Mechanisms of pathogenesis

Session 15

Virology Live

Fall 2021

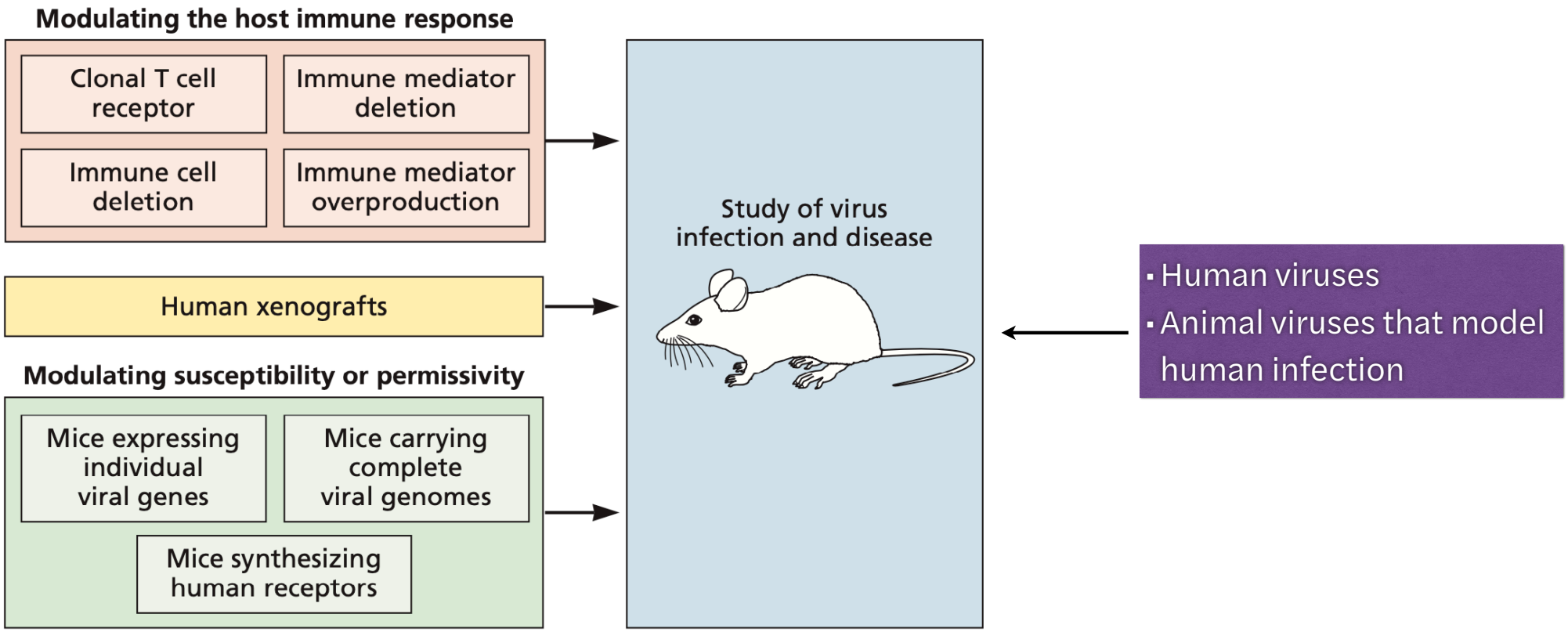
We have met the enemy and he is us.

–Walt Kelly

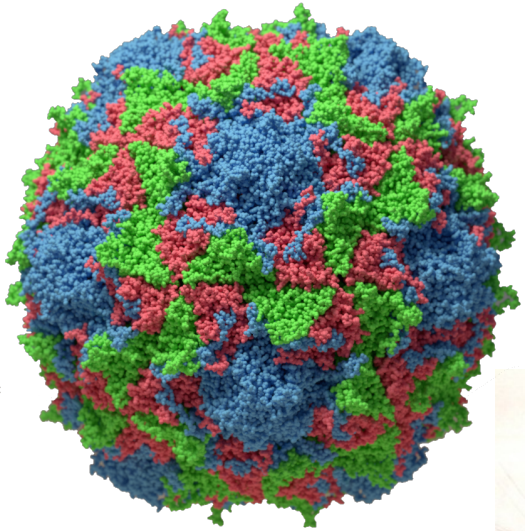
Pathogenesis

- The processes that lead to disease
- Often a collateral outcome of the parasitic nature of viruses
- Selective pressures that control evolution of viruses act only on their abilities to reproduce

Animal models: Mice lie, monkeys exaggerate



CD155 transgenic mice

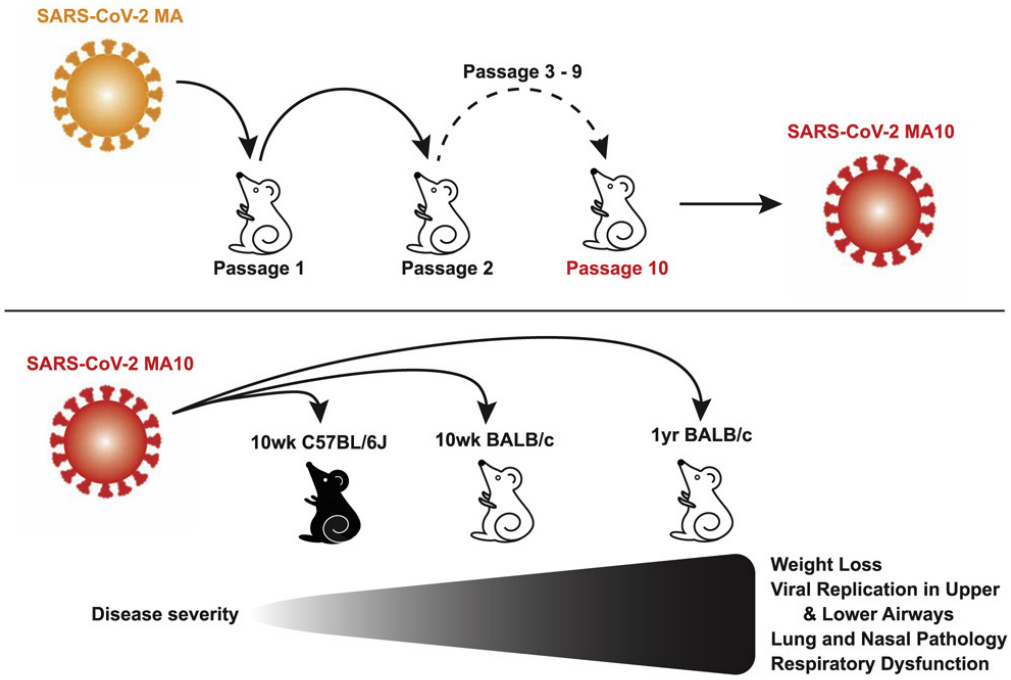


PVR/CD155

PVR-Tg

Animal models of COVID-19

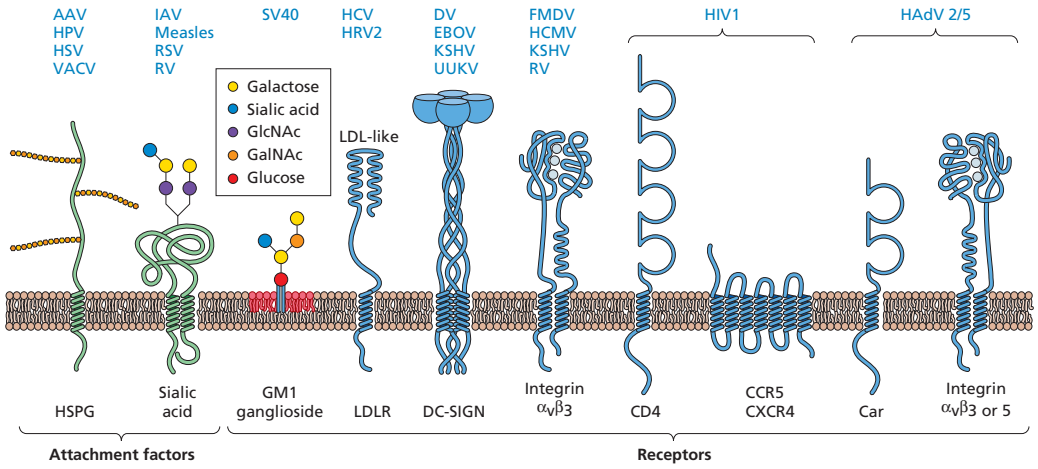
- Mice are not susceptible to infection
- Two amino acid changes in spike allow binding to mouse ACE2
- ACE2 transgenic mice
- Nonhuman primates
- Ferrets
- Hamsters



https://pubmed.ncbi.nlm.nih.gov/33031744/

Tissue tropism

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



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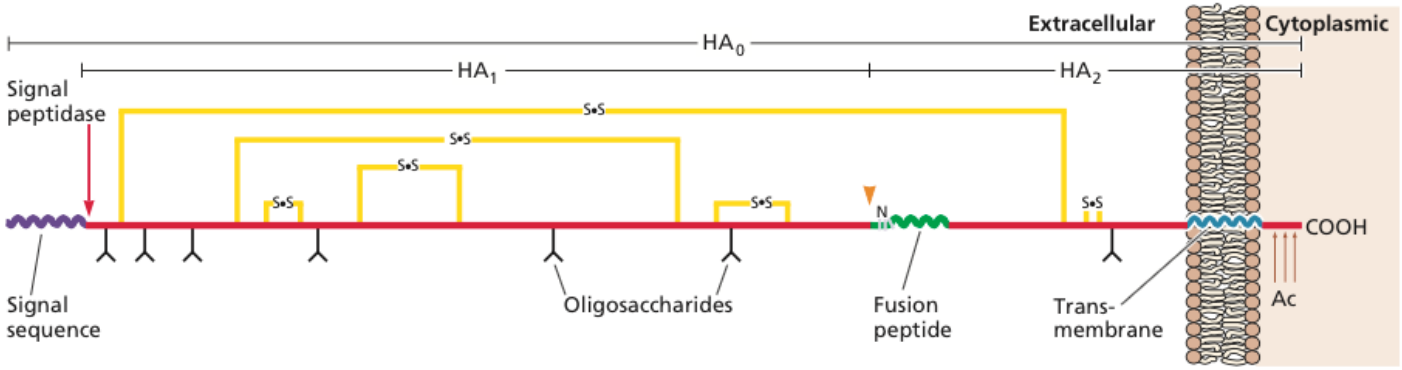
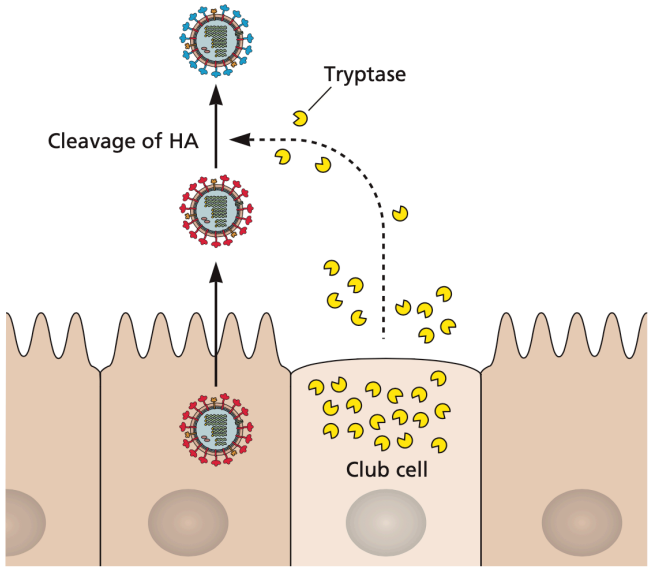
Glycoprotein cleavage as tropism determinant





- TMPRSS2 (transmembrane protease, serine 2), cleavage during entry

- Influenza H5N1 and furin - cleavage during assembly - broad tropism

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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.

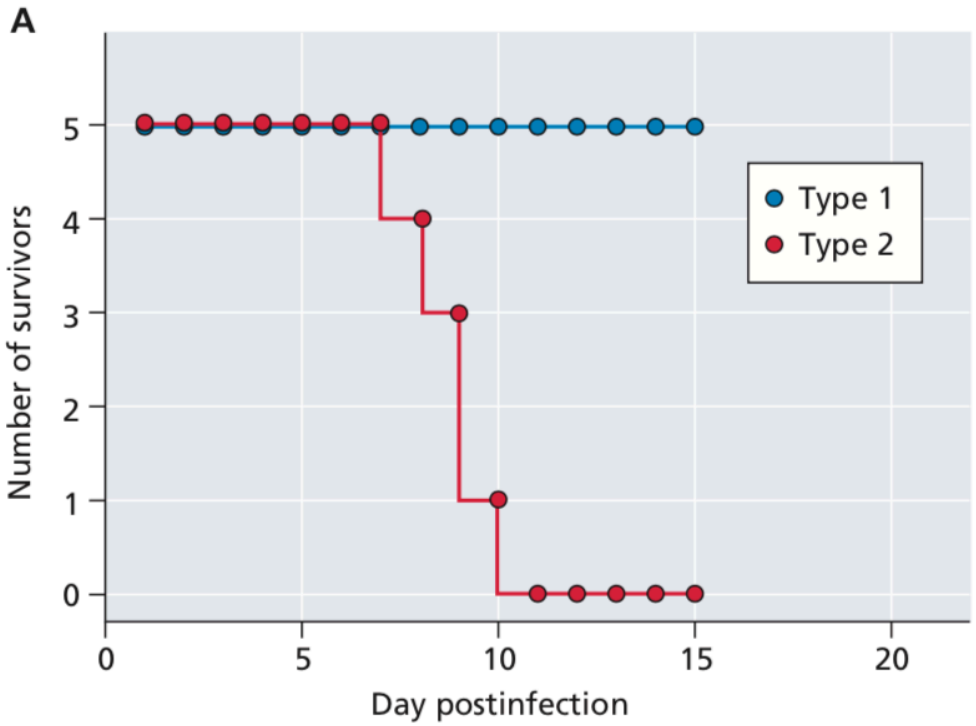
A. Susceptibility
B. Club cell tryptase
C. Permissivity
D. Tropism
E. All of the above

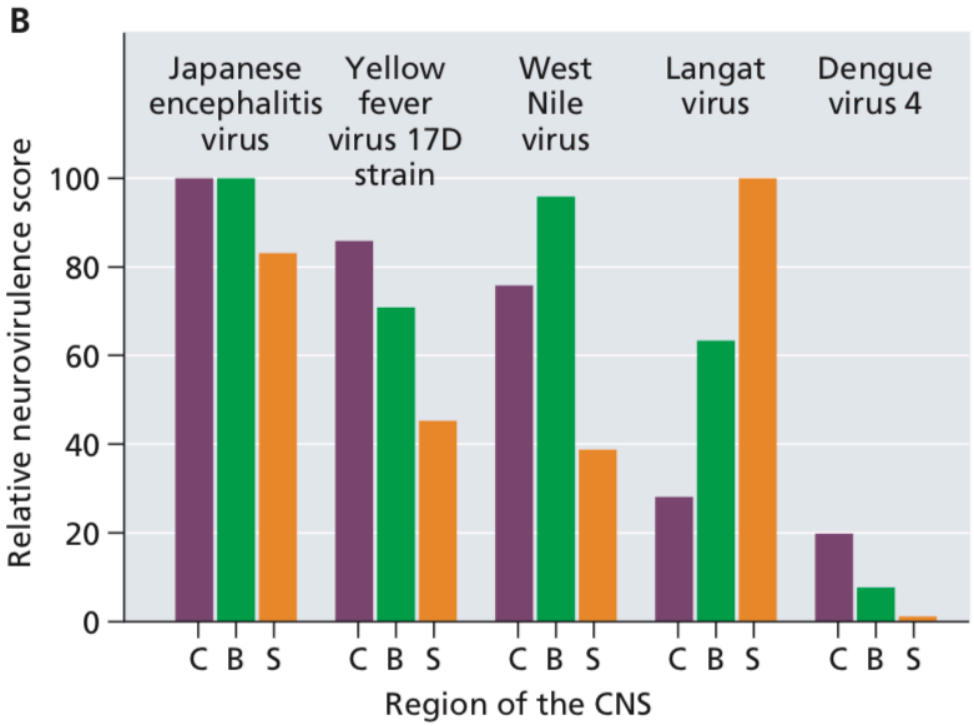
Viral virulence

- 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 only you can feel*
Signs = what others detect

Measuring viral virulence





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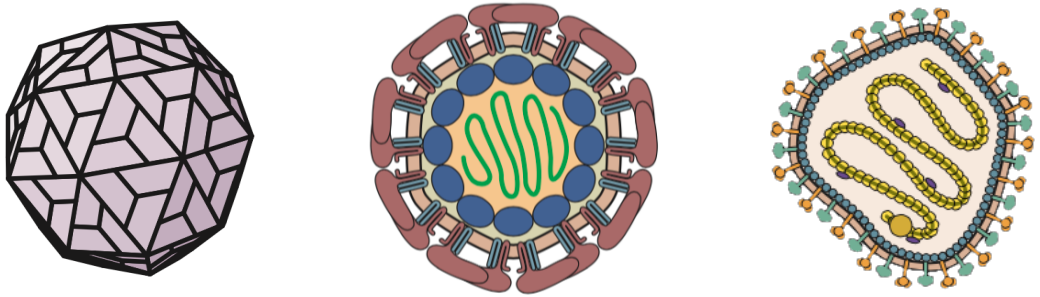
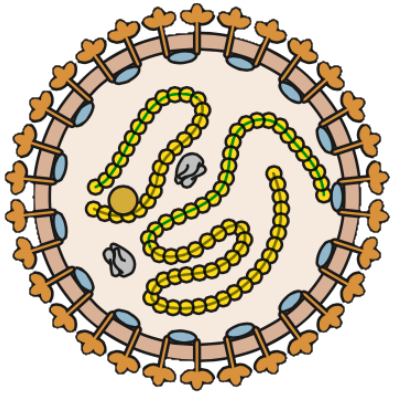
Viral virulence is a relative property



- Influenced by dose, route of infection, species, age, sex, and susceptibility of host
- Not correct to compare virulence of different viruses
- For similar viruses, assays must be the same
- Measuring virulence in humans is difficult

Virulence depends on route of inoculation



Lymphocytic choriomeningitis virus

Dose	Route	Outcome
100,000 PFU	Intraperitoneal	Survival
1 PFU	Intracranial	Death

It is not possible to conclude that one SARS-CoV-2 variant is more virulent than another simply from observations of hospitalization!

Progressive Increase in Virulence of Novel SARS-CoV-2 Variants in Ontario, Canada

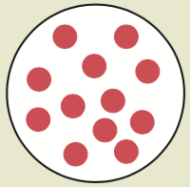
David N. Fisman, Ashleigh R. Tuite

doi: https://doi.org/10.1101/2021.07.05.21260050

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

Virus	Growth in cell culture	Effect on mice	Virulence phenotype
Wild type		 Reproduction	Neurovirulent
Mutation leading to a general defect in reproduction		 Poor reproduction	Attenuated
Mutation in a gene specifically required for virulence		 Poor reproduction	Attenuated

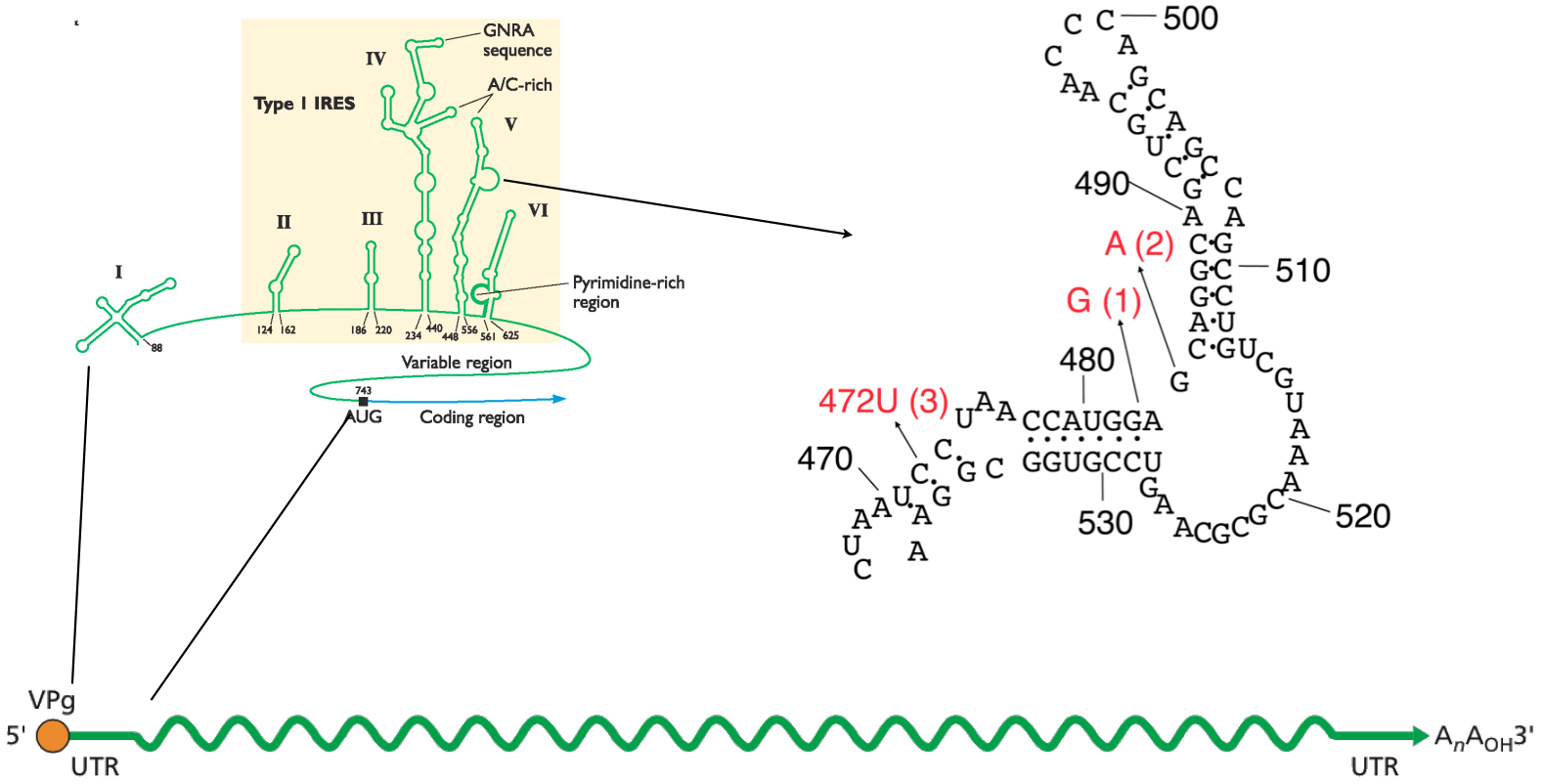
Four classes of viral virulence genes

- Genes/gene products that affect viral replication
- Genes encoding toxins
- Genes encoding modifiers of host defense mechanisms
- Gene/gene products that enable virus to spread in the host

Mutations in last two classes of genes often have no impact on virus reproduction in cells in culture, and may be called 'nonessential genes' which is a BAD NAME!

Viral virulence determinants need not encode proteins

Sabin vaccine strains of poliovirus contain a mutation in the 5'-noncoding region that reduces neurovirulence



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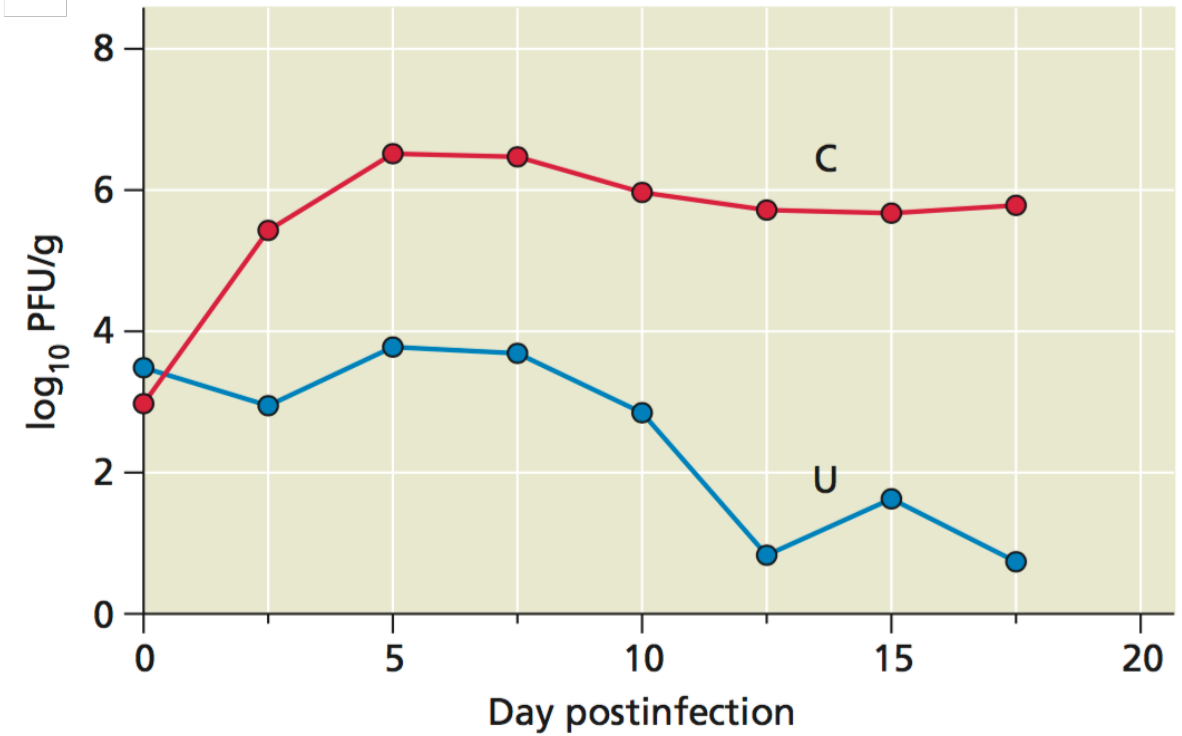
Poliovirus replication in mouse brain

Virus	Base at 472	LD_{50}
PRV7.3	U	$>2 \times 10^7$
PRV8.4	C	9×10^3



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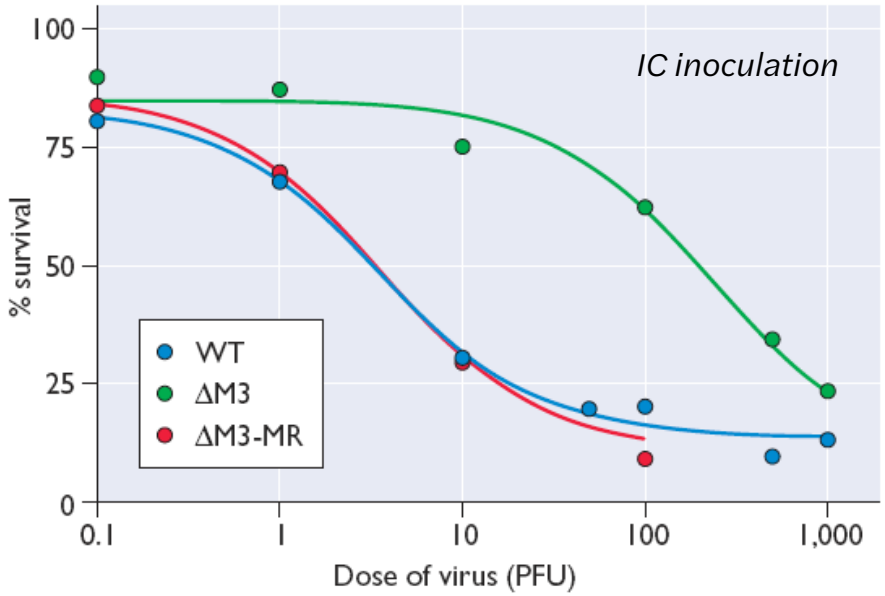
Viral gene products that modify host defense

- 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

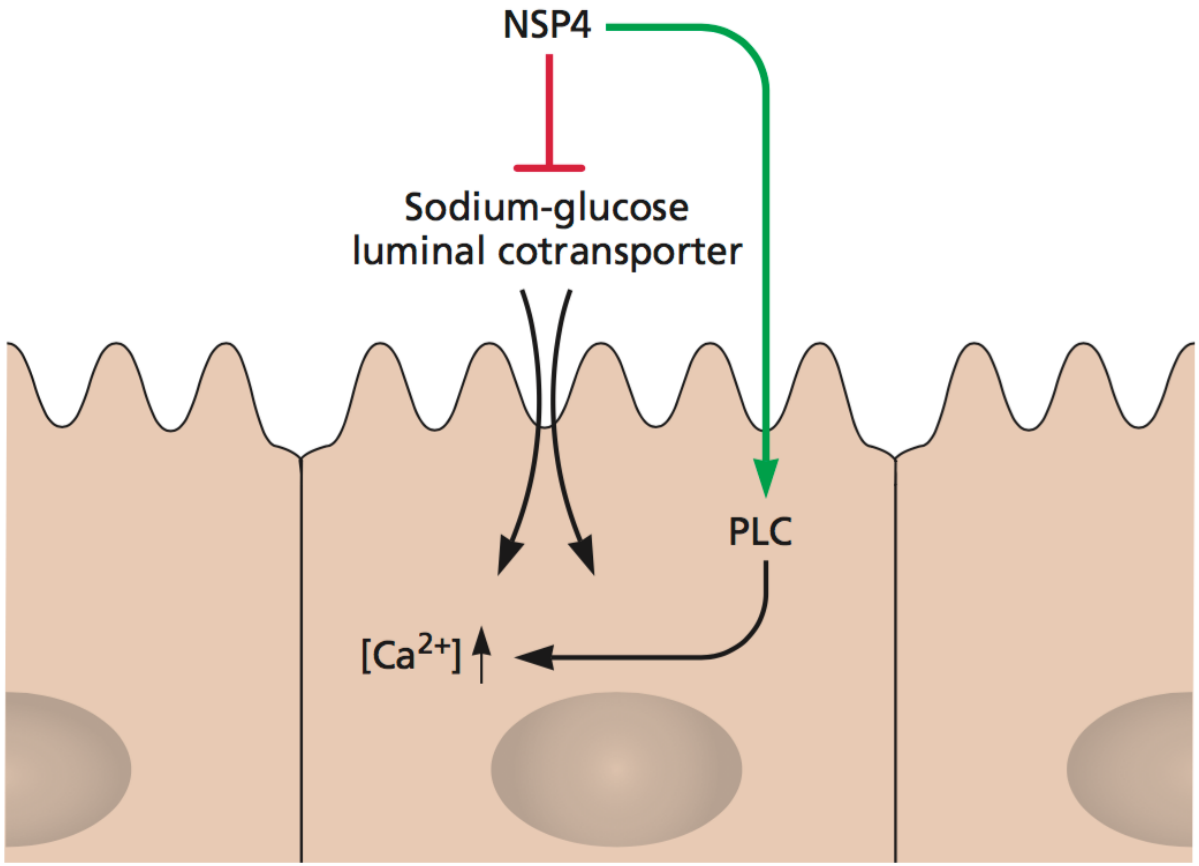
Viral virulence genes



Gammaherpesvirus 68 M3 gene encodes a chemokine receptor

Toxic viral proteins

NSP4 nonstructural glycoprotein of rotaviruses: viral enterotoxin



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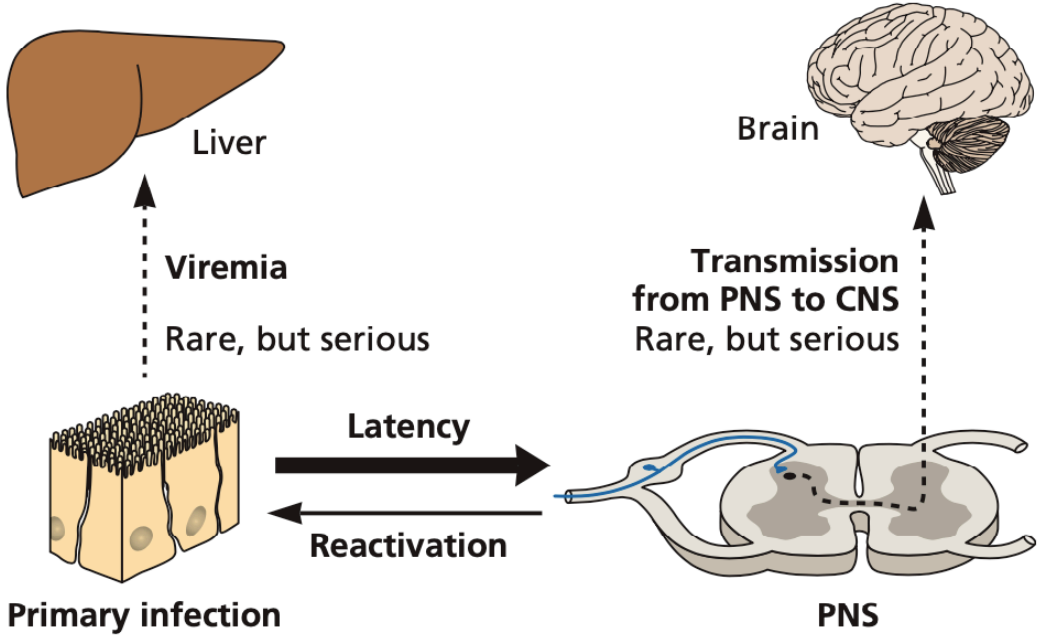
Which statement about viral virulence is wrong?

A. It can be influenced by dose, route of infection, species, age, sex, 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

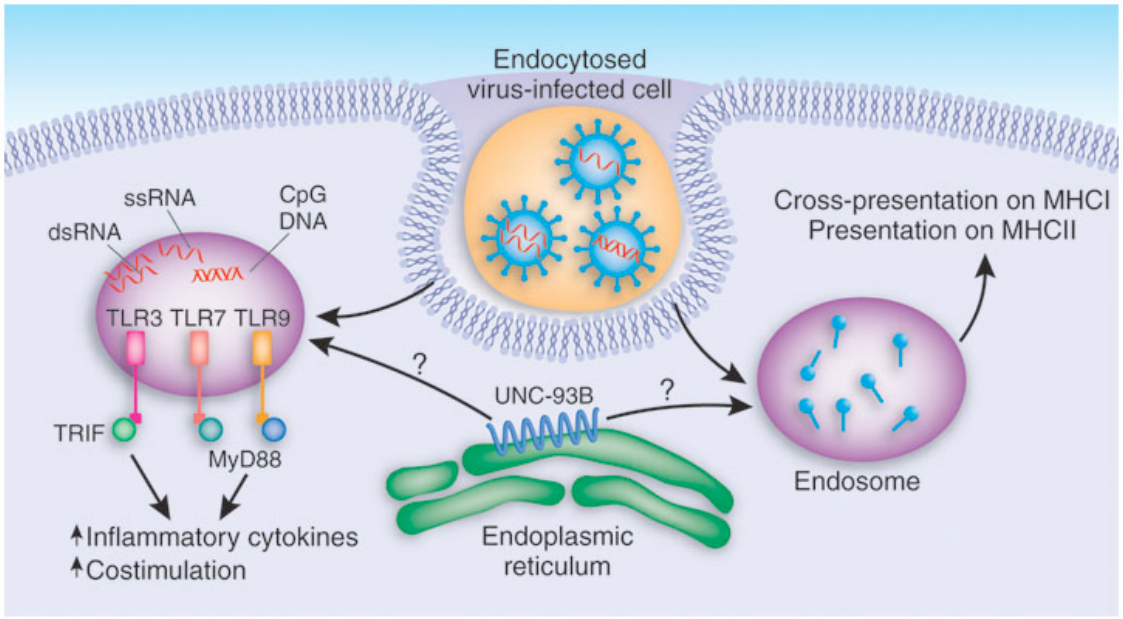
Cellular virulence determinants: Herpes simplex encephalitis



- 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)

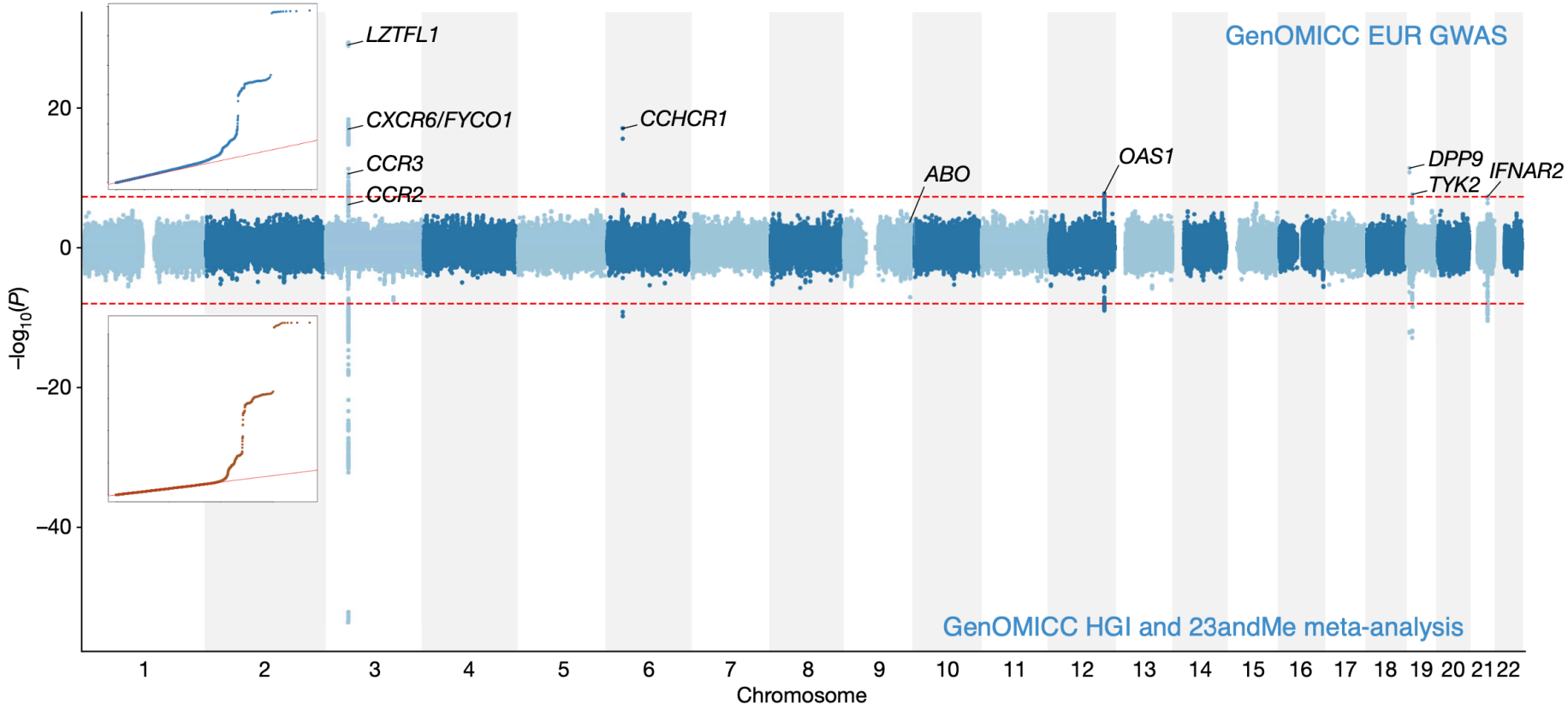
Cellular virulence determinants: Herpes simplex encephalitis

- Genome-wide association studies (GWAS) looking for single nucleotide polymorphisms (SNP) in patients
- Mutations in *TLR3*, *UNC-93B*, TRIF or TRAF3 predispose human carriers to HSV encephalitis



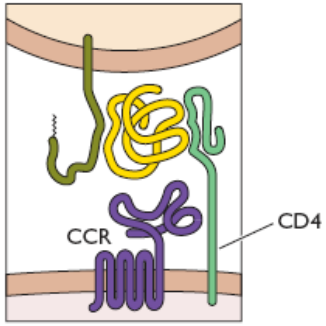
GWAS for critical COVID-19

GenOMICC (Genetics Of Mortality In Critical Care) genome-wide association study in 2,244 critically ill patients with COVID-19 from 208 UK intensive care units



https://doi.org/10.1038s41586-020-03065-y

Host genes that determine *susceptibility*

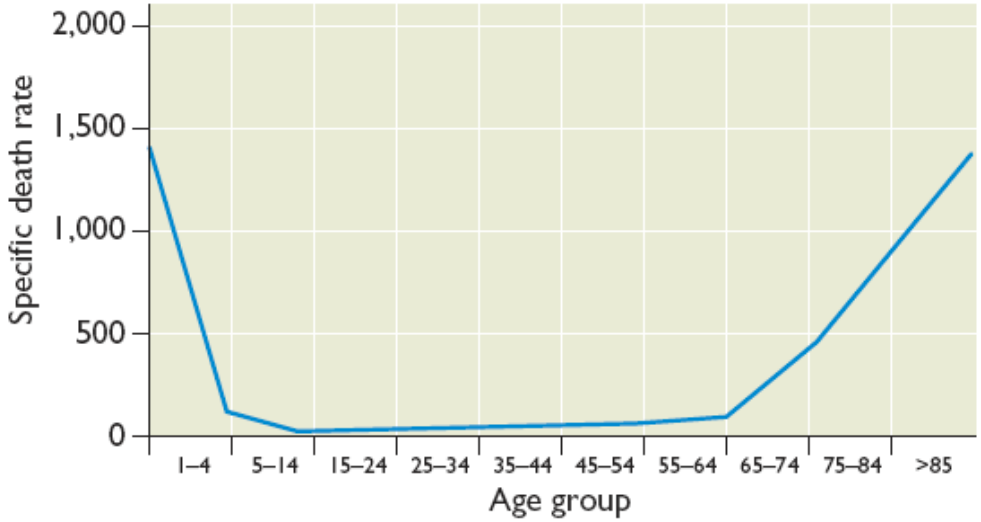


- Ccr5-delta32 mutation protects vs HIV-1 infection
- Present in 4-16% of European descent
- Stem cell therapy cured German AIDS patient
- And the London patient: http://www.virology.ws/2019/03/13/the-london-patient/

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)

Influenza, US, 1911-1915



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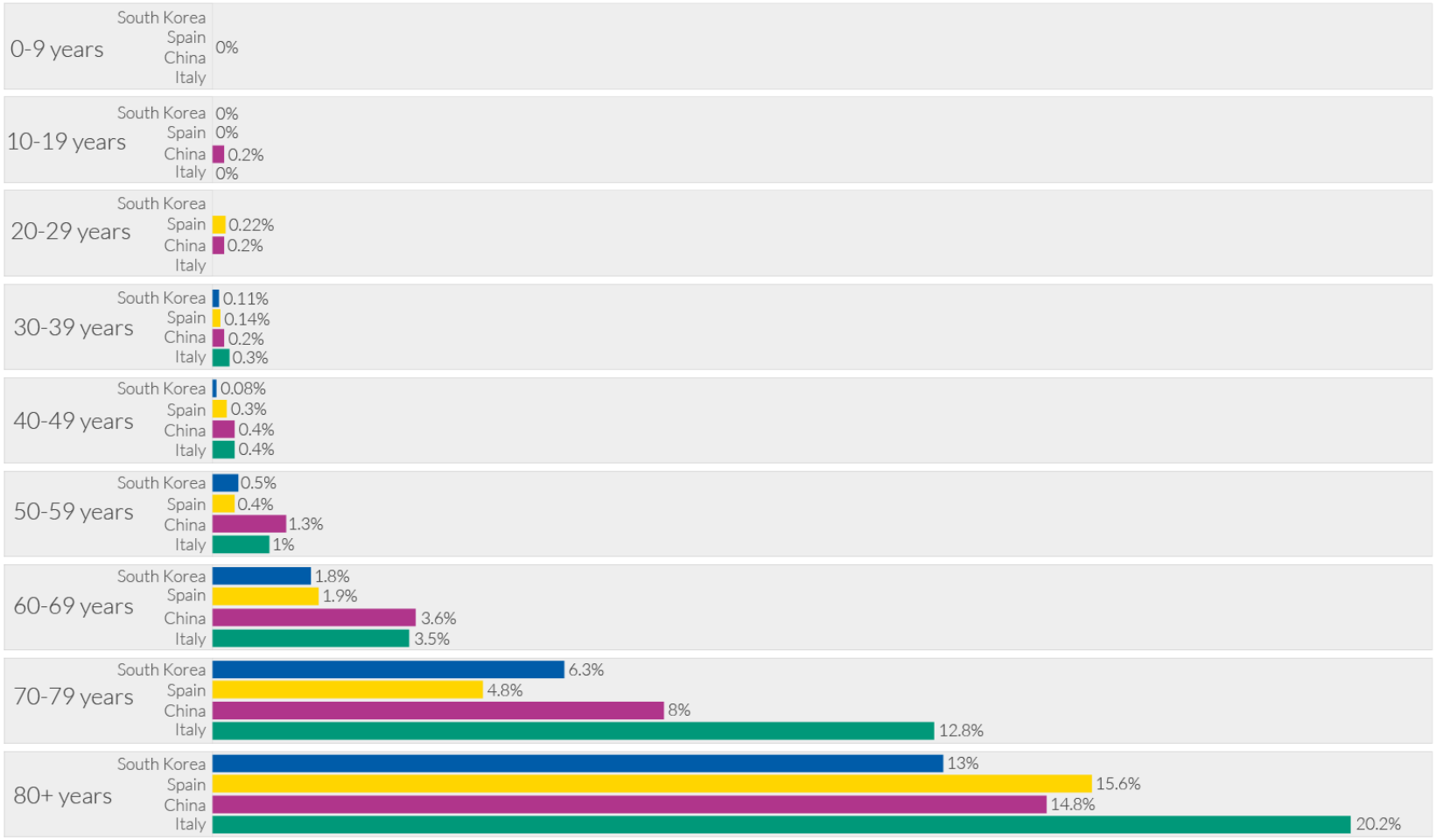
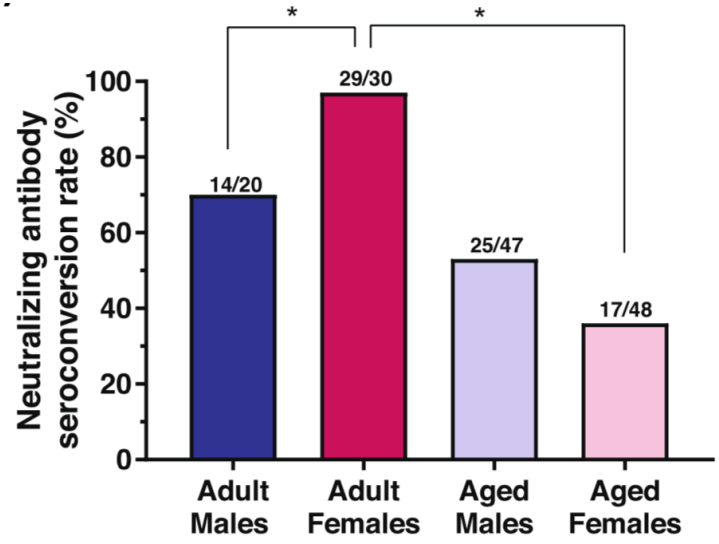
COVID-19 CFR by age



https://ourworldindata.org/mortality-risk-covid

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, COVID-19 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

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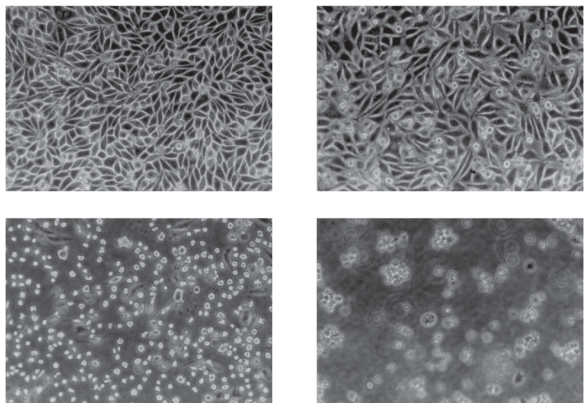
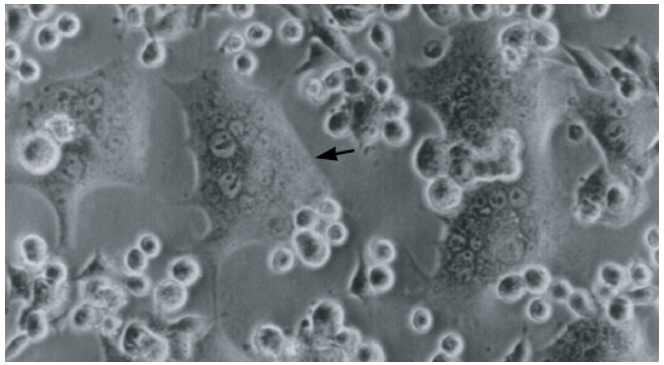
Which statement about determinants of viral virulence is incorrect:

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

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)





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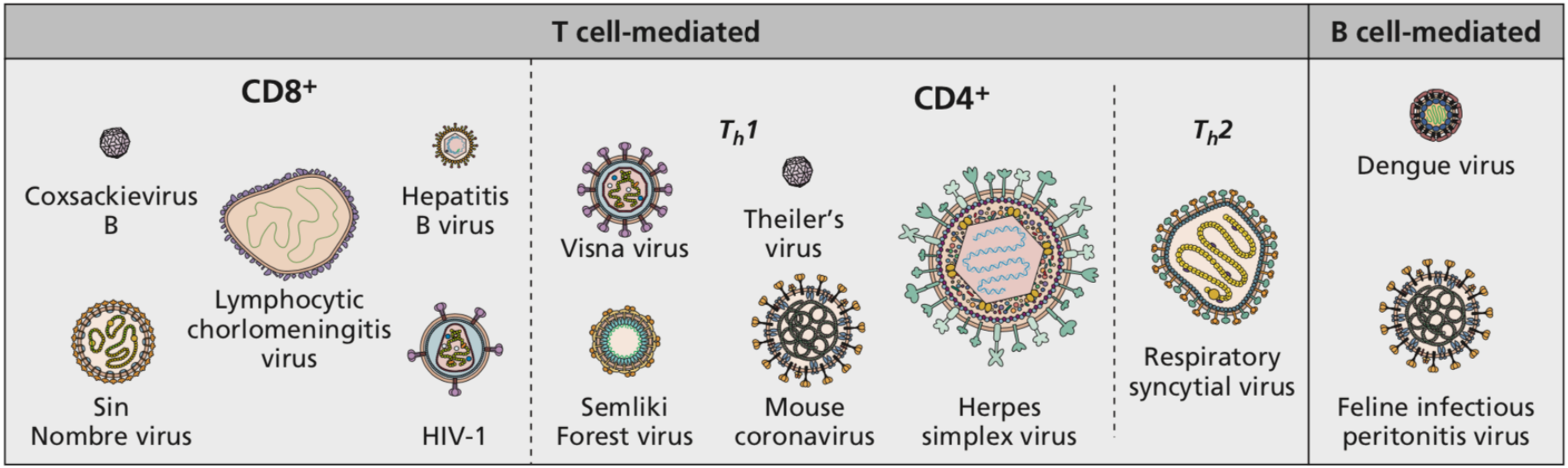
Immunopathology: Too much of a good thing

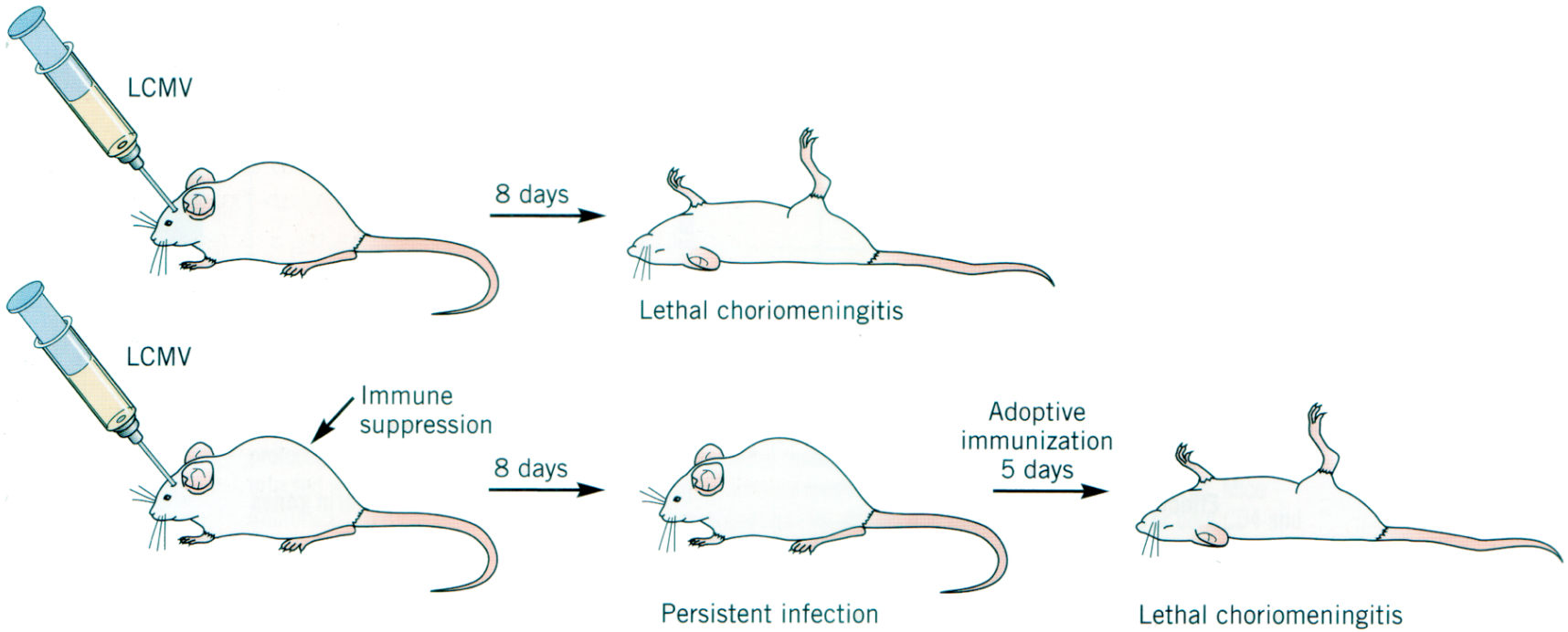
- Clinical signs & symptoms of viral disease (fever, tissue damage, aches, pains, nausea) are mainly a consequence of host response to infection
- Non-cytopathic viruses: disease is usually a consequence of the immune response

Immunopathology

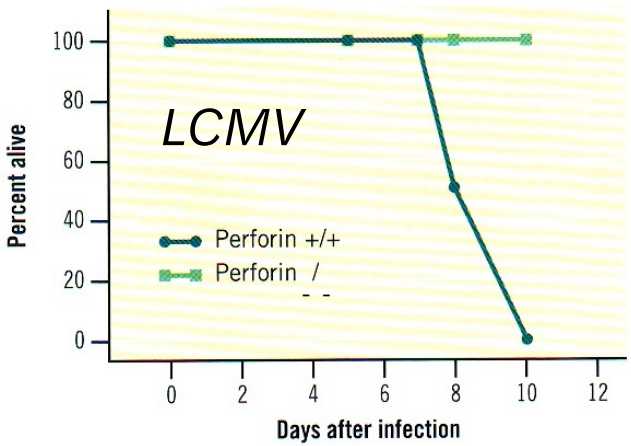


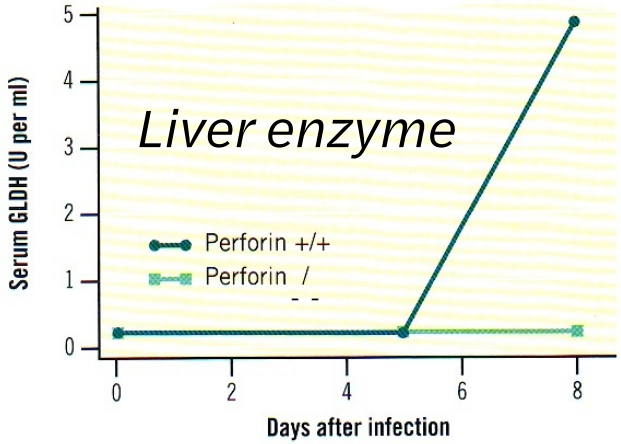
Also a consequence of over-exuberant innate immune responses

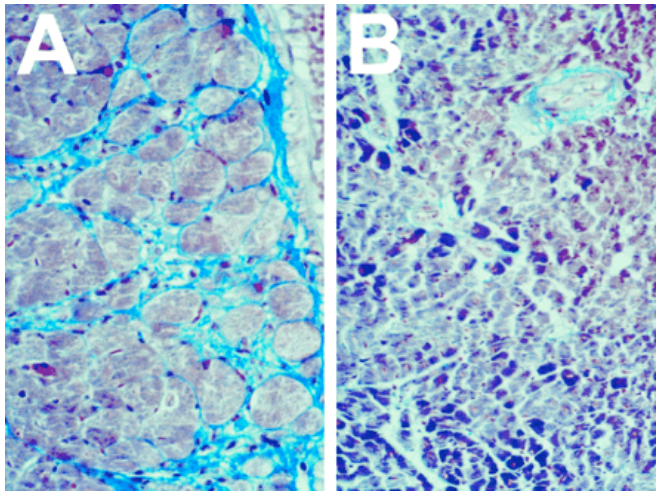
Viral disease mediated by CD8+ CTLs



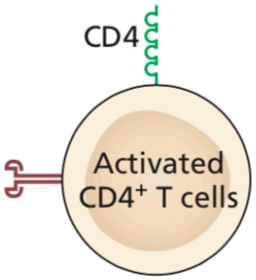
Lesions associated with CD8+ lymphocytes



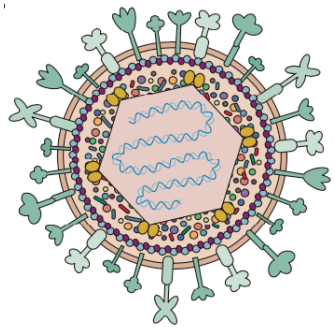




Lesions associated with CD4+ lymphocytes

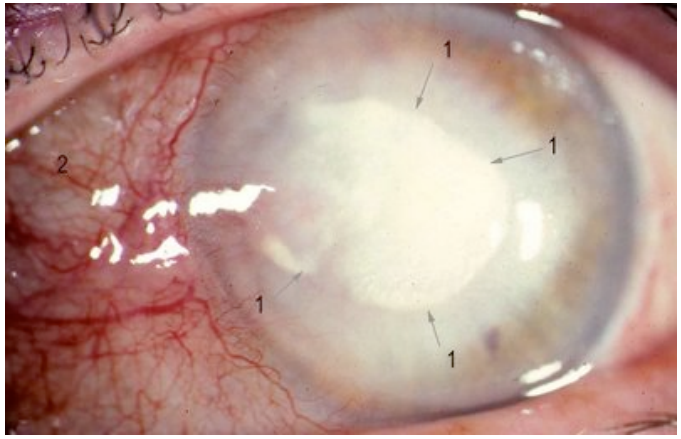


- Elaborate more cytokines than CD8+ T cells, and recruit and activate many nonspecific effector cells
- Most recruited cells are neutrophils and mononuclear cells, which are protective but cause tissue damage
- Immunopathology caused by release of proteases, reactive radicals, and cytokines (e.g. Tnf-α)

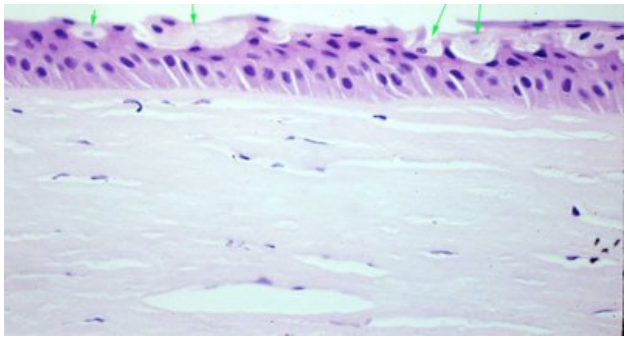


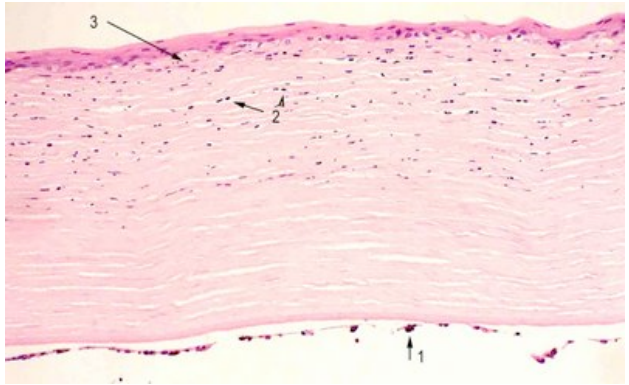
Lesions associated with CD4+ T cells

- 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

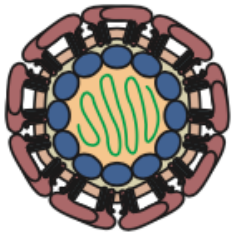




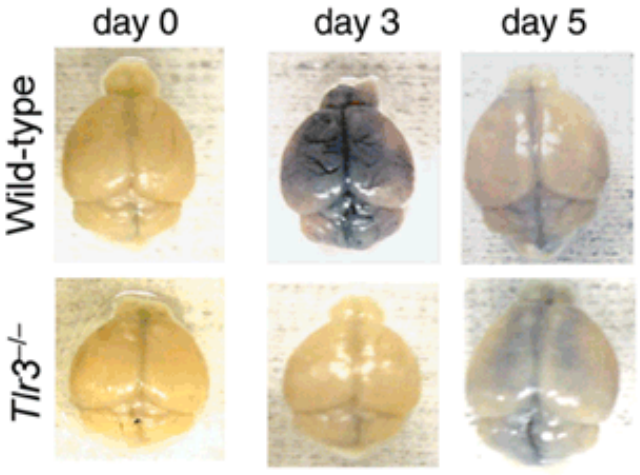
- Virus replicates in corneal epithelium, inflammation restricted to underlying uninfected stromal cells
- Secreted cytokines produced by infected cells in corneal epithelium recruit $CD4^+$ Th1 cells, produce cytokines which recruit neutrophils
- Cell infiltration + inflammatory mediators cause swelling 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



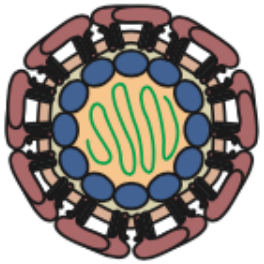
WNV infected



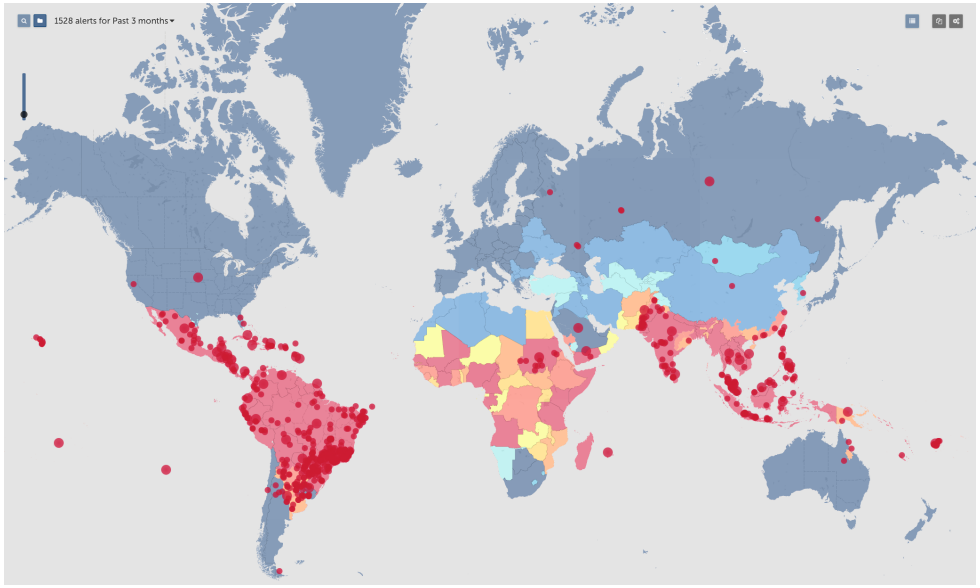
Poxes and rashes

- 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-ϒ
- Cytokines act locally to increase capillary permeability, influx of T cells

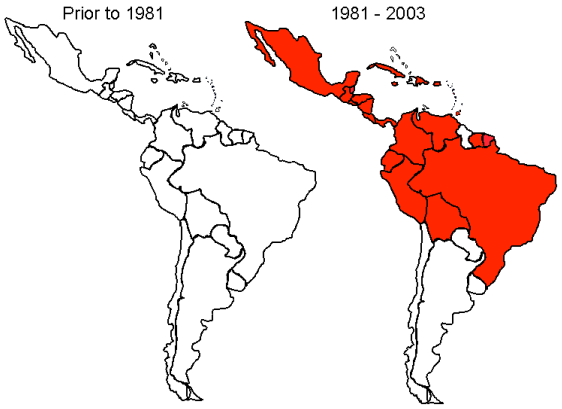
Dengue (breakbone fever)



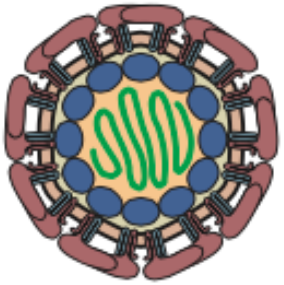
- Dengue virus, transmitted mainly by *Aedes aegypti*
- Endemic in the Caribbean, Central and South America, Africa and Southeast Asia - billions at risk
- 400 million infections/year
- Second only to malaria among insect-borne diseases



American Countries with laboratory confirmed dengue hemorrhagic fever, prior to 1981 and from 1981 to 2003



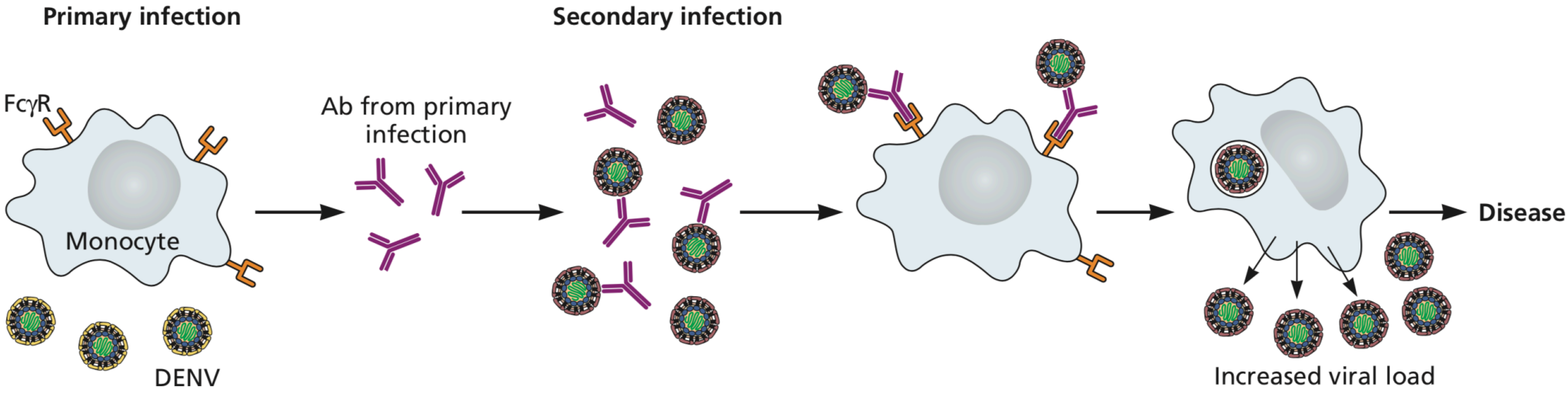
Source: WHO/PAHO/CDC, Aug. 2004



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

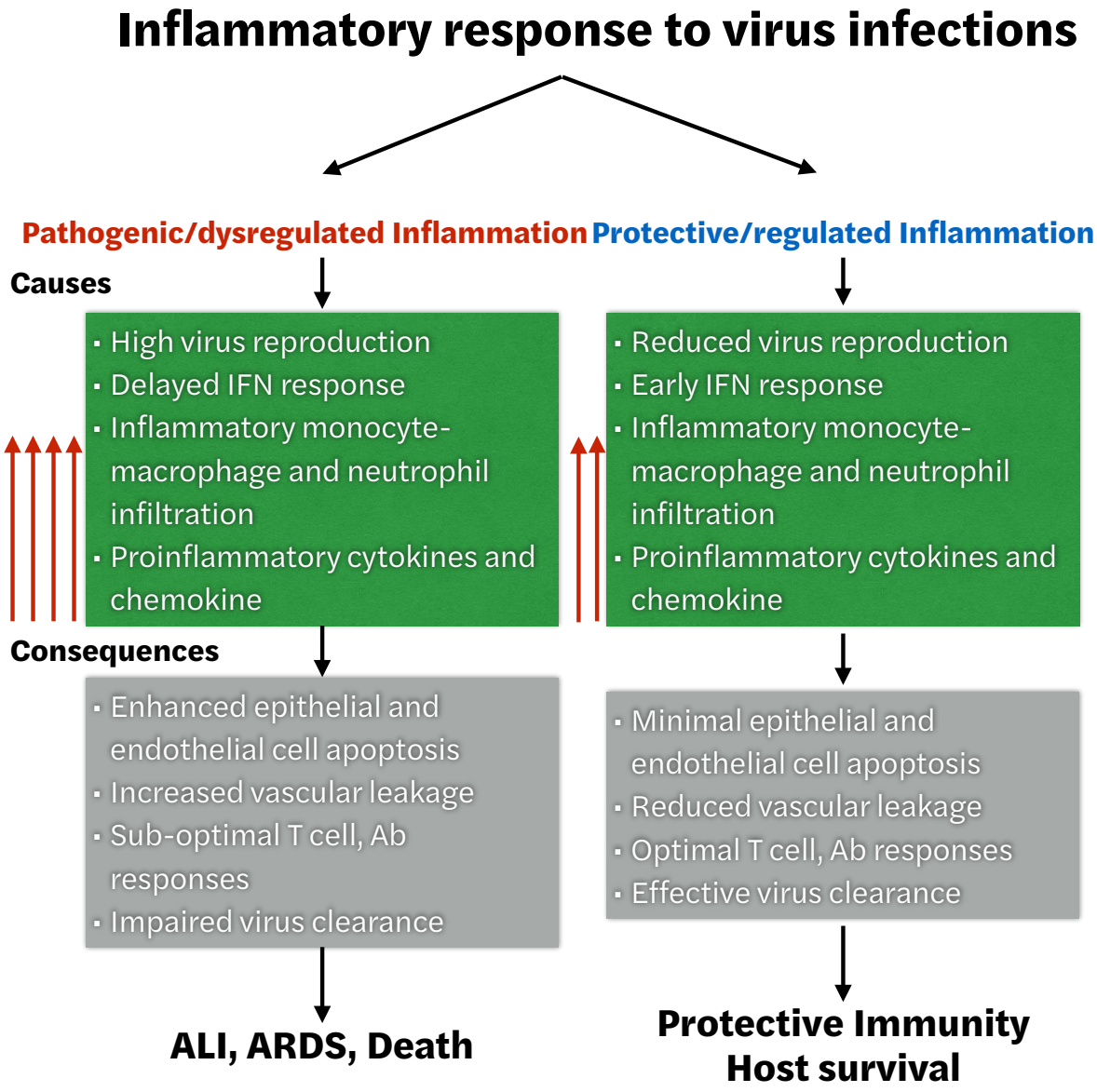
Dengue fever



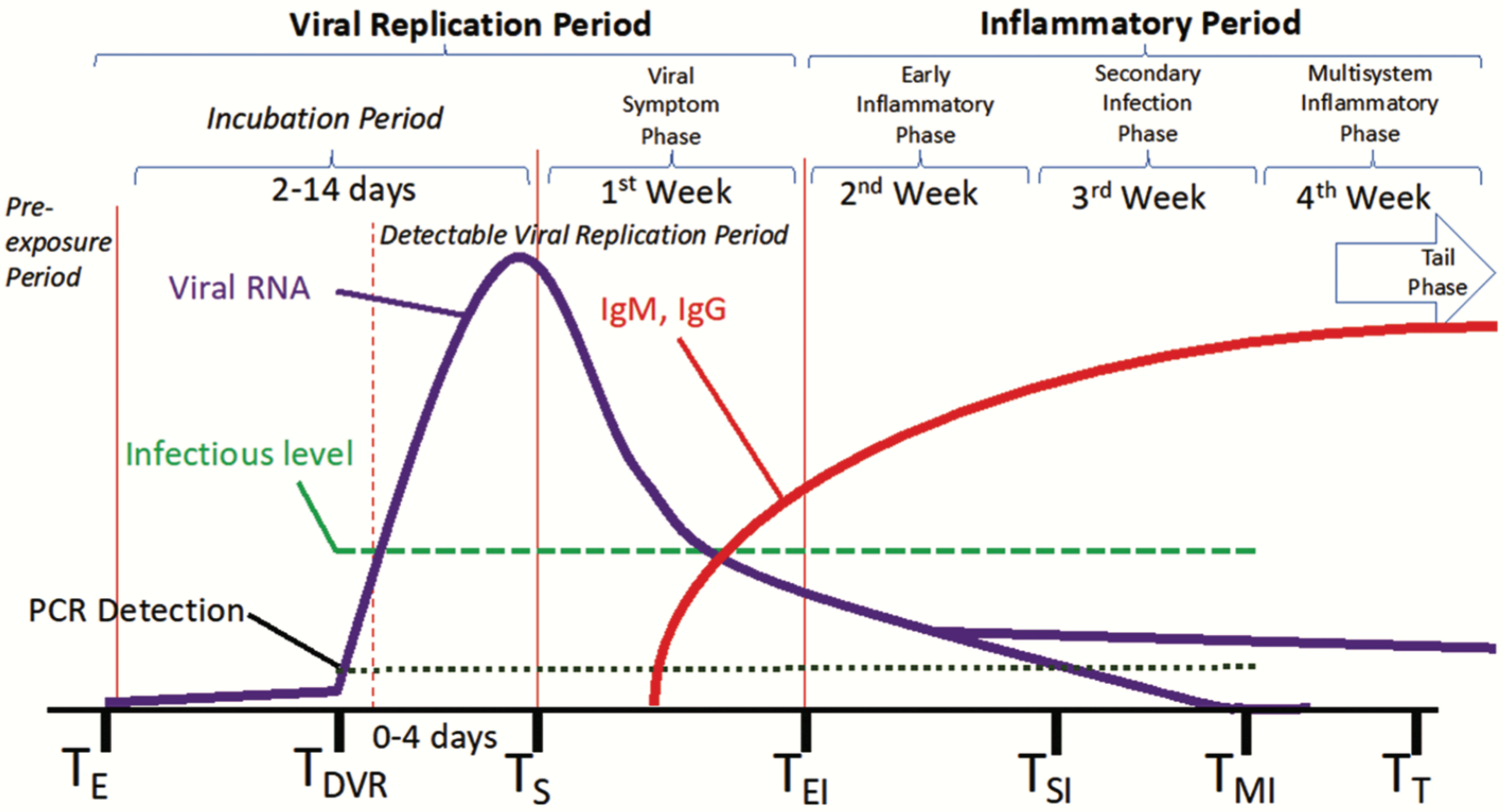
After secondary dengue infections, incidence of hemorrhagic fever and shock (severe dengue) 1/90 and 1/50

Immunopathology of SARS-CoV infections

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



Stages of COVID-19



https://pubmed.ncbi.nlm.nih.gov/33556957/

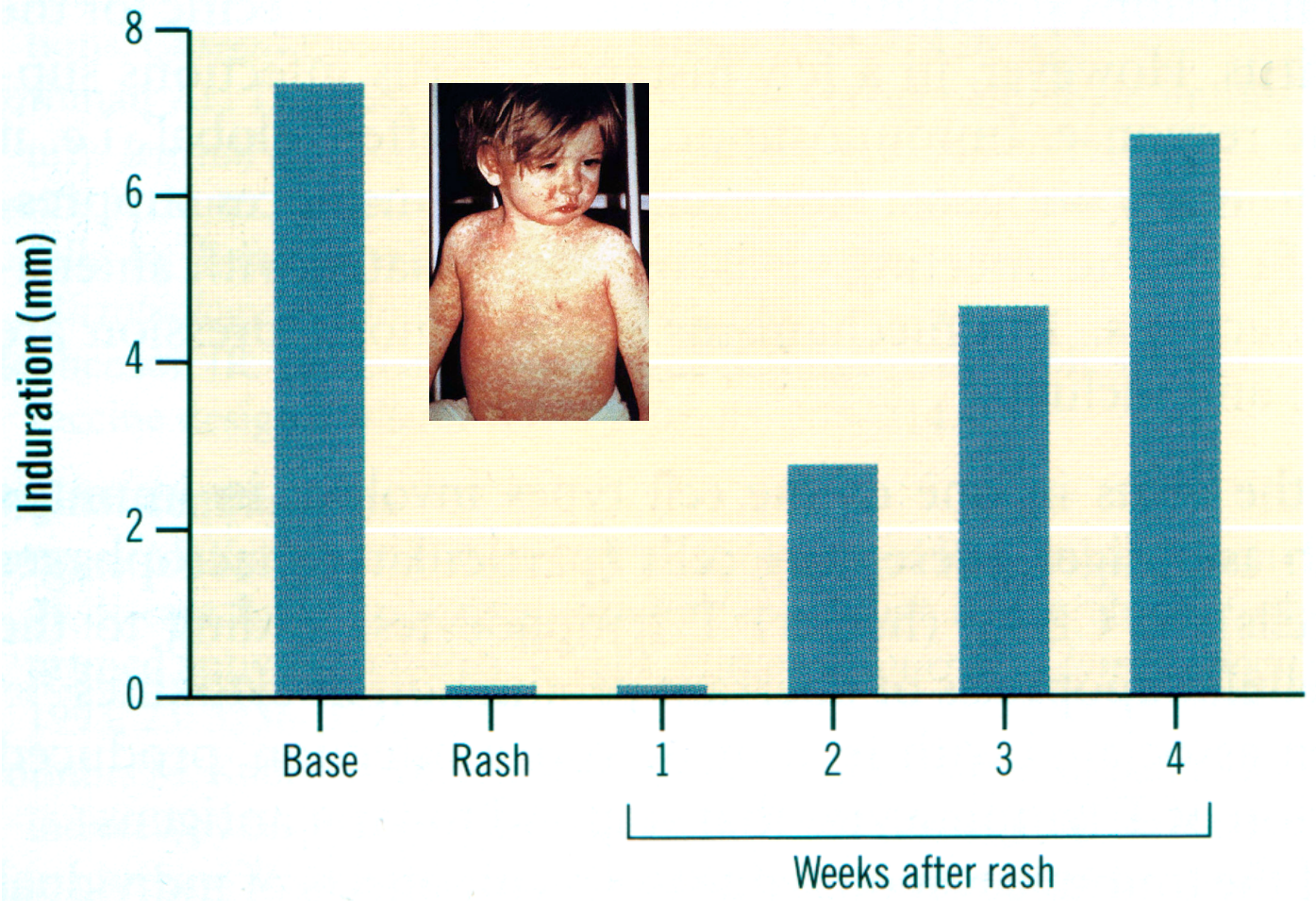
Immunosuppression

- 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)

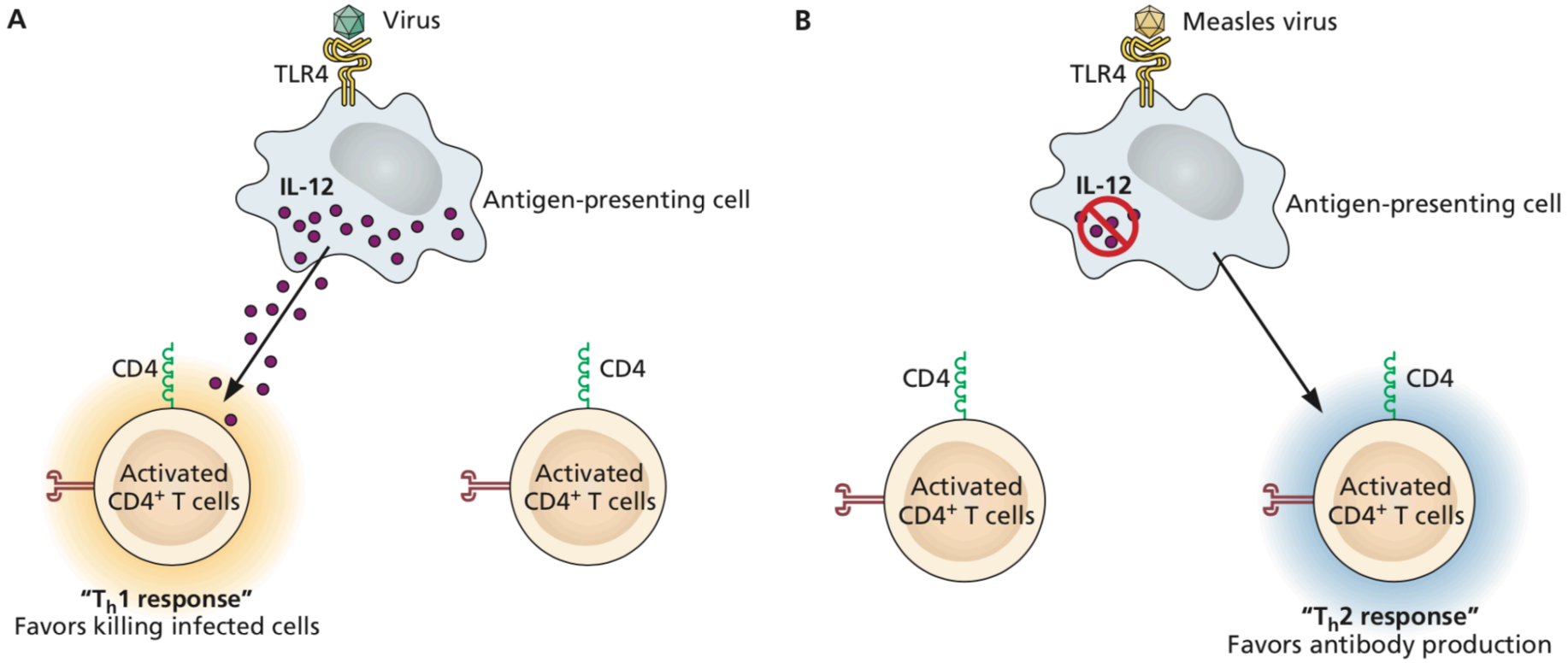
Immunosuppression during measles infection

TB test

Influx of T cells

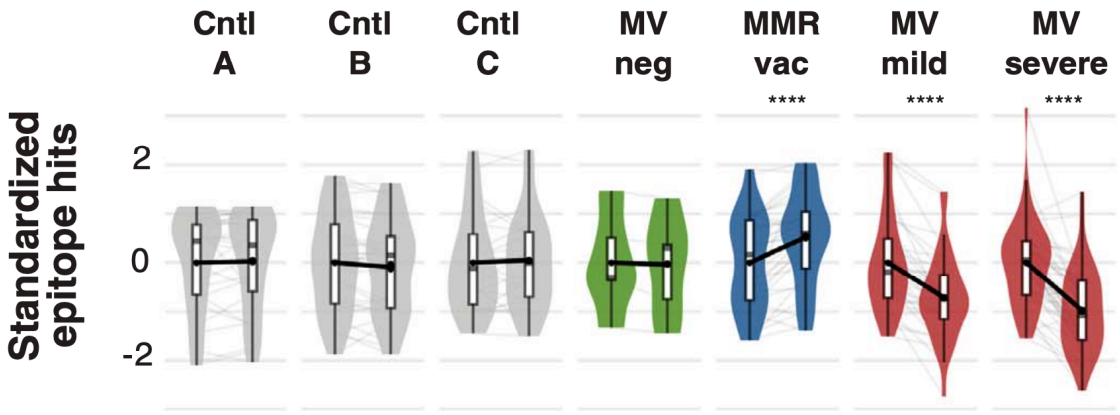


Measles virus immunosuppression

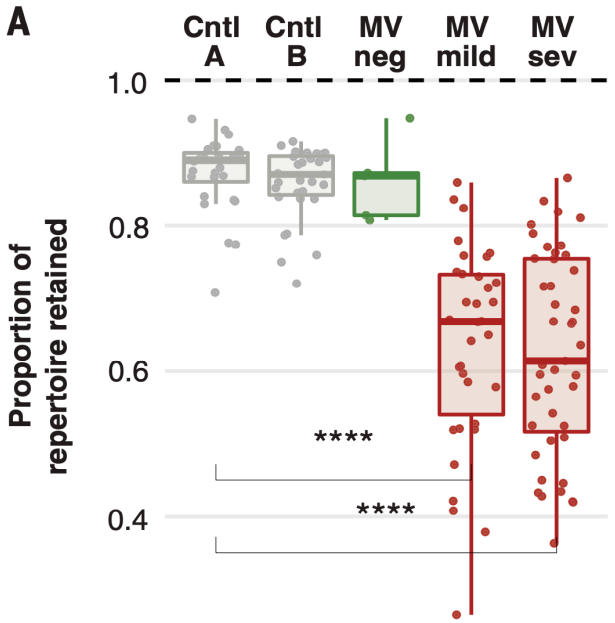


Measles infection erases immune memory

Infection of memory B cells



Serum reactivity from children against full proteomes of ~400 pathogenic human viruses



Proportion of total epitopes detected at time 1 that were retained at time 2

Mina et al., Science 366, 599–606 (2019)

Examples of immunosuppression

Virus	Disease	Cells infected	Manifestation
Measles	Measles	Monocytes, DC Thymic epithelial cells	Reduced T cells Enhanced infections
Rubella	Rubella	Lymphoid cells	Persistent rubella infection
HIV-1	AIDS	CD4+ T cells monocytes	Opportunistic infections Neoplasia

Next time: Acute infections