

Hepatitis A – Virus and Vaccination

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Historical Perspective

- 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 infected serum
 - Immunolectron 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

Virology

- Initially classified as a single stranded RNA enterovirus belonging to the Picornaviridae family
- Later, a new genus, Hepatovirus, created for HAV
- Inactivated at 85 deg C for 1 minute
- Survives in gastric acid (pH 3) and also
 - Dried faeces at room temperature for 4 weeks (17% survival rate)
 - Sea water (4% survival rate)
 - Live oysters at 5 days (12% survival rate)

Life Cycle

- Attachment to cell surface receptor, a glycoprotein named HAVCR1
 - First described in monkeys in 1996 and then in humans in 1998
 - Responsible gene known as TIM1, a susceptibility factor for asthma and allergy
- Once HAV enters a cell, cell host ribosomes bind to RNA and polysomes are formed
- HAV translated into a large polyprotein of 2,227 amino acids, organised into 3 regions, P1 – P3
 - P1 regions encode structural proteins, VP1 - VP4
 - P2 and P3 regions encode non-structural proteins, associated with viral replication

Genotypes

- Numerous HAV strains exist, with considerable nucleotide sequence variability (15% - 25% difference within the P1 region of the genome)
- 4 different genotypes associated with human infection (I,II,III, VII)
 - Genotypes IV, V, VI found exclusively in monkeys
- Despite nucleotide sequence heterogeneity, antigenic structure of human HAV highly conserved among strains

Pathogenesis

- Ingestion
- Survives gastric acidity
- Breaches small intestinal mucosa
- Carried by portal vein to liver
- Enters hepatocyte by glycoprotein receptor, HAVCR1/TIM-1
- Hepatocellular replication in cytoplasm
- Exported via
 - hepatic sinusoids
 - bile canaliculi

Infectivity of Body Fluids

Fluids	Comments
Stool	The main mode of infection (contaminated food or drinking water) HAV detectable during incubation period and for several weeks after onset of clinical symptoms HAV RNA detectable for up to 5 months after symptom onset
Blood	Viraemia detectable during incubation period Blood collected 3 and 11 days before clinical symptoms caused post-transfusion hepatitis in recipients
Bile	HAV detected in the bile of chimpanzees with acute infection
Urine	HAV detectable in low concentration during incubation period A urine sample infected 1/12 subjects after oral inoculation
Saliva	Salivary gland an extra-hepatic site of early HAV replication; nation-wide policy of disposable tableware in public eating places associated with reduced transmission in Taiwan

From: Sjogren M, Schiff's Diseases of the Liver , 10th Ed, 2007; Amado et al., Int J Exp Pathol 2010; 91:87-97; Su et al., BMC Public Health 2010;10:719

- Primarily transmitted enterically (faceo-oral)
 - Ingestion of contaminated food or water
 - Person-to-person contact through the household
 - +/- blood, urine, saliva
- Risk groups
 - Travelers to endemic areas
 - Crowded/unsanitary conditions
 - Day care workers
 - Institutionalised patients
 - Homosexual men with multiple sexual partners
 - Parenteral transmission has *rarely* been implicated

- Lack of cell injury in cell culture systems suggests that HAV is not directly cytopathic
- Immunologically-mediated liver injury more likely (as generally with HBV, HCV)

Strategies Limiting Innate Immune Response to HAV

- TLR3 and cytosolic RIG-1-like and MDA5 helicases (pattern recognition receptors) sense viral RNA and activate innate immune signaling pathways that induce IFN expression
- HAV interferes with both of these innate immune mechanisms via cleavage of essential adapter pathways

- HAV-infected chimpanzees show limited induction of IFN-stimulated genes compared with chimpanzees with acute resolving HCV infection
 - HAV RNA persists in liver for months, long after clearance from serum and faeces
 - HAV RNA detectable in liver for significantly longer (35 to >48 weeks) than HCV RNA in animals with acute HCV infection
- *Lanford et al. Proc Natl Acad Sci USA 2011;108:11223-8*

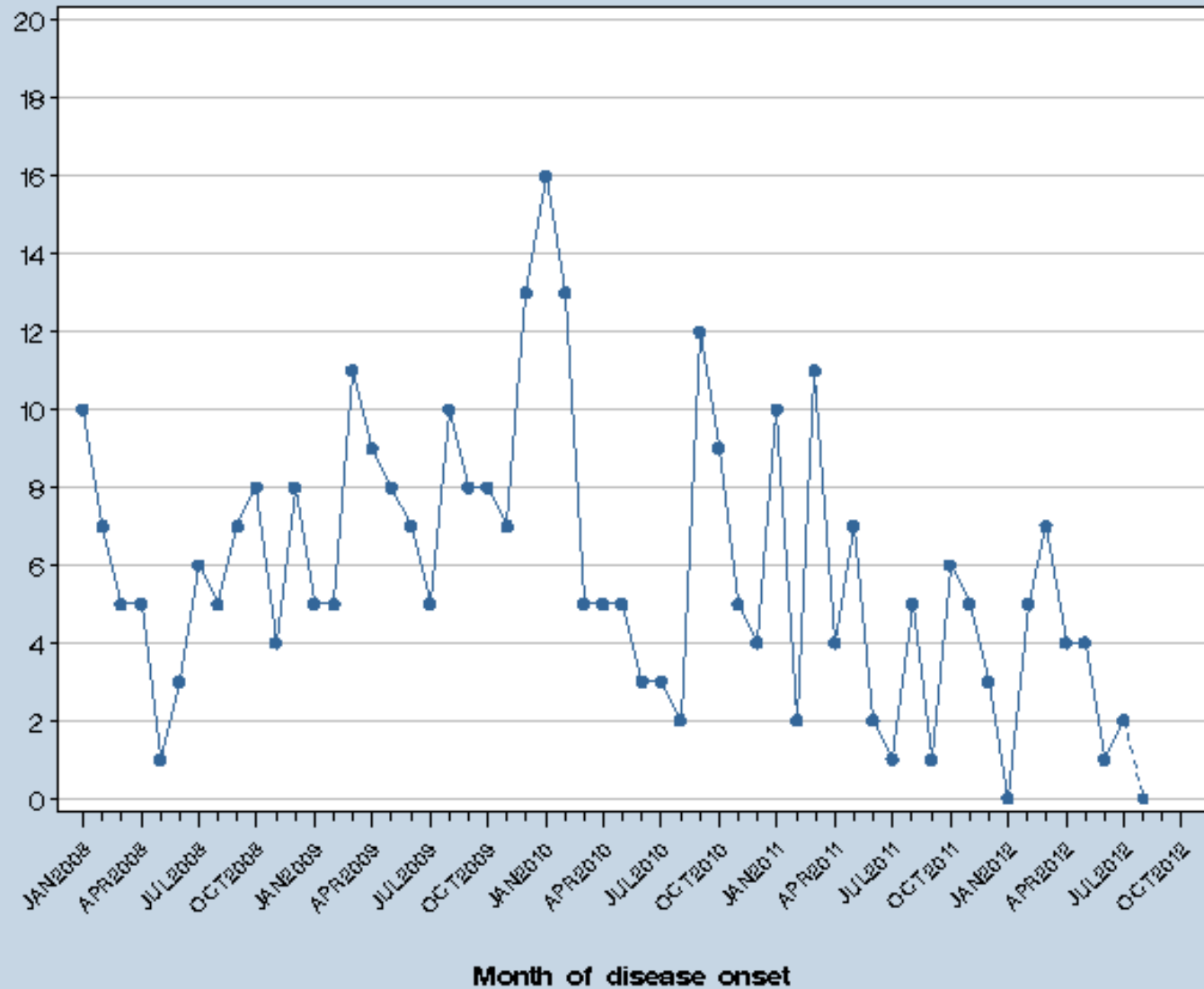
Epidemiology

- HAV remains a major cause of acute hepatitis world-wide, particularly those with poor sanitation, but also in industrialised nations
 - Annual incidences
 - 2-4 cases per 100,000 in Nordic countries
 - 7-10 cases per 100,000 in Germany, UK
 - 6 cases per 100,000 in Italy
- Particularly common in Africa, Asia, Latin America, where sero-prevalence rates approach 100%, mostly due to infection by age 5 years
- Sero-prevalence rates falling in industrialised countries, attributed to improvement in socio-economic conditions/sanitation

- HAV infection generally follows one of two epidemiological patterns
 - Countries with Poor Sanitation
 - Most children infected at an early age, but actual rates depend upon family income (presumed hygiene factors)
 - 95% of Bolivian school children from low-income families anti-HAV +ve compared to 56% children from high-income families (Gandolfo et al, 2003)
 - » Most cases of acute HAV infection in this setting remain asymptomatic and, since not recognised, potentially serve as a focal point for new cases (Vatev et al, 2011)
 - Industrialised Countries
 - Prevalence of HAV infection low in children and young adults
 - USA data: 10% in children/young adults but 37% in older adults (CDC 1996)

Hepatitis A notifications in NSW residents, by month of disease onset. January 2008 to August 2012

No. of notifications



Clinical Features

- Incubation period 2-6 weeks
- Prodromal symptoms
 - Non-specific
 - Fatigue, anorexia, nausea, vomiting, RUQ pain, arthralgia, myalgia, diarrhoea
 - Dark urine within 1-2 weeks of onset of prodromal symptoms in 90%
 - Usually decrease with onset of clinical jaundice
- Signs
 - RUQ tenderness/hepatomegaly 85%
 - Splenomegaly 15%
 - Cervical lymphadenopathy 15%

Clinical Patterns of HAV Infection

- **1. Asymptomatic, without jaundice**
 - Usual in children < 6 years
- **2. Symptomatic, with jaundice and self-limited to approximately 8 weeks**
 - Especially when older than 6 years
- **3. A more prolonged cholestatic hepatitis with jaundice**
 - <10%, resembles flucloxacillin-related hepatitis
- **4. Relapsing infection, with two or more peaks in jaundice over a 6 to 10 week period**
 - <10%
 - In a mouse model (*Dotzauer A et al. J Gen Virol 2012;93:754-760*)
 - Over a period of several weeks after infection, anti-HAV IgA-mediated transport of HAV to the liver enables enterohepatic recycling of HAV with endogenous re-infection
 - Terminated by highly avid IgG antibodies produced later in time-course of infection
- **5. Acute liver failure (ALF)**
 - <1%
 - Remains the main cause of ALF in developing countries
- **6. Autoimmune disease**
 - Rare reports

The Clinical Syndrome of Acute Liver Failure

Hepatic encephalopathy (incl. cerebral oedema)

Haemodynamic instability (vasodilatation, adrenal insufficiency)

Renal failure

Metabolic derangement

Coagulopathy

Susceptibility to infection

Severity of Clinical Syndrome

Degree of impairment of synthetic, bio-transformatory and endotoxin-scavenging activity (loss of functioning hepatocytes and Kupffer cells) due to initiating insult (INITIAL HIT)

+

Effects of cytokines released (a) from liver (activated Kupffer cells +/- stellate cells, sinusoidal endothelial cells, hepatocytes) and (b) due to complicating systemic infection

- Amplification of liver damage (SECOND HIT)
- Vasoactive effects, leading to multi-organ failure

versus

Capacity for liver regeneration (also cytokine-initiated)

Risk Factors for ALF Due to HAV

- ***Host-Related Factors***

- Age > 40 years

- » Muraoka 1990, Willner et al, 1998

- HAV-superinfection of chronic hepatitis C

- » Vento et al, 1998

- Strong immune response

- » Rezende et al. Hepatology 2003;38:613-618

- » Sasbon et al. Pediatr Crit Care Med 2010;11:385-389

- » Ajmera et al. J Viral Hepatitis 2011; 18:e167-174

- Cell surface receptor (TIM1/HAVCR1) polymorphism

- » Kim et al, 2011

- ***Viral Virulence Factors***

A polymorphism in TIM1 (HAVCR1), the gene encoding the HAV receptor, is associated with HAV-related ALF (Kim et al, J Clin Invest 2011; 121:1111-8)

- 30 Argentinian patients with HAV-related ALF and 102 healthy blood donor case-controls with past history of mild or asymptomatic HAV infection
- 6 amino acid insertion, 157insMTTTPV, in TIM1 associated with ALF

Genotype Frequency (%)

	n	Homozygous for Insertion	Heterozygous for Insertion	Homozygous for No Insertion
Patients with ALF	30	1 (3)	20 (67)	9 (30)
Case Controls	102	8 (8)	41 (40)	53 (52)

- *The first host genetic susceptibility factor shown to predispose to HAV-related ALF*
 - * *previously shown to protect against asthma/atopy*

TIM1 Insertion Polymorphism and ALF: Mechanistic Considerations

- TIM-1 protein containing the insertion polymorphism promotes more efficient HAV binding to its receptor
- HAV activates NK T cells via TIM-1, and TIM-1 protein containing the insertion polymorphism increases NK T cell cytolytic activity against HAV-infected hepatoma cells

» [Kim et al, J Clin Invest 2011; 121:1111-8](#)

ALF Due to HAV: Viral Virulence Factors

- 5' untranslated region of HAV genome sequenced in serum samples from 84 Japanese patients with HAV infection, including 12 with ALF
 - Fewer nucleotide substitutions in HAV genome between nucleotides 200 and 500 in those with ALF (mean 1.8 versus 5.4 in self-limited hepatitis), suggesting that nucleotide variation in central portion of 5' untranslated region may influence severity of infection
 - Fujiwara et al, Gut 2002;51:82-88
- Rates of nucleotide substitution did not vary significantly between ALF and non-ALF cases in a USA series
 - Ajmera et al. J Viral Hepatitis 2011;18:e167-174

ALF Due to HAV: Viral Virulence Factors

- Genotype IIIA infection associated with more severe clinical manifestations than genotype IA in Korean cohort
 - [Yun et al. J Med Virol 2011;83:2073-2081](#)
- Genotype IB found more frequently in ALF cases compared to non-ALF cases in a USA series
 - [Ajmera et al. J Viral Hepatitis 2011; 18:e167-174](#)
- Genotype other than IA associated with more severe illness in France
 - [Rezende et al. Hepatology 2003;38:613-618](#)

Autoimmune Disease

- Rare reports of HAV apparently triggering autoimmune hepatitis
- Genetic predisposition likely important
 - HLA-DRB1*12 significantly more frequent in AIH patients with +ve HAV IgM than in AIH patients with –ve HAV IgM in 25 children with AIH
 - » Elfaramawy et al. Ital J Pediatrics 2010;36:73

Diagnosis of HAV Infection

- IgM anti-HAV
 - Positive from the onset of symptoms and usually remains positive for around 4 months
 - Low-level positivity may persist for > 1 year
- HAV RNA
 - Limited to research laboratories
 - Documented in human sera for up to 21 days after the onset of illness
 - In patients with severe acute hepatitis, combination of detectable IgM anti-HAV and undetectable HAV RNA in serum early in clinical course may portend poor prognosis (presumably as a result of more vigorous immune response with increased liver damage)
 - Rezende et al. Hepatology 2003;38:613-618 (FRANCE)
 - Sasbon et al. Pediatr Crit Care Med 2010;11:385-389 (ARGENTINA)
 - Ajmera et al. J Viral Hepatitis 2011; 18:e167-174 (USA)

Immunisation

- Vaccination
- Post-exposure prophylaxis
 - HAV immunoglobulin
 - 0.02 mL/kg by IMI within 2 weeks of exposure
 - Considered safe, although risk of fever, myalgia, pain at injection site
 - Does not preclude active immunisation with vaccine

Vaccines

- Two human HAV strains, HM175 and CR326, used for production of commercially available vaccines
 - HM175 isolated from faeces of Australian patients in an outbreak of hepatitis A in 1978
 - CR326 isolated from Costa Rican patients
 - Nucleotide and amino acid sequences show 95% homology between the two strains
 - Inactivated vaccines prepared from these strains are thought to provide protection against all relevant human strains of HAV

Hepatitis A Virus Vaccination Regimens

Vaccine	HAV Strain	Age (y)	Dose (IMI)	Volume	Schedule (months)
Havrix (SmithKline)	HM175	2–18	720 U	0.5	0, 6-12
		>18	1440 U	1.0	0, 6-12
Vaqta (MSD)	CR326	2-18	25 U	0.5	0, 6-18
		>18	50 U	1.0	0, 6-18
Twinrix	HM175	≥18	720 U HAV 20 ug HBV	1.0	0,1,6

Both HAV vaccines safe and immunogenic, with detectable anti-HAV in serum for > 20 years;
Durable responses in HIV infection up to 10 years, especially with suppressed HIV RNA levels
(Crum-Cianflone et al, 2011)

6 HAV antigenic variants that likely escaped protective effects of available vaccines
recently isolated; completion of proper vaccination schedules likely critical to prevent
emergence of vaccine-escaping mutants
(Perez-Sautu et al, 2011)

Recommendations for Vaccination

- High risk groups
 - Children in endemic areas
 - Travelers to endemic areas
 - Homosexual men
 - Intravenous drug users
 - Patients with pre-existing chronic liver disease due to other aetiologies
- USA guidelines (2006): **All** children aged 12 – 23 months should receive HAV vaccination
 - Implementation of 1999 recommendation for routine childhood vaccination *in USA areas with consistently elevated HAV rates* reduced infection rates by 92%, from 12.0 cases per 100,000 in 1995 to 1.0 case per 100,000 in 2007, the lowest rate ever recorded