Hepatitis A: Mechanisms of Vaccine Induced Protection

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## Types of Viral Hepatitis

<table>
<thead>
<tr>
<th></th>
<th>A</th>
<th>B</th>
<th>C</th>
<th>D</th>
<th>E</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Source of virus</strong></td>
<td>feces</td>
<td>blood/blood-derived body fluids</td>
<td>blood/blood-derived body fluids</td>
<td>blood/blood-derived body fluids</td>
<td>feces</td>
</tr>
<tr>
<td><strong>Route of transmission</strong></td>
<td><strong>fecal-oral</strong></td>
<td><strong>percutaneous permucosal</strong></td>
<td><strong>percutaneous permucosal</strong></td>
<td><strong>percutaneous permucosal</strong></td>
<td><strong>fecal-oral</strong></td>
</tr>
<tr>
<td><strong>Chronic infection</strong></td>
<td>no</td>
<td>yes</td>
<td>yes</td>
<td>yes</td>
<td>yes</td>
</tr>
<tr>
<td><strong>Prevention</strong></td>
<td><strong>pre/post-exposure immunization</strong></td>
<td><strong>pre/post-exposure immunization</strong></td>
<td><strong>blood donor screening; risk behavior modification</strong></td>
<td><strong>pre/post-exposure immunization; risk behavior modification</strong></td>
<td><strong>ensure safe drinking water</strong></td>
</tr>
</tbody>
</table>
Acute Viral hepatitis by Type: 1982-1993
Pre-Vaccine

- 47% Hepatitis A
- 34% Hepatitis B
- 16% Hepatitis C
- 3% Non-ABC

Source: CDC Sentinel Counties Study on Viral Hepatitis
Hepatitis A Virus by Electron Microscopy

Feinstone, Kapikian, Purcell, 1973

Moritsugu, 1976
The Team
Structural Phylogenetic Analysis Reveals HAV to Be a ‘Primitive’ Picornavirus

Wang et al., Nature 2014 doi:10.1038/nature13806
HAV
Genome – Gene products – Processing - Assembly
Replication Cycle of HAV

Martin and Lemon, 2006
**HAV receptor**
- Expressed in liver, kidney, lung, T helper 2, and NKT cells
- Significant allergy determinant gene in man and mouse
- Inverse association between HAV infection and development of asthma?
- Polymorphism affect T cell function associated with chronicity in HCV and HIV infection

**HAVCR (TIM) family: phosphatydilserine receptors that regulate the immune response**
- Expressed in antigen presenting cells
- Mediates phagocytosis of apoptotic cells
- Involved in allergic responses (peanut allergy)
IFN-activating pathways disrupted by HAV 3C precursor-mediated proteolysis both MAVS and TRIF

Long incubation period
Persistent infection in cell culture

Qu et al., PLoS Pathog, 2011
Membrane Hijacking by Hepatitis A Virus

HEPATITIS A - CLINICAL FEATURES

- Rare complications:
  - Fulminant hepatitis
  - Cholestatic hepatitis
  - Relapsing hepatitis

- Jaundice by age group:
  - <6 yrs: <10%
  - 6-14 yrs: 40%-50%
  - >14 yrs: 70%-80%

- Incubation period:
  - Average 30 days
  - Range 15-50 days

- Chronic sequelae:
  - None
CLINICAL, VIROLOGIC AND SEROLOGIC EVENTS in HAV INFECTION
eHAv Circulates in Infected Humans and Chimpanzees while Virus Shed in Feces is Not Associated with Membranes

eHAV is Neutralized by Antibody Post-Endocytosis

Model for Post-Endocytic Neutralization of eHAV

Virologic and immunologic events in an acute HAV infection (Chimpanzee)

Pathogenesis: Is hepatitis A immune mediated?

How are HAV infections controled.?
WORLDWIDE PATTERNS of HAV ENDEMICITY
Age stratified prevalence of anti-HAV in different epidemiologic settings
# Global Patterns of Hepatitis A Virus Transmission

<table>
<thead>
<tr>
<th>Endemicity</th>
<th>Disease Rate</th>
<th>Peak age of infection</th>
<th>Transmission Pattern</th>
</tr>
</thead>
<tbody>
<tr>
<td>High</td>
<td>Low to High</td>
<td>Early childhood</td>
<td>Person to person</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>Outbreaks uncommon</td>
</tr>
<tr>
<td>Moderate</td>
<td>High</td>
<td>Late childhood/young adults</td>
<td>Person to person</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>Food and water borne outbreaks</td>
</tr>
<tr>
<td>Low</td>
<td>Low</td>
<td>Adults</td>
<td>Person to person</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>Food and water borne outbreaks</td>
</tr>
<tr>
<td>Very low</td>
<td>Very low</td>
<td>Adults</td>
<td>Travelers</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>Outbreaks uncommon</td>
</tr>
</tbody>
</table>
RISK FACTORS ASSOCIATED WITH HEPATITIS A 1990-2000, UNITED STATES

- Contact of day-care child/employee: 6%
- Other Contact: 8%
- Food- or waterborne outbreak: 4%
- Child/employee in day-care: 2%
- Men who have sex with men: 10%
- Injection drug use: 6%
- International travel: 5%
- Sexual or household contact: 14%
- Unknown: 46%

Source: CDC
Reported cases
Estimated infections

Age Distribution of Acute HAV Infections in the U.S.

Source: Armstrong & Bell, Pediatrics, 2002
Protection against hepatitis A

It’s the antibody, stupid!

(Paraphrased from Bill Clinton, 1992 Presidential campaign)
PREVENTING HEPATITIS A

- Hygiene
- Sanitation
- Immune globulin (pre- and post-exposure)
- Inactivated Hepatitis A vaccine (pre- and post-exposure)
Hepatitis A Prevention – Immune Globulin largely replaced by vaccine

- Pre-exposure
  - travelers to intermediate and high HAV-endemic regions who cannot take HAV vaccine

- Post-exposure (within 14 days)
  - Routine
    - household and other intimate contacts – vaccine now considered as good as IG

Selected Situations
- institutions (e.g., day care centers)
- common source exposure (e.g., food prepared by infected food handler)
HAV Vaccine Principles

- One serotype
- Growth in cell culture
- Low level of serum antibody alone is protective
HAV in Cell Culture

Characteristics of HAV in Cell Culture

- Primary isolation requires long incubation period
- Adaptation Through passage
- Host restriction to Primate cells + a few others
- HAV remains largely cell associated
- No cytopathic effect
- Virus establishes persistent infections

HAV in AGMK cells by Immunofluorescence
Attenuation of HAV after Serial Passage in 1° AGMK Cells

Karron et al., 1992
Characteristics of Live HAV Vaccine

- Proper attenuation is difficult to achieve
- Poor Response to oral administration
- Requires multiple i.m. or s.c. dose to achieve adequate immune response
- Antibody responses generally low but durable
- Risk of reversion to virulence?
- Cold chain requirement?

Two live HAV vaccines in use in China/India
Principles of killed HAV vaccine

- Produced in cell culture
- Virus attenuated in humans – safety factor
- Purified
- Inactivated by formalin
- Adjuvanted – Alum - Virosomes
- Single dose provides at least short term immunity
- Two doses provide protection > 20 years
KILLED HEPATITIS A VACCINES

- Highly immunogenic
  - 97%-100% of children, adolescents, and adults have protective levels of antibody within 1 month of receiving first dose; essentially 100% have protective levels after second dose

- Highly efficacious
  - In published studies, 94%-100% of children protected against clinical hepatitis A after equivalent of one dose
Efficacy of a Single Dose of HAV Vaccine (Merck)

HAV Vaccine n=519
Placebo n=518

From Werzberger et al., 1992
Efficacy of a 2 Dose Inactivated HAV Vaccine (GSK)

- **n = 40,119 Thai schoolchildren**

Clinically apparent cases were monitored between months 5-10
Efficacy = 97%

From Innes et al., 1992
HAV ANTIBODY TITERS

Durability of Vaccine Response

Theeten et al. Vaccine 2015
Worldwide HAV Vaccine Strategies

- **Developing Countries**
  - Probably no general use vaccine at this time

- **Transition Countries**
  - Focus vaccine primarily on children - universal

- **Developed Countries**
  - Mixed strategy for universal childhood vaccination, high risk individuals, high incidence areas emphasis on children, community outbreaks (children)

- **Highly Developed - Very low incidence countries**
  - High risk individuals ie. travelers
Children in regions with high rates of hepatitis A (e.g., Alaska Natives, American Indians)

Persons at increased risk for infection

- Travelers to intermediate and high HAV-endemic countries
- Homosexual and bisexual men
- Intravenous drug users
- Persons with chronic liver disease (increased Health risk)
HA Vaccine U.S. - Modified Strategy 1999

- Children in regions with high rates of hepatitis A (e.g., Alaska Natives, American Indians)
- Children in communities, counties, states with consistently high disease rates
- Persons at increased risk for infection
  - Travelers to intermediate and high HAV-endemic countries
  - Homosexual and bisexual men
  - Intravenous drug users
  - Persons with chronic liver disease (increased Health risk)
HA Vaccine Strategy US: 2006 - Present

- Vaccine approved for 12 mo. old children
  - Universal childhood vaccination at 12 mos
  - Continue vaccination of high risk individuals
Universal Childhood Vaccination

- **Benefits**
  - established delivery system
  - vaccination before risk period
  - potential to interrupt transmission

- **Other issues & considerations**
  - immunogenicity in infants – maternal antibody
  - development of combination vaccines
  - duration of protection
  - cost-effectiveness
Hepatitis A Rates in the US: 1952-2007

Vaccine licensed
Hepatitis A Rates by County in the US

1987–1997

2007

* per 100,000 population

Rate*  
0–4  
5–9  
10–19  
≥20

CDC data
Effect of Universal Childhood Vaccine in Argentina
Single dose HA vaccine given at 12 months
~95% Coverage Beginning July 2005

Vacchino, 2008
# Change in HA Incidence by Age Group in Argentina After UI of 12 Month Old Children

<table>
<thead>
<tr>
<th>Age (yrs)</th>
<th>Pre- UI (1998-2002)</th>
<th>2007</th>
<th>% decline</th>
</tr>
</thead>
<tbody>
<tr>
<td>&lt;1</td>
<td>32.2</td>
<td>6.1</td>
<td>81.2</td>
</tr>
<tr>
<td>1</td>
<td>67.9</td>
<td>11.5</td>
<td>83.1</td>
</tr>
<tr>
<td>2-4</td>
<td>201.3</td>
<td>26.1</td>
<td>87.1</td>
</tr>
<tr>
<td>5-9</td>
<td>248.8</td>
<td>28.2</td>
<td>88.7</td>
</tr>
<tr>
<td>10-14</td>
<td>108.6</td>
<td>17.9</td>
<td>83.6</td>
</tr>
<tr>
<td>15-49</td>
<td>20.6</td>
<td>4.4</td>
<td>78.8</td>
</tr>
<tr>
<td>50+</td>
<td>5.9</td>
<td>4.7</td>
<td>20.7</td>
</tr>
<tr>
<td>Overall</td>
<td>88.5</td>
<td>10.2</td>
<td>88.0</td>
</tr>
</tbody>
</table>

Vacchino, 2008
Argentina
Effect of UI on Rate of Severe Hepatitis A

Hospitalized

Fulminant Hepatic Failure

No HA cases had been vaccinated

↓89%

↓100%

Pena et al., 2009
Summary

- HAV and HAV pathogenesis remain areas for study
- Serum antibody alone sufficient for protection
- HAV vaccines were first approved 22 years after the virus was identified
- Understanding of the epidemiology of HA has led to rational vaccine use strategies
- Childhood vaccination programs can have a profound impact on HA rates in the entire population
- HA can be controlled by vaccination and could potentially be eliminated
HAV Pathogenesis:
Disruption of IFN Signaling by HAV

HAV 3ABC Localizes to Mtch surface and cleaves MAVS

Yang et al., PNAS, 2007

HAV 3ABC protease precursor is localized to the mitochondrial surface through the transmembrane domain in 3A. The cysteine protease, 3C\textsubscript{PRO} cleaves the mitochondrial antiviral signaling protein (MAVS) disrupting the interferon signaling pathway. Disruption of the IFN pathway may result in

a) the prolonged incubation period observed in HAV infections and

b) in the ability of the virus to establish persistent infections \textit{in vitro}

From Seth et al, Cell Research, 2006
Overall structure.
Strategies for Use of HAV Vaccine

- Developing Countries
- Transition
- Developed World
HA Incidence in U.S. by Race and Ethnicity

Vaccine introduced
Live attenuated HAV vaccine - Questions

- Are there extrahepatic site of replication?
- What is the mechanism of hepatic injury; viral or immune mediated?
- Can limited replication produce an adequate immune response?
- Relevance of animal models to human attenuation?
- What degree of hepatic injury would be acceptable?
## Clinical Manifestations of 8647 Hospitalized Patients 1988 Shanghai Epidemic (primarily 18-40 yo’s)

<table>
<thead>
<tr>
<th>Symptom</th>
<th>%</th>
<th>Clinical Findings</th>
<th>%</th>
<th>Complications</th>
<th>%</th>
</tr>
</thead>
<tbody>
<tr>
<td>Jaundice</td>
<td>84</td>
<td>Hepatomegaly</td>
<td>87</td>
<td>Cholestasis</td>
<td>1.6-5.3</td>
</tr>
<tr>
<td>Weight loss</td>
<td>82</td>
<td>Splenomegaly</td>
<td>9</td>
<td>Upper gastrointestinal bleeding</td>
<td>0.5-1.2</td>
</tr>
<tr>
<td>Malaise</td>
<td>80</td>
<td>Skin rashes</td>
<td>3</td>
<td>Thrombocytopenic purpura</td>
<td>&lt;0.1 (6 cases)</td>
</tr>
<tr>
<td>Fever</td>
<td>76</td>
<td>Mild edema</td>
<td>2</td>
<td>Guillain-Barr? syndrome</td>
<td>&lt;0.1 (4 cases)</td>
</tr>
<tr>
<td>Nausea</td>
<td>69</td>
<td>Petechia</td>
<td>2</td>
<td>Pure red cell aplasia</td>
<td>&lt;0.1 (3 cases)</td>
</tr>
<tr>
<td>Vomiting</td>
<td>47</td>
<td>Cardiac arrhythmias</td>
<td>0.8</td>
<td>Autoimmune hemolytic anemia</td>
<td>&lt;0.1 (2 cases)</td>
</tr>
<tr>
<td>Abdominal pain</td>
<td>37</td>
<td></td>
<td></td>
<td>Transverse myelitis, optic neuritis</td>
<td>&lt;0.1 (1 case each)</td>
</tr>
<tr>
<td>Arthralgia</td>
<td>6</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Yao G, 1991
Mean HA Vaccine Coverage in Children 12-24 Months of Age

Information Systems Sentinel Sites, 2006-2009. MMWR, 2010
eHAV is Resistant to Neutralization by Anti-capsid mAbs and Polyclonal Post-convalescent Serum

Infra-red Immunofluorescence Focus Reduction Assay

VP1pX is protected in eHAV particles but rapidly degrades to VP1 upon detergent treatment.
HAV Pathogenesis: Disruption of IFN Signaling by HAV

HAV 3ABC protease precursor is localized to the mitochondrial surface through the transmembrane domain in 3A. The cysteine protease, 3C\textsuperscript{PRO} cleaves the mitochondrial antiviral signaling protein (disrupting the interferon MAVS) signaling pathway. Disruption of the IFN pathway may result in

a) the prolonged incubation period observed in HAV infections and

b) in the ability of the virus to establish persistent infections \textit{in vitro}

From Moore and Ting, 2008

Yang et al., PNAS, 2007