Mechanisms of pathogenesis

Lecture 15
Biology 4310
Virology
Spring 2020

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
CD155 transgenic mice

PVR

PVR-Tg
Tissue tropism

- The spectrum of tissues infected by a virus
  - Enterotropic, neurotropic, hepatotropic
- Ranges from limited to pantropic
- Some determinants: Susceptibility, permissivity, accessibility, defense
Glycoprotein cleavage as tropism determinant

- Influenza H5N1 and furin - cleavage during assembly
- TMPRSS2 (transmembrane protease, serine 2), endosome transmembrane protease - cleavage during entry
S cleavage and zoonotic potential of SARS-CoV-2

Furin cleavage site
Not present in RaTG13, a bat SARS-like CoV isolated in 2013, the closest known isolate to SARS-CoV-2

S cleaved by endosomal cathepsins, TMPRSS for SARS-CoV, SARS-CoV-2
Insertion of multiple basic amino acids at the HA cleavage site allows influenza virus to infect many organs. This means that the _____ of the virus has changed.

A. Susceptibility  
B. Club cell tryptase  
C. Permissivity  
D. Tropism  
E. All of the above
Viral virulence

- Capacity of a virus to cause disease in a host
- Virulent vs avirulent or attenuated virus (e.g. attenuated vaccines)
- Virulence can be quantitated:
  - Virus titer
  - Mean time to death
  - Mean time to appearance of signs
  - Measurement of fever, weight loss
  - Measurement of pathological lesions (poliovirus); reduction in blood CD4+ lymphocytes (HIV-1)

- Many signs/symptoms* of disease are caused by immune response!

*Symptoms = what you can feel
Signs = what others detect
Measuring viral virulence

A

Number of survivors

Day postinfection

B

Relative neurovirulence score

Region of the CNS

Japanese encephalitis virus
Yellow fever virus 17D strain
West Nile virus
Langat virus
Dengue virus 4

0
10
20
30
40
50
60
70
80
90
100

C
B
S
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>Intraperitoneal</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><img src="wild_type.png" alt="Image" /></td>
<td>Reproduction</td>
<td>Neurovirulent</td>
</tr>
<tr>
<td>Mutation leading to a general defect in reproduction</td>
<td><img src="mutation.png" alt="Image" /></td>
<td>Poor reproduction</td>
<td>Attenuated</td>
</tr>
<tr>
<td>Mutation in a gene specifically required for virulence</td>
<td><img src="mutation2.png" alt="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
Viral virulence determinants need 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 × 10$^7$</td>
</tr>
<tr>
<td>PRV8.4</td>
<td>C</td>
<td>9 × 10$^3$</td>
</tr>
</tbody>
</table>
Viral 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

Gammaherpesvirus 68 M3 gene encodes a chemokine receptor

IC inoculation
Toxic viral proteins

*NSP4 nonstructural glycoprotein of rotaviruses: viral enterotoxin*
Cellular virulence determinants: 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)
Cellular virulence determinants: Herpes simplex encephalitis

- GWAS, SNPs
- Mutations in *TLR3*, *UNC-93B*, TRIF or TRAF3 predispose human carriers to HSV encephalitis
Mda-5 inborn errors and severe rhinovirus infection

A

(+)-ssRNA viruses

(-)-ssRNA viruses

DNA viruses

Bacteria

- Respiratory fluid sampling

- Rhinovirus

- hCoV-HKU1

- hCoV-OC43

- hCoV-NL63

- Influenza B

- Influenza A

- RSV

- PIV4

- Adenovirus

Haemophilus influenzae
Mycoplasma pneumoniae
Staphylococcus aureus
Streptococcus viridans,
Acinetobacter spp., Enterococcus
daecalis, & Escherichia coli

Proband

WT/WT K365E/WT ?/? K365E/K365E

K365E/WT

Card Hel1 Hel2i Hel2 Pincer CTD

Father

Mother

Sister

Brother

Mda-5
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
- And now the London patient: http://www.virology.ws/2019/03/13/the-london-patient/
Other determinants of virulence: Age

- Very young and very old humans most susceptible to disease
- Young - immaturity of immune response
- Old - less elastic alveoli, weaker respiratory muscles, diminished cough reflex; reduced rate of production of new immune cells (bone marrow diminishes with age)
Host determinants of virulence

- In general, males/men are slightly more susceptible to viral infections than females/women (but not always)
- Elevated humoral immunity in females compared with males is phylogenetically conserved - reproductive success?
- Female antibody responses correlate with elevated estradiol
- Pregnancy: hepatitis A, B, E, influenza more lethal

npj Vaccines (2019)4:29 ; https://doi.org/10.1038/s41541-019-0124-6
Other determinants

- 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
  Cigarette smoking increases susceptibility to respiratory infections

- Air pollution increases respiratory disease

- Stress causes increased susceptibility
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
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)
Immunopathology: Too much of a good thing

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

<table>
<thead>
<tr>
<th>CD8⁺</th>
<th>T cell-mediated</th>
<th>CD4⁺</th>
<th>B cell-mediated</th>
</tr>
</thead>
<tbody>
<tr>
<td>Coxsackievirus B</td>
<td>Visna virus</td>
<td>Theiler's virus</td>
<td>Dengue virus</td>
</tr>
<tr>
<td>Lymphocytic choriomeningitis virus</td>
<td>Semliki Forest virus</td>
<td>Mouse coronavirus</td>
<td>Respiratory syncytial virus</td>
</tr>
<tr>
<td>Sin Nombre virus</td>
<td>HIV-1</td>
<td>Herpes simplex virus</td>
<td>Feline infectious peritonitis virus</td>
</tr>
</tbody>
</table>

*Also a consequence of over-exuberant innate immune responses*
Viral disease mediated by CD8+ CTLs

1. LCMV injection
2. Immune suppression
3. Persistent infection
4. Adoptive immunization
5. Lethal choriomeningitis
Lesions associated with CD8+ lymphocytes

**LCMV**

**Liver enzyme**

![Graphs showing percent alive and liver enzyme levels over days after infection for Perforin +/- genotypes.]

A: Histological image of infected tissue.

B: Histological image of uninfected tissue.
Lesions associated with CD4+ lymphocytes

- 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, inflammation restricted to underlying uninfected stromal cells
- Secreted cytokines produced by infected cells in corneal epithelium recruit CD4+ Th1 cells, produce cytokines which recruit neutrophils
- Cell infiltration + inflammatory mediators cause swelling and damage of corneal cells
TLR3 and West Nile virus encephalitis

- *tlr3*⁻/⁻ mice more resistant to WNV lethal infection, have impaired cytokine production
- TNF-α 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-Y
- 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

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
After secondary dengue infections, incidence of hemorrhagic fever and shock syndrome 1/90 and 1/50
Immunopathology of SARS-CoV infections

- Fever, cough, pneumonia, acute lung injury, acute respiratory distress
- Contribution of dysregulated inflammatory cytokine production ("cytokine storm")

Inflammmatory response to virus infections

Pathogenic/dysregulated Inflammation

- High virus reproduction
- Delayed IFN response
- Inflammatory monocyte-macrophage and neutrophil infiltration
- Proinflammatory cytokines and chemokine

Consequences

- Enhanced epithelial and endothelial cell apoptosis
- Increased vascular leakage
- Sub-optimal T cell, Ab responses
- Impaired virus clearance

ALI, ARDS, Death

Protective/regulated Inflammation

- Reduced virus reproduction
- Early IFN response
- Inflammatory monocyte-macrophage and neutrophil infiltration
- Proinflammatory cytokines and chemokine

Consequences

- Minimal epithelial and endothelial cell apoptosis
- Reduced vascular leakage
- Optimal T cell, Ab responses
- Effective virus clearance

Protective Immunity
Host survival
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

TB test

Influx of T cells

Virology Lectures 2020 • Prof. Vincent Racaniello • Columbia University
Measles virus immunosuppression

**A**
- Virus
- TLR4
- IL-12
- Antigen-presenting cell
- CD4
- Activated CD4+ T cells
- "Th1 response"
  - Favors killing infected cells

**B**
- Measles virus
- TLR4
- IL-12
- Antigen-presenting cell
- CD4
- Activated CD4+ T cells
- "Th2 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&lt;br&gt;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&lt;br&gt;monocytes</td>
<td>Opportunistic infections&lt;br&gt;Neoplasia</td>
</tr>
</tbody>
</table>
Next time: Acute infections