up to 30 years**



OCCULT HBV INFECTION

- In otherwise unexplained chronic hepatitis, low levels of HBV DNA may be found in serum of
 - 30-35% HBsAg -ve, IgG anti-HBc -ve, anti-HBs -ve subjects (apparently un-exposed to HBV)
 - up to 60% HBsAg -ve, IgG anti-HBc +ve, anti-HBs +ve subjects (HBV infection apparently resolved)
 - HBV DNA found in 0 - 17% HBsAg -ve blood donors with normal LFT's (highest in endemic areas)

OCCULT HBV INFECTION

- **HBV DNA detection rates even higher in liver tissue than serum (up to 94%)**



OCCULT HBV INFECTION: INFECTIVITY

- **Clearly established**
 - **Transmission of hepatitis by injection of HBV DNA-positive sera into chimps**
 - **Post-transfusion transmission of hepatitis in humans**
 - **Vertical transmission of hepatitis**
 - **Infection arising from HBsAg -ve organ donors**



THE SEARCH FOR ADDITIONAL HEPATITIS VIRUSES

- Many “viral discovery programs”, particularly linked to industry
- These led, sequentially, to discoveries of
 - GB virus C (Hepatitis G virus) *Linnen et al., 1996*
 - TT virus family *Nishizawa et al., 1997*
 - SEN virus *Sottini et al., 2001*



GB VIRUS C/HEPATITIS G VIRUS

- In the 1950's (before discovery of any hepatitis virus), acute hepatitis occurred in a surgeon with initials "GB"
- GB serum inoculated into marmoset monkeys and caused hepatitis in that animal model
- Debate ensued regarding whether the GB agent represented a human hepatitis agent or a reactivated monkey virus and interest waned



GB VIRUS C/HEPATITIS G VIRUS

- In recognition that a non-A, non-E human hepatitis virus may exist, investigators at Abbott Laboratories resurrected the GB story in the 1990's
 - Frozen samples from inoculated monkeys were obtained and the full GB genome sequenced (GBV-A); a further two GB agents became apparent (GBV-B and GBV-C)
 - GBV-A a primary monkey virus
 - GBV-C a primary human virus
 - GBV-B might be able to infect both species
 - Linnen et al subsequently reported the cloning of HGV from an immunoreactive complementary DNA clone
 - Sequence analysis of these 4 viruses showed that GBV-C and HGV are strain variants of the same virus
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GB Virus C/Hepatitis G Virus

- Globally distributed RNA virus, member of the Flaviviridae family
- Mainly transmitted parenterally but sexual and vertical modes also described
- Does not replicate in the liver
- **Does not cause hepatitis or any other pathology**



TT VIRUS

- Discovered by representational difference analysis of serum obtained from 5 Japanese patients with post-transfusion non-A, non-E hepatitis
- Name derived from initials of first patient in whom detected
- Widely and highly prevalent DNA virus
- Member of *Circoviridae* family
- Transmitted by both enteral and parenteral routes
- Vertical transmission described
- Replicates in the liver
- Does not cause hepatitis or any other pathology



SEN VIRUS

- DNA virus, first isolated from plasma of IVDU with HIV infection
- Member of *Circoviridae* family
- Phylogenetic analysis shows existence of 8 strains
- SENV-D and/or SENV-H found by PCR in:
 - 1.8% US blood donors, 20% healthy donors in Japan
 - 11/12 (92%) transfusion-associated non A-E hepatitis cases in NIH prospective study, compared with 55/225 (24%) transfused patients who did not develop hepatitis
 - Persistent infection with biochemical evidence of chronic hepatitis in 2/11 (18%) *Umemura et al., 2001*
 - ***TO PROVE CAUSALITY, NEED TO ESTABLISH INTRAHEPATIC LOCALISATION AND REPLICATION AT VERY LEAST***
- No association between SEN virus and acute liver failure, chronic hepatitis, cirrhosis or hepatitis-associated aplastic anaemia found in separate studies from US, Japan, Germany, Greece