Highlights in the Discovery of Hepatitis A Virus

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Global Hepatitis A Meeting
November 29-December 1, 2007
Miami Beach, Florida
Known Characteristics of the Two Forms of Viral Hepatitis - 1947

<table>
<thead>
<tr>
<th>Characteristic</th>
<th>Hepatitis A</th>
<th>Hepatitis B</th>
</tr>
</thead>
<tbody>
<tr>
<td>Incubation Period</td>
<td>15-40 days</td>
<td>60-160 days</td>
</tr>
<tr>
<td>Mode of infection</td>
<td>Fecal/oral</td>
<td>Parenteral</td>
</tr>
<tr>
<td>Immunity</td>
<td>Homologous</td>
<td>Homologous</td>
</tr>
<tr>
<td>Value of IG prophylaxis</td>
<td>Good</td>
<td>None</td>
</tr>
<tr>
<td>Heat resistance</td>
<td>56°C X 30 min</td>
<td>56°C X 60 min</td>
</tr>
<tr>
<td>Ether resistance</td>
<td>10% X 2hr @40°C</td>
<td>Triple extraction @ -20°C</td>
</tr>
</tbody>
</table>
The Search for the Hepatitis A Virus

- Human transmission → Multiple Studies
- Cell culture → Detroit 6 cells
- Marmoset Inoculation → GB Virus/Berlin Agent
- Gel diffusion → Milan Antigen/Fecal Ag
- IEM → Fecal antigen
- IEM → HAV
Human Transmission Studies

- MacCallum et al. – 1944: Transmission to volunteers,
- Neefe and Stokes et al. – 1945: Separation of A and B, homologous immunity, stool infectivity, protection by gamma globulin, disinfection of infectious hepatitis
- Havens – 1945: Fecal excretion, period of infectivity, homologous immunity, viral characterization
- Krugman et al. – 1958: Natural history of viral hepatitis and separation of two forms of hepatitis, development of standardized reagents, MS-1 & MS-2
Saul Krugman
Detroit 6 Cells

- First studied by Rightsel and McLean at Parke-Davis who observed CPE in cultures inoculated with plasma from icteric patients. 1956-1966

- Lack of specificity of the system was demonstrated by a series of experiments in Australia by Cole, Cross and Marmion et al. 1965-1966

- Melnick, Boggs et al. Using MS-1 material identified the “Kirk hepatitis virus” in D-6 cell. They ultimately showed this to be a parvovirus cell culture contaminant. 1971
Animal Models

• Deinhardt-1967, began a series of marmoset (Saguinus sp) inoculations using sera from icteric patients.

• GB, a 34 yo surgeon developed hepatitis and his serum on the 3rd day of jaundice transmitted hepatitis to 4/4 marmosets and was passaged several times

• Parks and Melnick later showed that this was likely an indigenous marmoset agent.

• A similar virus was identified in Germany termed the Berlin Agent

• Later - 1969 Deinhardt used MS-1 serum and showed true transmission to marmosets.
Fritz Deinhardt & Friends
Gel Diffusion Immunoprecipitation

- MILAN ANTIGEN – Salvatore Del Prete et al. 1970
- Identified an antigen in the serum from patients with short incubation hepatitis using serum from multiply-transfused patient - reacted with both AuAg+ serum and AuAg- sera
- Shown by Taylor et al. to be abnormal serum lipoprotein
Gel Diffusion Immunoprecipitation

- **Fecal Antigen** – Ferris, et al. 1970
- Originally described by gel immuno-precipitation using hemophiliac sera. Then fecal antigen was purified and used to raise antibody in rabbits.
- Ultimately, particles were observed by IEM using rabbit antiserum
IEM for HAV

1. Extract stools
2. Low speed clarification
3. Incubate stool filtrate with antibody
4. High speed centrifugation
   - Resuspend pellet and stain

Filtration

IEM for HAV
Norwalk Virus IEM

Kapikian et al., J. Virol. (1972) 10:1075
IEM Approach to the Search for HAV in Purcell’s Lab

• We knew that we should look in stools, not serum
• We used stool samples and sera from well characterized outbreaks and volunteer studies
• Stool filtrates were screened with ISG as the antibody for candidate virus-like particles
• All particles that were identified by ISG were then evaluated for HAV specificity by testing with paired HAV sera under code
Virus-like Particles in Stool from Patient in Hepatitis Outbreak on Palau Island, Micronesia
MS-1 HAV Aggregated by Immune Globulin
HAV by IEM

A. MS-1 convalescent

B. Holy Cross Convalescent

C. H. C. Convalescent

D. ISG

E. ISG

F. ISG
Anti-HAV by IEM

0+

1+

2+

3-4+
## Serologic Proof of HA-Ag

<table>
<thead>
<tr>
<th>Patient No.</th>
<th>Hepatitis A antigen</th>
<th>Hepatitis B antigen</th>
<th>Norwalk gastroenteritis antigen</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>First</td>
<td>Second</td>
<td>First</td>
</tr>
<tr>
<td>1</td>
<td>0</td>
<td>1-2</td>
<td>-</td>
</tr>
<tr>
<td>2</td>
<td>0</td>
<td>1-2</td>
<td>+</td>
</tr>
<tr>
<td>3</td>
<td>0</td>
<td>3-4</td>
<td>-</td>
</tr>
<tr>
<td>4</td>
<td>0</td>
<td>3-4</td>
<td>-</td>
</tr>
<tr>
<td>5</td>
<td>0</td>
<td>1-2</td>
<td>-</td>
</tr>
<tr>
<td>6</td>
<td>0</td>
<td>3-4</td>
<td>-</td>
</tr>
<tr>
<td>7</td>
<td>0-1 (acute)</td>
<td>3-4</td>
<td>-</td>
</tr>
<tr>
<td>8</td>
<td>0 (acute)</td>
<td>3</td>
<td>-</td>
</tr>
<tr>
<td>9</td>
<td>0 (acute)</td>
<td>3</td>
<td>-</td>
</tr>
<tr>
<td>10</td>
<td>0</td>
<td>2</td>
<td>-</td>
</tr>
<tr>
<td>11</td>
<td>0</td>
<td>3</td>
<td>-</td>
</tr>
<tr>
<td>12</td>
<td>1-2 (acute)</td>
<td>3-4</td>
<td>-</td>
</tr>
<tr>
<td>13</td>
<td>0</td>
<td>0</td>
<td>-</td>
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<tr>
<td>14</td>
<td>0</td>
<td>0</td>
<td>-</td>
</tr>
<tr>
<td>15</td>
<td>0</td>
<td>0</td>
<td>-</td>
</tr>
<tr>
<td>16</td>
<td>2-3</td>
<td>2-3</td>
<td>-</td>
</tr>
</tbody>
</table>
## Serologic comparison of HA-Ag and Fecal Ag by IEM

<table>
<thead>
<tr>
<th>ANTISERUM</th>
<th>Antibody Score When Incubated With</th>
<th>HA Ag</th>
<th>Fecal Ag</th>
</tr>
</thead>
<tbody>
<tr>
<td>Human Convalescent (MS-1)</td>
<td></td>
<td>3+</td>
<td>0</td>
</tr>
<tr>
<td>Rabbit Anti-Fecal Ag</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>F1</td>
<td></td>
<td>0-1+</td>
<td>3+</td>
</tr>
<tr>
<td>125</td>
<td></td>
<td>2-3+</td>
<td>3+</td>
</tr>
<tr>
<td>521</td>
<td></td>
<td>0</td>
<td>3+</td>
</tr>
</tbody>
</table>
Characterization of HAV
Density of HAV by IEM
Characterization of HAV
Purified HAV
Characterization of HAV
HAV Proteins – Western Blot
HAV Infection of Chimpanzee

- **Chimp 753**
- **Liver Biopsy**
- **Anti-HA**
- **IEM**
- **ALT (IU/L)**
- **IgA HA**
- **SPRIA P/N**

**Number of HA Ag Particles Visualized in 5 Squares of a 400 Mesh Grid**

**Time Before and After Inoculation**

**DAYS**

**MONTHS**
Cryoelectron Microscopy of HAV

Holland Cheng in Martin and Lemon, 2006