

Viral Pathogenesis

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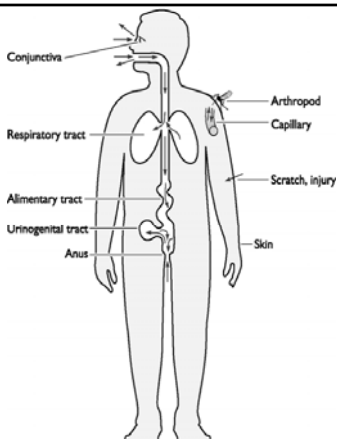
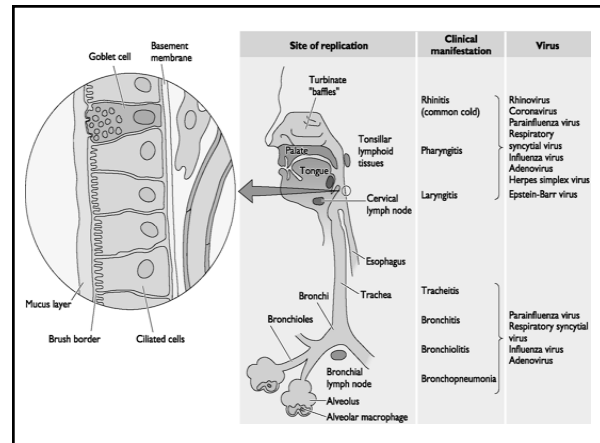
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Respiratory tract

- Most common route of viral entry
- Absorptive area of lung: 140 m²; ventilation rate 6 L/min
- Barriers to infection: swallowing; ciliary action from lower tract; macrophages in alveoli (no cilia or mucus); IgA
- Viruses enter by aerosolized droplets from cough or sneeze, or contact with saliva
- Large droplets lodge in nose; smaller in airways or alveoli

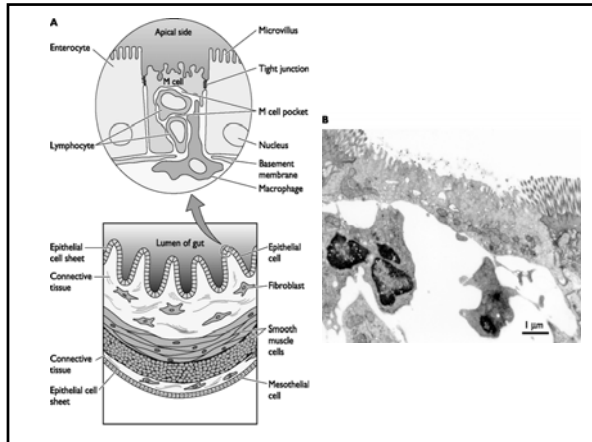
Viral Pathogenesis

- **Pathogenesis:** the process by which one organism causes disease in another
- Two components of viral disease:
 - Effects of virus replication on the host
 - Effects of host response on virus and the host
- The goal of studies on pathogenesis is to identify the viral and host genes that influence the production of disease
- Animal models



Alimentary tract

- Eating, drinking, social activities introduce viruses into the alimentary tract
- Designed to mix, digest, absorb food, so contents are always in motion; good opportunities for virus-cell interactions
- Extremely hostile environment: stomach is acidic, intestine is alkaline; presence of digestive enzymes, bile detergents, mucus, antibodies, phagocytic cells
- Viruses have evolved to infect are resistant: enteroviruses; reovirus (require proteases); enteric coronavirus (enveloped!)



Skin

- Outer layer of dead, keratinized cells cannot support viral infection; entry usually occurs by breaks or punctures
- Skin abrasions; insect or animal bites; needle punctures
- Epidermis is devoid of blood or lymphatics; local replication
- Dermis and sub-dermal tissues are highly vascularized; infection may spread

Urogenital tract

- Protected by mucus, low pH
- Minute abrasions from sexual activity may allow viruses to enter
- Some viruses produce local lesions (HPV)
- Some viruses spread from urogenital tract (HIV, HSV)

Viral Spread

- After replication at the site of entry, viruses may remain localized: virus spreads within the epithelium and is contained by tissue structure and immune system
- Some viruses spread beyond the primary site: **disseminated**; if many organs are infected, **systemic**
- Physical and immune barriers must be breached

Eye

- Sclera and conjunctiva are route of entry
- Every few seconds eyelid passes over sclera, washing away foreign particles; little opportunity for infection
- Infection usually occurs after injury: grit, ophthalmologic procedures, improperly sanitized swimming pools
- Localized infection: conjunctivitis
- Disseminated infection: EV70 spread to CNS
- HSV-1 can infect cornea, blindness may result, virus spread to sensory ganglia

Viral Spread

- Below the epithelium is the basement membrane; integrity can be compromised by epithelial inflammation and destruction
- Below basement membrane are subepithelial tissues, where virus encounters tissue fluids, lymphatic system, and phagocytes; all play roles in clearing and spreading infection
- Role of directional release of virus from polarized cells

Viral Spread

A Influenza/apical

B measles/apical

C VSV/basolateral

- Apical release facilitates virus dispersal; virus usually does not invade underlying tissues
- Basolateral release provides access to underlying tissues and may facilitate systemic spread
- Sendai virus: apical release from respiratory tract, local infection; mutant that is released from both apical and basal surfaces causes disseminated infection

Pathogenesis of mousepox

- Frank Fenner
- First to demonstrate how disseminated viral infections develop from local multiplication to primary and secondary viremia to target organs

Day 0: Skin invasion, Multiplication

Day 1: Regional lymph node, Multiplication

Day 2: Bloodstream: Primary viremia

Day 3: Spleen and liver: Multiplication, Necrosis

Day 4: Bloodstream: Secondary viremia

Day 5: Skin: Focal infection, Multiplication

Day 7: Swelling of foot: Primary lesion

Day 9: Early rash: Papules

Day 10: Sparse rash: Macules

Hematogenous Spread

Labels: Epithelium at body surface, Basement membrane, Lymphatic capillary, Postcapillary venule, Vein, Great vein, Thoracic lymph duct, Efferent lymphatic, Germinal center, Marginal sinus.

- Viruses that produce disseminated infection often do so by entering the blood
- Viruses may enter blood directly through capillaries, by replicating in endothelial cells, or through vector bite
- Virus in the extracellular fluids is taken up by lymphatic capillaries, which are more permeable than circulatory capillaries, then spread to blood
- Once in blood, virus has access to almost every tissue
- In lymph nodes, viruses encounter lymphocytes and other immune cells, and may replicate in them; may also spread infection to distant tissues
- Other viruses spread freely in the blood

Neural spread

- Many viruses spread from primary site of infection by entering local nerve endings
- For some viruses (rabies, alpha herpesviruses) neural spread is definitive characteristic of pathogenesis
- For other viruses (poliovirus, reovirus) invasion of the CNS is an infrequent diversion from normal replication and hematogenous spread

Viremia

Y-axis: Relative virus titer (0-4)

X-axis: Days after infection (0-14)

Primary viremia peaks at day 1.

Secondary viremia peaks at day 10.

- Presence of infectious virus in the blood
- Active viremia: results from virus replication
- Passive viremia: results from virus introduced into the blood without replication
- Diagnostic value
- Practical problems (blood supply)

Labels: Spinal cord, Dorsal root ganglion, Sensory pseudounipolar neuron, Motor neuron, Motor end plate, Axon, Perineural Lymphatics, Endoneurial space, Schwann cell, Sensory nerve ending.

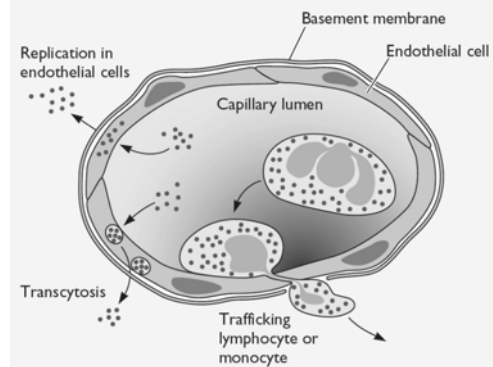
Arrows: Anterograde, Retrograde.

Poliovirus
Yellow fever virus
Herpes simplex virus
Rabies virus

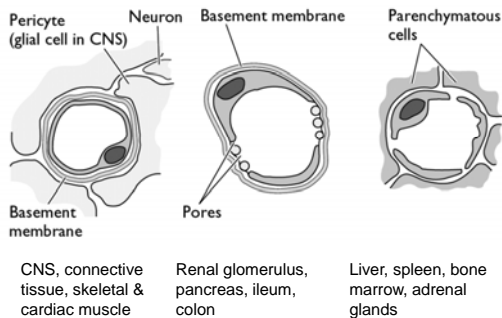
Infections of the CNS

- A **neurotropic** virus can infect neural cells; infection may occur by neural or hematogenous spread from a peripheral site
- A **neuroinvasive** virus can enter the CNS after infection of a peripheral site
- A **neurovirulent** virus can cause disease of nervous tissue
- HSV: low neuroinvasiveness, high neurovirulence
- Mumps: high neuroinvasiveness, low neurovirulence
- Rabies: high neuroinvasiveness, high neurovirulence

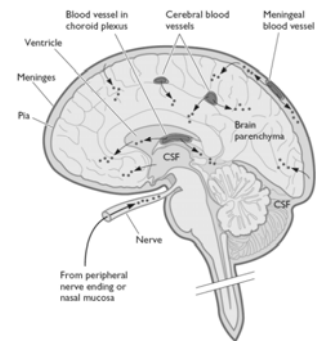
Tissue invasion: blood-brain junction



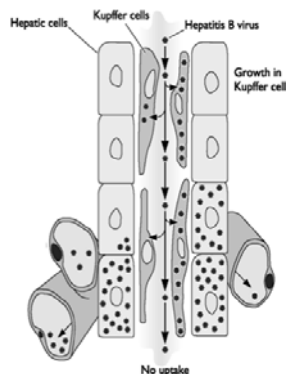
Tissue invasion



Tissue invasion: CNS



Tissue invasion: Liver



Tissue Tropism

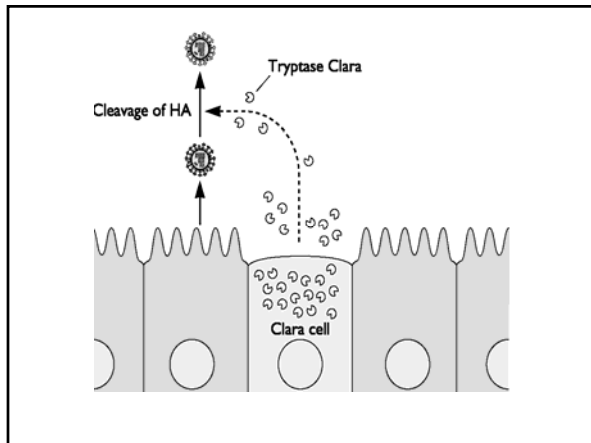
- The spectrum of tissues infected by a virus
 - e.g. an enteric virus replicates in the gut and not in the lung; a neurotropic virus replicates in cells of the nervous system and not in hematopoietic cells
- The tropism of some viruses is limited; other viruses are pantropic, e.g. can replicate in many organs
- What are the determinants of viral tropism?

Determinants of Tissue Tropism

- Cell receptors for viruses
 - e.g. HIV-1 & CD4; EBV & CR2 but not poliovirus or influenza virus
- Cellular proteins that regulate viral transcription
 - e.g. JC papovavirus replicates in oligodendrocytes because the viral enhancer is active only in this cell type
- Cell proteases
 - e.g. cleavage of influenza virus HA by serine proteases

What makes viruses virulent?

- A major goal of virology is to identify viral and host genes that determine virulence
- Virulence genes are usually identified by mutation: deletion or disruption of one of these genes results in a virus that causes reduced or no disease in a specified system
- Viral genes affecting virulence fall into four classes:
 - Those that affect the ability of the virus to replicate
 - Those that modify the host's defense mechanisms
 - Those that enable the virus to spread in the host
 - Those which have intrinsic cell killing effects



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

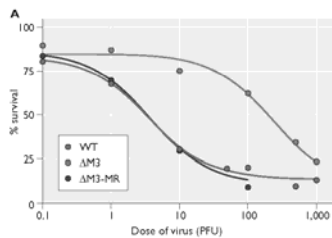
Viral virulence

- The capacity of a virus to cause disease in an infected host
- A virulent virus causes significant disease, while an avirulent or attenuated virus causes reduced or no disease
- Virulence can be quantitated:
 - LD₅₀ (Lethal Dose 50%; amount of virus needed to kill 50% of infected animals)
 - The mean time to death
 - The mean time to appearance of symptoms
 - Measurement of fever, or weight loss
 - measurement of pathological lesions (poliovirus); reduction in blood CD4+ lymphocytes (HIV-1)

Genes that modify the host's defense mechanisms

- Virokines (secreted proteins that mimic cytokines, growth factors, or similar extracellular immune regulators) and viroceptors (homologs of host receptors or cell surface immune molecules)
- Mimic normal cellular molecules critical to host defense
 - sabotage the body's innate and adaptive defenses
 - Not required for growth in cell culture
 - Most have been found in large DNA viruses (pox, herpes, adenovirus)
- Examples:
 - Soluble cytokine receptor - bind cytokines, block action
 - Proteins that bind key proteins in complement cascade
 - Proteins that affect MHC-1 antigen presentation

A herpesvirus gene that binds cytokines: effect on survival in mice



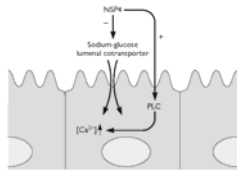
Mechanisms of cell injury by viruses

Non-cytolytic viruses: disease usually a consequence of the immune response: immunopathology

Proposed mechanism	Virus
CD8 ⁺ T-cell mediated	Coxsackievirus B
	Lymphocytic choriomeningitis virus
	Sin Nombre virus
	Human immunodeficiency virus type 1 Hepatitis B virus
CD4 ⁺ T-cell mediated	Th1
	Th2
	Th17
	Th22
	Th25
B-cell mediated (antibody)	Dengue virus
	Feline infectious peritonitis virus

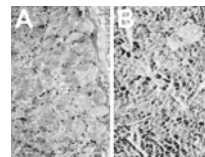
Toxic viral proteins

- NSP4 nonstructural glycoprotein of rotaviruses: a viral enterotoxin
- When expressed in cells, causes increase in intracellular calcium.
- When fed to young mice, causes diarrhea by potentiating chloride secretion. Thus, NSP4 triggers a signal transduction pathway in intestinal mucosa



Mechanisms of cell injury by viruses

- Lesions associated with **CD8⁺ T cells**: myocarditis caused by coxsackievirus B
- Hypothesis: tissue damage due to cytotoxicity of CD8⁺ T cells; perforin knockout mice develop less severe disease
- CD8⁺ T cells may also release proteins that recruit inflammatory cells which elaborate proinflammatory cytokines



How do viruses injure cells?

- Infection of cultured cells by cytolitic viruses: cytopathic effects
- Many viruses cause inhibition of host protein and RNA synthesis, which leads to loss of membrane integrity, leakage of enzymes from lysosomes, cytoplasmic degradation
- Syncytium formation by enveloped viruses (parainfluenza, HIV)
- Virus infection can induce *apoptosis* (programmed cell death)

Lesions associated with B cells: Dengue

- Caused by Dengue virus, transmitted mainly by bites of *Aedes aegypti* mosquitoes
- Endemic in the Caribbean, Central and South America, Africa and Southeast Asia
- 50 million infections/year
- Primary infection is usually asymptomatic, but may result in standard symptoms of virus infection: *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

Dengue Fever

- In 1/14,000 *primary* infections, people get **Dengue Hemorrhagic Fever (DHF)**, a life threatening disease.
- Patients produce antibodies to virus, but there are four serotypes, and no cross-protection
- Non-protective antibodies can **enhance** the infection of peripheral blood monocytes by ***F_c-receptor mediated uptake*** of antibody coated virus particles. Infected macrophages release cytokines, causing severe symptoms
- After *secondary* dengue infections, (i.e. infections of people with antibody to Dengue virus), the incidence of DHF 1/90.

Cell injury associated with free radicals

- Superoxide (O_2^-) and nitric oxide (NO) are produced during the inflammatory response
- NO is made by nitric oxide synthase, an interferon-inducible enzyme
- Low concentrations of NO have a protective effect, high concentrations cause tissue damage by reacting with O_2^- to form peroxynitrite, which is much more reactive than either radical

