We have met the enemy and he is us.
—WALT KELLY
Animal models: Mice lie, monkeys exaggerate

- Human viruses in animals
- Animal viruses that resemble human infection
Viral virulence

- Capacity of a virus to cause disease in a host
- Virulent vs avirulent or attenuated virus
- Virulence can be quantitated:
  - Mean time to death
  - Mean time to appearance of symptoms
  - Measurement of fever, weight loss
  - Measurement of pathological lesions (poliovirus); reduction in blood CD4+ lymphocytes (HIV-1)
Measuring viral virulence

**Graph 1:**
- **Y-axis:** Number of survivors
- **X-axis:** Day postinfection
- **Legend:**
  - Type 1
  - Type 2

**Graph 2:**
- **Y-axis:** Relative neurovirulence score
- **X-axis:** Region of the CNS
- **Legend:**
  - Japanese encephalitis virus
  - Yellow fever virus 17D strain
  - West Nile virus
  - Langat virus
  - Dengue virus 4
Viral virulence is a relative property

- Influenced by dose, route of infection, species, age, gender, and susceptibility of host
- Cannot compare virulence of different viruses
- For similar viruses, assays must be the same
Virulence depends on route of inoculation

*Lymphocytic choriomeningitis virus*

<table>
<thead>
<tr>
<th>Dose</th>
<th>Route</th>
<th>Outcome</th>
</tr>
</thead>
<tbody>
<tr>
<td>100,000 PFU</td>
<td>Intraoperitoneal</td>
<td>Survival</td>
</tr>
<tr>
<td>1 PFU</td>
<td>Intracranial</td>
<td>Death</td>
</tr>
</tbody>
</table>
Which statement about viral virulence is wrong?

A. It can be influenced by dose, route of infection, species, age, gender, and susceptibility of host
B. It can be quantitated by measurement of fever
C. Ebola virus is more virulent than human papillomavirus
D. It is the capacity of a virus to cause disease in a host
E. When comparing virulence, the assays must be the same
Viral virulence

- Major goal of virology is to identify viral and host genes that determine virulence
- Virulence genes usually identified by mutation: a virus that causes reduced or no disease in a specified system
# Identifying virulence genes

<table>
<thead>
<tr>
<th>Virus</th>
<th>Growth in cell culture</th>
<th>Effect on mice</th>
<th>Virulence phenotype</th>
</tr>
</thead>
<tbody>
<tr>
<td>Wild type</td>
<td>![Cell culture image]</td>
<td>Reproduction</td>
<td>Neurovirulent</td>
</tr>
<tr>
<td>Mutation leading to a general defect in reproduction</td>
<td>![Cell culture image]</td>
<td>Poor reproduction</td>
<td>Attenuated</td>
</tr>
<tr>
<td>Mutation in a gene specifically required for virulence</td>
<td>![Cell culture image]</td>
<td>Poor reproduction</td>
<td>Attenuated</td>
</tr>
</tbody>
</table>
Viral virulence genes

- Viral replication
- Invasiveness
- Tropism
- Modify the host defense mechanisms
- Enable the virus to spread in the host
- Intrinsic cell killing effects
Virulence determinants may not encode proteins

Sabin vaccine strains of poliovirus contain a mutation in the 5’-noncoding region that reduces neurovirulence
Poliovirus replication in mouse brain

<table>
<thead>
<tr>
<th>Virus</th>
<th>Base at 472</th>
<th>LD$_{50}$</th>
</tr>
</thead>
<tbody>
<tr>
<td>PRV7.3</td>
<td>U</td>
<td>$&gt;2 \times 10^7$</td>
</tr>
<tr>
<td>PRV8.4</td>
<td>C</td>
<td>$9 \times 10^3$</td>
</tr>
</tbody>
</table>

**Graph:**
- X-axis: Day postinfection
- Y-axis: log$_{10}$ PFU/g
- Red line: PRV7.3
- Blue line: PRV8.4
- Points labeled: C, U
Gene products that modify host defense

- Immune modulators
  - Apoptosis, autophagy, intrinsic proteins (Apobec3G)
  - Virokines and viroceptors
  - Complement binding proteins
  - Modifiers of MHC I, II pathways
- Often not required for growth in cell culture
Viral virulence genes

Gammarpesivirus 68 M3 gene encodes a chemokine receptor
Toxic viral proteins

NSP4 nonstructural glycoprotein of rotaviruses: viral enterotoxin
miRNAs

- miR-122, liver-specific miRNA required for HCV replication
- Anti-miR-122 reduces HCV RNA in humans
Mechanisms of cell injury by viruses

- Cytolytic viruses: cytopathic effects (apoptosis, necrosis, pyroptosis)
- Viroporins
- Viral inhibition of host protein and RNA synthesis, leads to loss of membrane integrity, leakage of enzymes from lysosomes, cytoplasmic degradation
- Syncytium formation by enveloped viruses (parainfluenza, HIV)
Role of the microbiome in viral replication

Poliovirus infection of TgPvr mice
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Which statement about determinants of viral virulence is incorrect:

A. Virulence genes can encode viral proteins
B. Virulence genes can encode cellular proteins
C. They are the same in all viruses
D. They can be found in untranslated regions
E. They may encode immune modulators
Immunopathology: Too much of a good thing

- Clinical symptoms of viral disease (fever, tissue damage, aches, pains, nausea) are a consequence of host response to infection
- Non-cytopathic viruses: disease is usually a consequence of the immune response
## Immunopathology

<table>
<thead>
<tr>
<th>Mechanism</th>
<th>Virus</th>
</tr>
</thead>
<tbody>
<tr>
<td>CD8$^+$ T cell mediated</td>
<td>Coxsackievirus B</td>
</tr>
<tr>
<td></td>
<td>HIV-1</td>
</tr>
<tr>
<td></td>
<td>Hepatitis B virus</td>
</tr>
<tr>
<td>CD4$^+$ T cell mediated - Th1</td>
<td>Measles virus</td>
</tr>
<tr>
<td></td>
<td>Herpes simplex virus</td>
</tr>
<tr>
<td>CD4$^+$ T cell mediated - Th2</td>
<td>Respiratory syncytial virus</td>
</tr>
<tr>
<td>B cell mediated (antibody)</td>
<td>Dengue virus</td>
</tr>
</tbody>
</table>
Viral disease mediated by CD8$^+$ CTLs
Lesions associated with CD8 cells

**Graphs**

- **LCMV**
  - Y-axis: Percent alive
  - X-axis: Days after infection
  - Two lines: Perforin $+/+$ and Perforin $^-/-$

- **Liver enzyme**
  - Y-axis: Serum GGT (U per ml)
  - X-axis: Days after infection
  - Two lines: Perforin $+/+$ and Perforin $^-/-$
Lesions associated with CD4+ T cells

- Elaborate more cytokines than CD8+ T cells, and recruit and activate many nonspecific effector cells
- Most recruited cells are neutrophils and mononuclear cells, which are protective but cause tissue damage
- Immunopathology caused by release of proteases, reactive radicals, and cytokines (e.g. Tnf-α)
Lesions associated with CD4+ T cells

- Herpes stromal keratitis, one of the most common causes of blindness in developed countries; almost entirely immunopathological (CD4+ Th1 cells)
- Repeated infections cause opacity and reduced vision
Lesions associated with CD4+ T cells

- Virus replicates in corneal epithelium, but CD4+ Th1 inflammation restricted to underlying uninfected stromal cells
- Stromal cells damaged by secreted cytokines produced by infected cells in corneal epithelium
TLR3 and West Nile virus encephalitis

- Tlr3\(^{-/-}\) mice more resistant to WNV lethal infection, have impaired cytokine production
- TNF-\(\alpha\) compromises blood-brain barrier
Poxes and rashes

- Many virus infections produce characteristic rash (measles, smallpox, varicella zoster)
- Th1 cells and macrophages activated by original infection home in on infected foci in skin
- These cells produce cytokines such as IL-2 and IFN-γ
- Cytokines act locally to increase capillary permeability, influx of T cells
Dengue fever (breakbone fever)

- Dengue virus, transmitted mainly by *Aedes aegypti*
- Endemic in the Caribbean, Central and South America, Africa and Southeast Asia - billions at risk
- 400 million infections/year
- Second only to malaria among insect-borne diseases
American Countries with laboratory confirmed dengue hemorrhagic fever, prior to 1981 and from 1981 to 2003

Prior to 1981

1981 - 2003

Source: WHO/PAHO/CDC, Aug. 2004
Dengue fever

- Primary infection asymptomatic or acute febrile illness with severe headache, back and limb pain and rash. Severe aches and pains in the bones.
  - Normally self-limiting, patients recover in 7-10 days
  - In 1/14,000 primary infections: dengue hemorrhagic fever, life threatening disease
  - Internal bleeding leads to fatal dengue shock syndrome
- Antibodies to virus made; four serotypes, no cross-protection
Dengue fever

After secondary dengue infections, incidence of hemorrhagic fever and shock syndrome 1/90 and 1/50
Immunosuppression

- Global reduction of the immune response caused by virus infection
- Mechanisms
  - Replication in one or more cells of immune system
  - Perturbation of cytokine homeostasis and intracellular signaling
  - Viral proteins acting as viroceptors or virokines (immune modulators)
Immunosuppression during measles infection
Measles virus immunosuppression

"T_h1 response" Favors killing infected cells

"T_h2 response" Favors antibody production
## Examples of immunosuppression

<table>
<thead>
<tr>
<th>Virus</th>
<th>Disease</th>
<th>Cells infected</th>
<th>Manifestation</th>
</tr>
</thead>
<tbody>
<tr>
<td>Measles</td>
<td>Measles</td>
<td>Monocytes, DC Thymic epithelial cells</td>
<td>Reduced T cells Enhanced infections</td>
</tr>
<tr>
<td>Rubella</td>
<td>Rubella</td>
<td>Lymphoid cells</td>
<td>Persistent rubella infection</td>
</tr>
<tr>
<td>HIV</td>
<td>AIDS</td>
<td>CD4+ T cells monocytes</td>
<td>Opportunistic infections Neoplasia</td>
</tr>
</tbody>
</table>
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Which of the following is an example of B-cell mediated immunopathology?

1. CD8⁺ T cells that cause tissue damage
2. Poxes and rashes
3. Dengue shock syndrome
4. HIV-1 associated opportunistic infections
5. All of the above
Host genes that determine susceptibility

- Ccr5-delta32 mutation protects vs HIV-1 infection
- Present in 4-16% of European descent
- Stem cell therapy cured German AIDS patient
- HIV gets the zinc finger: http://www.virology.ws/2014/03/19/hiv-gets-the-zinc-finger/
Host genes that determine susceptibility

*Herpes simplex encephalitis*

- Rare and potentially fatal CNS infection, ~1 case/250,000/yr
- 70% mortality if untreated
- Two peaks of incidence: 6 mo - 3 yr (primary infection) and >50 yr (reactivation from latency)
Human determinants of susceptibility

- GWAS, SNPs
- Mutations in *TLR3, UNC-93B, TRIF* or *TRAF3* predispose human carriers to HSV encephalitis
Other determinants of susceptibility: Age

- Very young and very old humans most susceptible to disease
- Young - immaturity of immune response; greater freedom from immunopathology
  - LCM i.c. adult mice lethal; infant mice survive (T cell response)
- Old - less elastic alveoli, weaker respiratory muscles, diminished cough reflex
Influenza, US, 1911-1915
Other determinants of susceptibility

• Males slightly more susceptible to viral infections than females

• Pregnancy: hepatitis A, B, E, influenza more lethal, polio more common

• Malnutrition increases susceptibility because physical barriers and immune response are compromised
  - Why measles is 300 times more lethal in developing countries than Europe, N. America
Other determinants

- Cigarette smoking increases susceptibility to respiratory infections
- Air pollution increases respiratory disease
- Stress causes increased susceptibility