Human Viral Pathogens
Bioscience in the 21st Century
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What we are going to discuss

- Viruses as human pathogens
- What defines a virus
- Influenza
- Smallpox
- Polio
- Ebola
- Zika
Why study viruses?

- Some make people sick
- There are a whole lot of them and we don’t know most of them
Sir F. Macfarlane Burnet
Former Director of the Walter and Eliza Hall Institute
Sir F. Macfarlane Burnet was an Australian virologist/immunologist who shared the 1960 Nobel prize for Medicine and Physiology.

He made several seminal discoveries in the 1930s including that there were multiple distinct strains of polio virus not just one.
Burnet wrote in the introduction to the third edition of his book *Natural History of Infectious Disease* published in 1962:

“...the late 20th century would be witness to the virtual elimination of infectious disease as a significant factor in social life”.

To write about infectious disease... "is almost to write of something that has passed into history."
How could he have been so wrong?

- Think of when he wrote *i.e.*, 1962
- What was happening in medicine in the mid 1960s?
  - Fleming discovers penicillin 1929 and widespread use occurred in the second world war
  - Polio vaccine licensed 1955
  - Measles vaccine licensed 1963
  - Mumps vaccine licensed 1967
  - Rubella vaccine licensed in 1969
- Evidently antibiotic could kill all bacteria and vaccines could prevent all viral disease
There are a lot more viruses than many people thought

- Most estimates suggest there are around $10^{31}$ virus particles in the world
- A significant number of those exist in the ocean
- Est. $10^6$-$10^8$ particles per ml in the oceans
- There are a lot of milliliters in the ocean and $10^{31}$ is a BIG number
When Were Viruses Discovered?

- In 1879 Adolph Mayer transmitted a disease of tobacco plants with an extract from diseased leaves.
- In 1892 Dimitri Ivanovksy transmitted the tobacco disease with a filtrate i.e., a “filterable agent”
- Ivanovksy wasn’t convinced he was on to anything and speculated the disease was caused by a toxin, not a new form of pathogen.
- A Dutch scientist diluted the filtrate and showed the agent recovered full infectivity which a toxin could not do.
Also in 1898 Freidrich Loeffler and Paul Frosch demonstrated foot and mouth disease was transmitted by a filterable agent – the first animal virus.

In 1900 Walter Reed showed yellow fever was transmitted by a filterable agent – the first human virus.

In 1906 Negri showed smallpox was caused by a filterable agent.

In 1908 Ellerman and Bang transmitted leukemia in chickens with a filtrate of the serum – the first retrovirus.
What is a Virus?

- From the Latin
- slimy liquid, poison, stench
Peter Medawar, who shared the 1960 Nobel Prize with Burnet defined viruses as:

“...a piece if nucleic acid surrounded by bad news.”
What makes viruses unique?

- The mechanism of viral replication separates them from all other replicating elements.
- Other microorganisms replicate via binary fission.
- Viruses essentially kill themselves during replication.
- They go through an “Eclipse” phase.
Unique aspect of viral replication

ONE STEP GROWTH CYCLE

**YIELD (BURST SIZE)**

**HOURS AFTER ADSORPTION**

**VIRIONS PER CELL**

- 1000
- 100
- 10
- 1
- 0.1

- 1
- 7
- 10
- 15
- 20
Bacterial and Viral genomes
Influenza virus
Death rates in the US 1900-2000

- 40 States Have Health Departments
- Influenza Pandemic
- Last Human to Human Transmission of Plague
- First Continuous Municipal Use of Chlorine in Water in the United States
- First Use of Penicillin
- Salk Vaccine Introduced
- Start of the AIDS Epidemic
The 1918 Flu killed more Americans than died in:

- World War I
- World War II
- The Korean War
- The Vietnam War
- The Iraq/Afghanistan conflicts
Influenza typically kills ca. 20,000 people per year in the US

- Last year ca. 110,000,000 individuals in the US were vaccinated
- December 4-10 is National Influenza Vaccination Week
- The goal is for a higher rate than last year (40%)
- So far the 2016/7 flu season looks good
Influenza Positive Tests Reported to CDC by U.S. Clinical Laboratories, National Summary, 2016-2017 Season
Pneumonia and Influenza Mortality from the National Center for Health Statistics Mortality Surveillance System

Data through the week ending November 12, 2016, as of November 30, 2016
FluView
A Weekly Influenza Surveillance Report Prepared by the Influenza Division

Number of Influenza-Associated Pediatric Deaths by Week of Death: 2013-2014 season to present

- 2013-2014
  Number of Deaths Reported = 111

- 2014-2015
  Number of Deaths Reported = 148

- 2015-2016
  Number of Deaths Reported = 87

- 2016-2017
  Number of Deaths Reported = 0

Week of Death

- Deaths Reported Previous Week
- Deaths Reported Current Week
Who was Benjamin Waterhouse and What did he have to do with Virology? Or the College Rhode Island? Or Harvard?

Gilbert Stuart, ca., 1776
Early in 1799 Dr. John Lettsom, a prominent physician in London, sent his friend Waterhouse a copy of Jenner’s 1798 manuscript *An inquiry into the Causes and Effects of the Variollae Vaccinae*

Waterhouse corresponded with Jenner and received some of the vaccine by 1800.

He vaccinated his five year old son, Daniel.

Waterhouse vaccinated all his children and several servants in 1800.

He tested his experiment by sending his subjects to the Smallpox Hospital in Brookline to be exposed.

They returned with nothing but a sore arms.

Waterhouse believed smallpox was vanquished.

He was wrong.
What Happened Next?

- Smallpox killed an estimated 300-500 million people in the 20th century
- 1967 the World Health Organization begins a smallpox eradication program
- 1977 the last natural case of smallpox, Ali Maow Maalin occurs in Somalia
- In 1980, 182 years after Jenner inoculates James Phipps, the WHO announces the end of smallpox
- We should have done better
Polio virus has likely existed for centuries

Egyptian stone tablet from the 18th dynasty (1580-1350 B.C.)
Polio virus
Epidemic polio is a disease of the 20th century.

Why?
The first polio epidemic occurred in Vermont in 1894.

There were 132 cases of paralytic disease.

Clean water _ca._ 1900.

No longer drinking your neighbor’s feces.

Therefore first exposure to polio is later in life.

Risk of paralytic polio increases >10 fold in older people.

Newborns are like recipients of the Salk vaccine for 12-14 months because of maternal IgG.
Unfortunately this is a hypothesis that has been experimentally confirmed.

In the 1960s, as we helped the "developed world" by cleaning water supplies.
Key events in Polio

- In 1908 Landsteiner shows infantile paralysis caused by a “filterable agent” and produces disease in monkeys with human material.
- 1931 Burnett showed there were multiple forms of poliovirus which did not “cross react”.
- 1949 John Enders cultivates poliovirus in non-neuronal cells in tissue culture.
- 1954 Enders and colleagues received the Nobel Prize in Medicine.
- 1954 Salk inactivated polio vaccine.
- 1960 Sabin live attenuated vaccine replaced the Salk vaccine.
The Sabin vaccination worked but.....

Vaccine-associated paralytic polio
Attenuation of the Sabin strains

A Derivation of Sabin type 3 attenuated poliovirus

Type 3 P3/Leon/37 (isolate from fatal paralytic case)

21 passages in vivo (intracerebrally in monkeys)
8 passages in vitro (monkey testicle cultures)
39 passages in vitro (monkey kidney cultures)
3 plaque purifications (monkey kidney cultures)
3 passages in vitro (preparative, monkey kidney cultures)

P3/Leon 12a,b KP3/56 Sabin vaccine strain

B Determinants of attenuation in the Sabin vaccine strains

<table>
<thead>
<tr>
<th>Virus</th>
<th>Mutation (location/nucleotide position)</th>
</tr>
</thead>
<tbody>
<tr>
<td>P1/Sabin</td>
<td>5'-UTR (480)</td>
</tr>
<tr>
<td></td>
<td>VP4 (935)</td>
</tr>
<tr>
<td></td>
<td>VP3 (2438)</td>
</tr>
<tr>
<td></td>
<td>VP1 (2795)</td>
</tr>
<tr>
<td></td>
<td>VP1 (2079)</td>
</tr>
<tr>
<td>P2/Sabin</td>
<td>5'-UTR (481)</td>
</tr>
<tr>
<td></td>
<td>VP1 (2908)</td>
</tr>
<tr>
<td>P3/Sabin</td>
<td>5'-UTR (472)</td>
</tr>
<tr>
<td></td>
<td>VP3 (2034)</td>
</tr>
</tbody>
</table>

C Reversion of P3/Sabin

<table>
<thead>
<tr>
<th>Amino acid change</th>
<th>Nucleotide change</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ser</td>
<td>GAGA&lt;sub&gt;α&lt;/sub&gt;</td>
</tr>
<tr>
<td>Lys Thr</td>
<td></td>
</tr>
</tbody>
</table>

P3/Leon/37 (Parent) GCG TGC CAG
P3/Leon 12a,b (Vaccine) CGA AGA
P3/1119 (Revertant)  CAA CAA
A couple of thoughts before leaving polio

- The first *ca.* 140 million doses of the Salk vaccine were contaminated with SV40, a DNA tumor virus.
- Hilary denies it!
Tim Flanigan in Liberia
former Brown Chief of Infectious Disease

http://www.timothypflaniganmd.com/
Prior Filovirus outbreaks

- **Marburg**
  - Year 1975: 3 (33%) cases in Zimbabwe
  - Year 1980: 2 (50%) cases in Kenya
  - Year 1987: 1 (100%) case in Zaire
  - Year 1998/00: 149 (83%) cases in Zaire
  - Year 2005: 252 (90%) cases in Angola

- **Ebola**
  - Year 1976: 284 (53%) cases in Sudan
  - Year 1976: 318 (88%) cases in Zaire
  - Year 1977: 1 (100%) case in Zaire
  - Year 1979: 34 (65%) cases in Sudan
  - Year 1994: 44 (64%) cases in Gabon
  - Year 1994: 1 (0%) case in Ivory Coast
  - Year 1995: 315 (77%) cases in Zaire (Kikwit)
  - Year 1995/96: 37 (57%) cases in Gabon
  - Year 1996/97: 60 (75%) cases in Gabon
  - Year 2000: 425 (53%) cases in Uganda
  - Year 2002: 122 (81%) cases in Gabon/Congo
  - Year 2003/04: 178 (89%) cases in Zaire
  - Year 2004: 17 (41%) cases in Sudan
  - Year 2005: 12 (75%) cases in Zaire

*Now called Democratic Republic of Congo*
What about 2014-6

- Guinea, Liberia, Sierra Leone
- 26,646 cases
- 11,323 deaths (as of 3/30/16) far more than all the prior outbreaks together

Why?

- The virus got into large cities rather than remote villages
- Recent data suggests a mutation in the envelope glycoprotein facilitated interspecies transfer
Reston made the news in 1989!

- On October 2, 1989, 100 cynomolgus macaques (*Macaca fascicularis*) from Ferlite Farms in Mindanao Island, Philippines were flown from Manila, through Amsterdam to New York, and then transported by truck to Hazleton Research Products' (HRP) Reston Primate Quarantine Unit in Reston.
- Hazelton is a major supplier of primates for biomedical research.
- There had not been any African species quarantined in the Reston unit for many years.
- Several of the monkeys died.
- Serology indicated Ebola.
The company euthanized all the monkeys
Six workers seroconverted
They but presented no symptoms
This variant is highly pathogenic for nonhuman primates but not humans
Ebola has killed thousands of gorillas and there was consideration several years ago about vaccinating gorillas to prevent extinction
What happened in Philadelphia in the summer and early fall of 1793?

- Philadelphia was at the time the US capitol
- Ca. 15% of the population died
- The government was essentially evacuated
- New Jersey and Maryland banned residents of Philadelphia from entering
- By the end of October the epidemic had ended
October 23: temperature falls to 50°F (mosquitoes perish)

Infectious disease arrives from Santo Domingo

Cases ~10,000
Deaths ~5,000

What about Memphis in 1878

- In late July Yellow fever was killing people in Vicksburg and New Orleans
- On August 1 a New Orleans steamboat worker became ill in Memphis
- He died in a quarantine hospital on August 5
- On August 13 the owner of the inn where he had stayed died and the epidemic was on
- In August the local paper advised “Avoid patent medicines and bad whisky”
Over half the population fled the city

Of the 20,000 remaining 14,000 were black and 6,000 were Caucasian

70% of the Caucasian died by the first frost

10% of the blacks died

Why the difference?

This fact was well known and was in part responsible for the slave trade
So what did Walter Reed do?
Reed was:

- A major in the US Army
- The youngest graduate of the University of Virginia medical program at age 17 in 1869
- Reed directed a military Typhoid Board in 1898 demonstrating the importance of sanitation
- Reed observed that more soldiers died of yellow fever in the Spanish American war than from combat
- In 1900 he was appointed to chair a board to study infectious diseases in Cuba
Reed cont.

- Reed was sent to Cuba in 1901
- He was able to disprove a theory for a bacterial cause of yellow fever
- In 1901 Reed showed yellow fever was caused by a “filterable” agent
- Reed then turned to Findlay’s mosquito hypothesis
- He was assisted by three physicians: Dr. James Carroll a bacteriologist, Dr. Jesse W. Lazear an expert on mosquitoes and Dr. Aristedes Agramonte a pathologist
- Carroll allowed himself to be bitten by a mosquito and developed yellow fever but there were not the correct controls
Lazear then “allowed” private William Dean to be bitten by a mosquito under more controlled conditions.

- He developed yellow fever.
- Both Dean and Carroll recovered.
- Lazar then allowed himself to be bitten.
- He developed yellow fever and after several days died.
- Following his death Reed established Camp Lazear where individuals were exposed to mosquito under very controlled conditions.
- These studies ultimately proved Findlay correct about the role of the mosquito in yellow fever.
- What was not appreciated by Findley or Reed initially was the intrinsic replication cycle in the mosquito of 10-20 days before it could transmit the infection.
Zoonoses

- From the Greek *zoon* animal and *nosos* disease
- Diseases *transmitted* between vertebrate animals and humans
- Unless you can eliminate the virus in the reservoir it is impossible to eradicate these diseases
Yellow fever virus has a vaccine

- In 1937 a relatively safe live attenuated vaccine for yellow fever was developed.
- While over 500 million doses of the vaccine have been distributed, yellow fever still infects 200,000 people per year, resulting in 30,000 deaths.
About to bite
Biting
Virus replicate in vectors

1. Infectious blood meal ingested.
2. Virus infects and multiplies in mesenteronal epithelial cells.
3. Virus released (escapes) from mesenteronal epithelial cells.
4. a. Virus infects salivary glands after secondary amplification in other cells/tissues.
   b. Virus infects salivary glands without secondary amplification in other cells/tissues.
5. Virus released from salivary gland epithelial cells and is transmitted by feeding.
### Flaviviruses

<table>
<thead>
<tr>
<th>Virus</th>
<th>Vector</th>
<th>Disease</th>
</tr>
</thead>
<tbody>
<tr>
<td>Dengue virus</td>
<td>Aedes mosquitoes</td>
<td>Mild systemic; breakbone fever, dengue hemorrhagic fever, dengue shock syndrome</td>
</tr>
<tr>
<td>Yellow fever virus</td>
<td>Aedes mosquitoes</td>
<td>Hepatitis, hemorrhagic fever</td>
</tr>
<tr>
<td>Japanese encephalitis virus</td>
<td>Culex mosquitoes</td>
<td>Encephalitis</td>
</tr>
<tr>
<td>West Nile virus</td>
<td>Culex mosquitoes</td>
<td>Fever, encephalitis, hepatitis</td>
</tr>
<tr>
<td>St. Louis encephalitis virus</td>
<td>Culex mosquitoes</td>
<td>Encephalitis</td>
</tr>
<tr>
<td>Russian spring-summer</td>
<td>Ixodes, Dermacentor ticks</td>
<td>Encephalitis</td>
</tr>
<tr>
<td>encephalitis virus</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Powassan virus</td>
<td>Ixodes ticks</td>
<td>Encephalitis</td>
</tr>
<tr>
<td>Hepatitis C virus</td>
<td>None</td>
<td>Hepatitis (see “Hepatitis viruses,” next page)</td>
</tr>
</tbody>
</table>

### Disease mechanisms

- Viruses are cytolitic
- Viruses cause viremia, systemic infection
- Nonneutralizing antibodies can facilitate infection of monocytes/macrophages via Fc receptors

### Diagram

- Mucosal surfaces
  - Lymph node
    - Primary viremia
      - Blood
        - Vascular endothelium
        - Macrophage
        - Liver
        - Spleen
        - Lymph nodes
      - Secondary viremia
        - Encephalitis
        - Yellow fever hepatitis
        - Hemorrhagic fever
What about Zika?

- First described in monkeys in the Zika Forest in Uganda in 1947 and later detected in *Aedes africanus*.
- First human cases (three) were reported in Nigeria in 1954.
- Zika is related to Dengue and West Nile virus.
- In 2007, an outbreak of Zika occurred on Yap Island in Micronesia. This was the first report outside Africa or Asia.
- An estimated 5005 of the 6892 Yap residents over the age of 3 were infected in this epidemic.
- There were no deaths and the illness was mild but the investigators conclude “Public health officials should be aware of the further expansion of Zika virus transmission.”

*Duffy et al., N. Engl J. Med* 2009;360:2536-43
Zika was first reported in Brazil in 2015
By early 2016 it is estimated that 500,000-1,3000,000 people in Brazil have been infected
During the epidemic there was a 40 fold increase in the incidence of children born with microcephaly
In August over 1000 pregnant women in Puerto Rico were reported to be infected with Zika
A report in MMWR published December 2 of this year examined 13 children born to infected mothers in Brazil and who appeared normal at birth
By five months all children had brain abnormalities on neuroimaging and 11 had microcephaly
What we need to know with Zika

- Does it have a mammalian reservoir?
- How long does the viremic phase last in humans?
- How long does Zika persist in “immunologically privileged” sites? It is known that Ebola can persist in such sites for a year.
- Does the current outbreak represent a new strain of the virus?