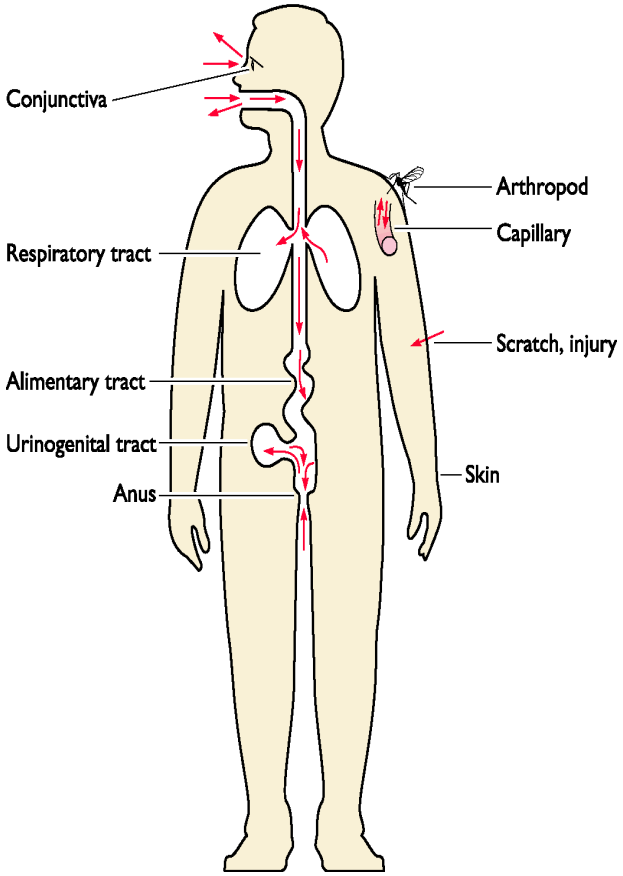
VIROLOGY

Mechanisms of Viral Pathogenesis

The nature of host-virus interactions

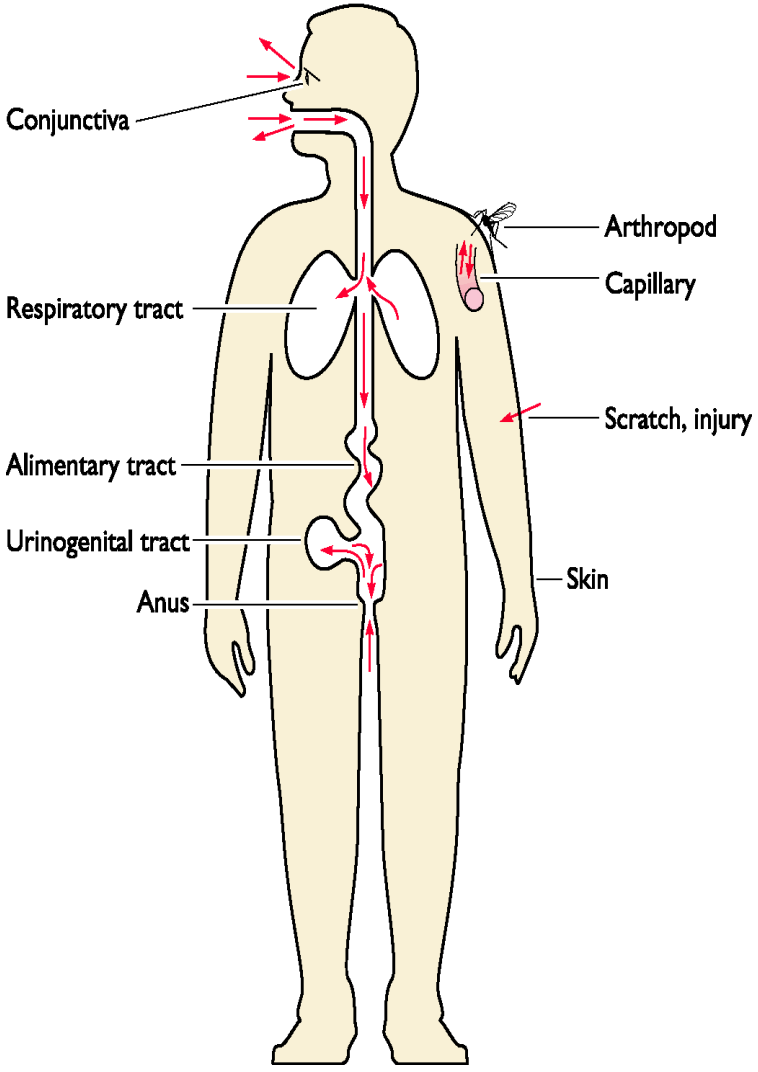


The virus genome must establish itself in a host population to endure

Thus, viruses cannot be avoided; virus infections are a fact of life

All individuals inevitably encounter viruses that infect then and make them unwell

The nature of host-virus interactions



Viral Pathogenesis refers to the series of events that occur during viral infection of a host

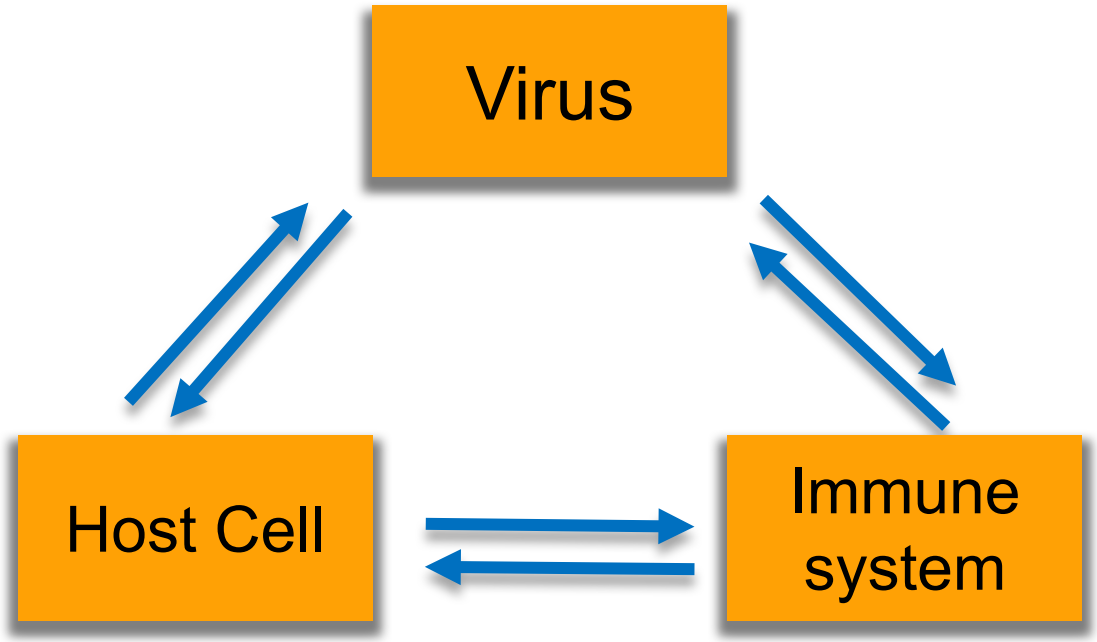
Viral pathogenesis:
is a disease the outcome of all viral infections?

- The outcome of a viral infection depends from the the characteristics of the virus, the virus-host interactions, and from the host defense responses

- It is the ***sum*** of the effects on the host of the ***virus replication*** and of the ***host responses***

Viral pathogenesis:
is a disease the outcome of all viral infections?

- *Pathogenesis*: the process of producing a disease
- The outcome of all viral infections is determined by the three-way interaction between virus, host cell and immune system



VIRAL PATHOGENESIS

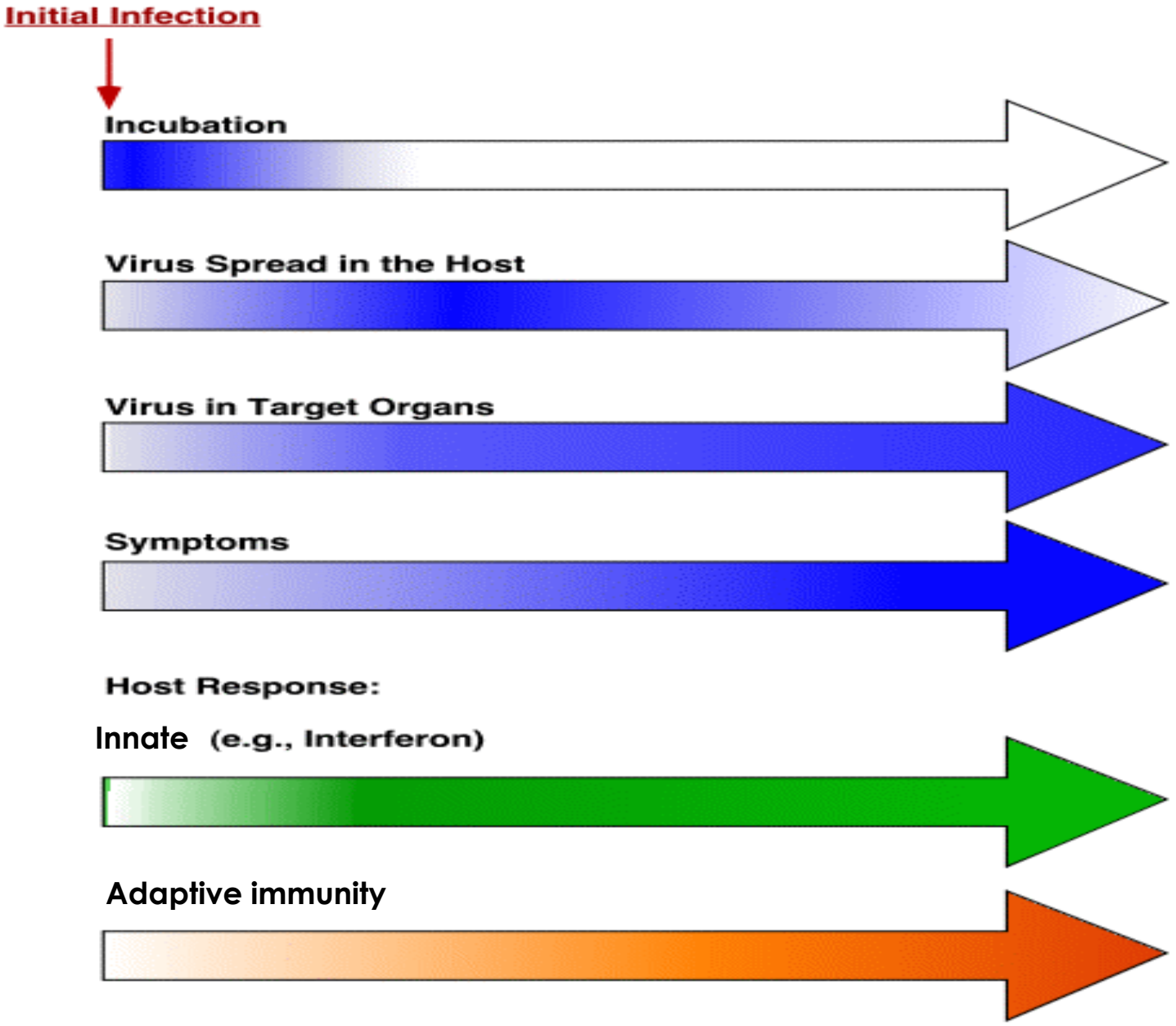
The process by which viruses cause disease

- Viral entry
- Viral spread
- Tissue invasion
- Tropism
- Virus shedding and transmission

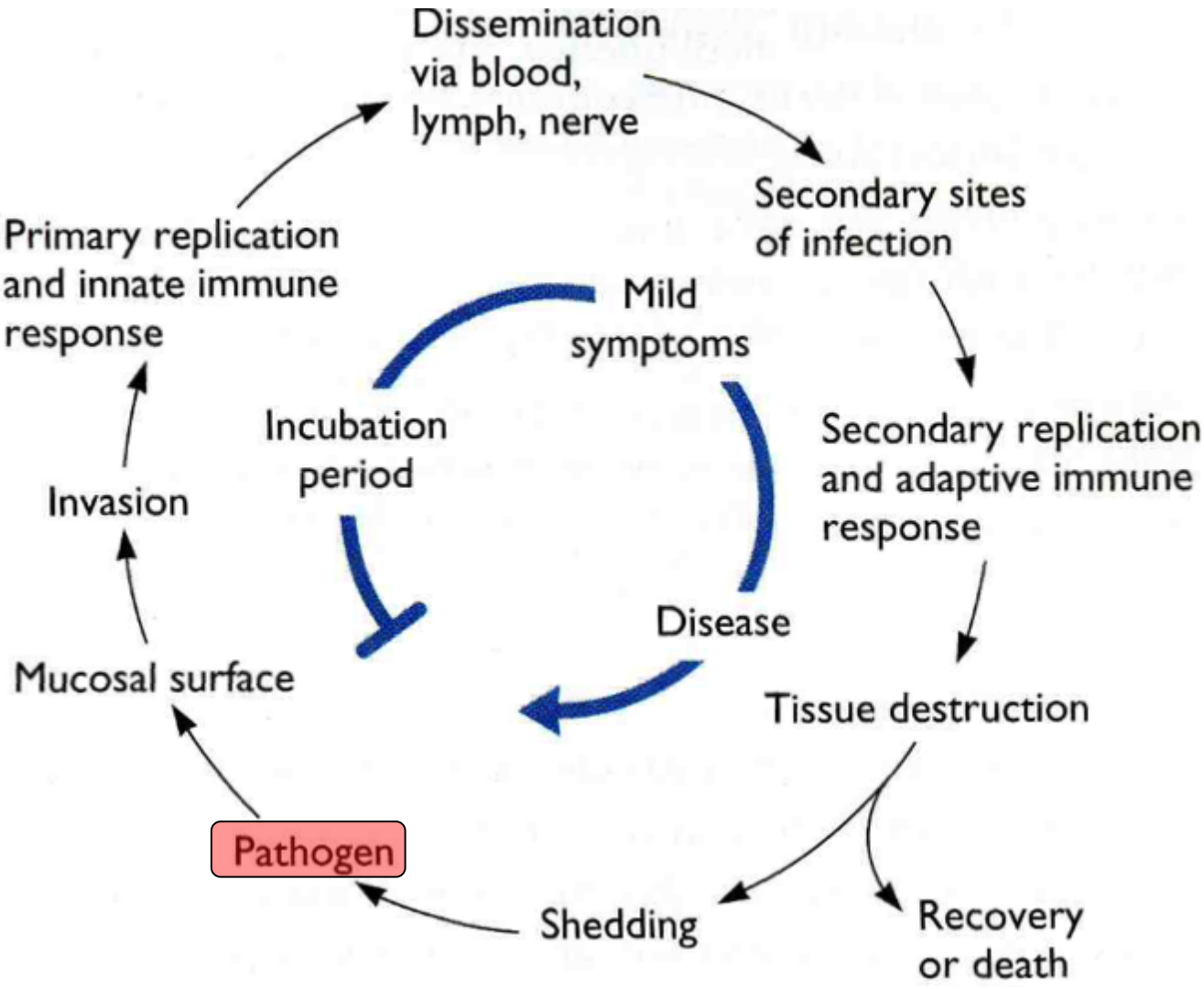
- The host defense

- Disease

Viral pathogenesis: *time course of typical infection*



Views of viral pathogenesis



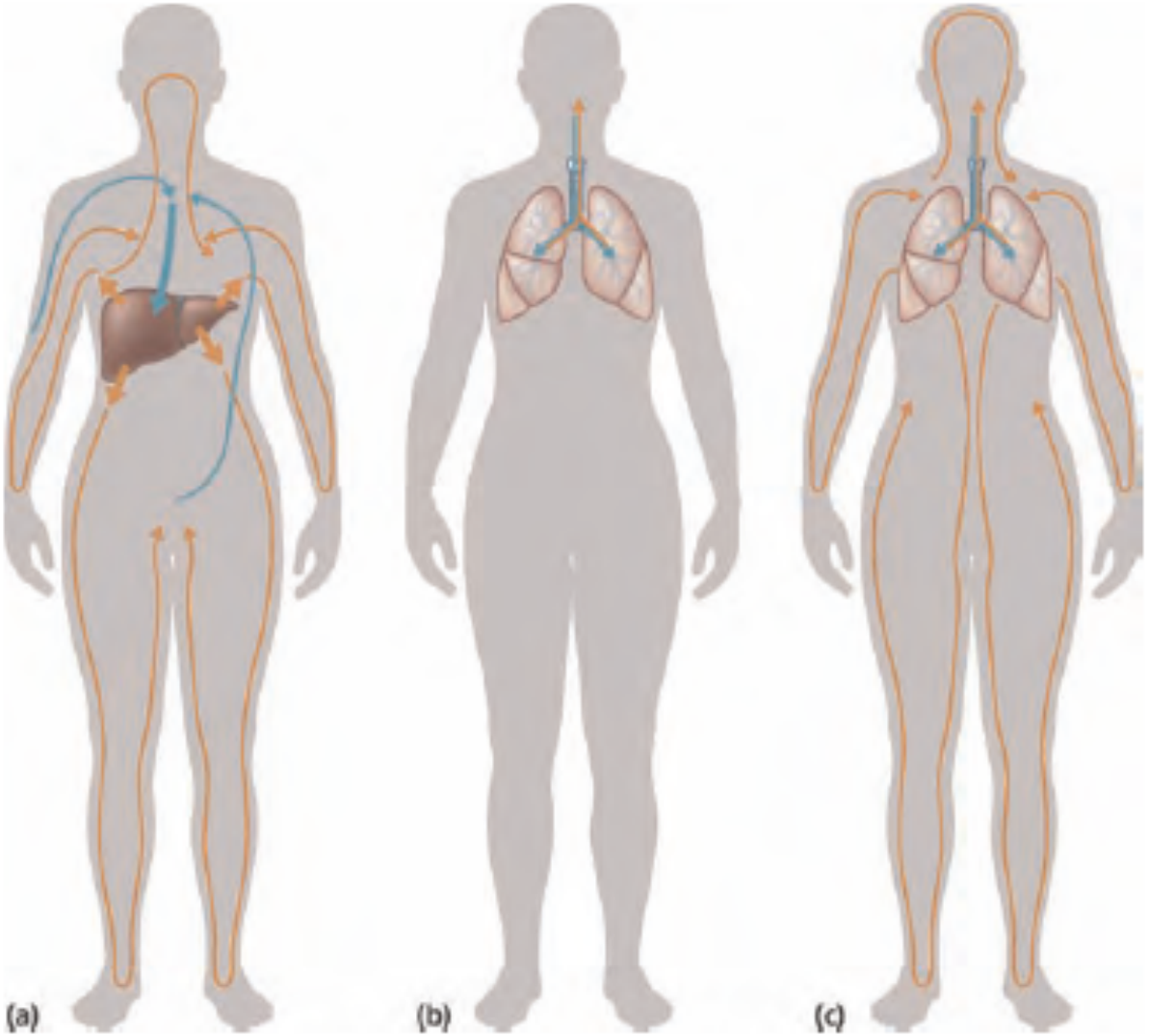
Infection viewed as a series of steps with a predictable outcome

Viral pathogenesis:
is a disease the outcome of all viral infections?

Determinants of viral disease:
the nature of the disease

- viral cell and tissue tropism
- type of target tissues (replication sites)
- pathways of viral entry
- viral spread to the replication sites
- cells permissivity to virus replication
- virulence of viral strain

Three scenarios for the relationship between the route of entry of a virus and the nature of the disease



Viral pathogenesis:
is a disease the outcome of all viral infections?

Determinants of viral disease:
the severity of the disease

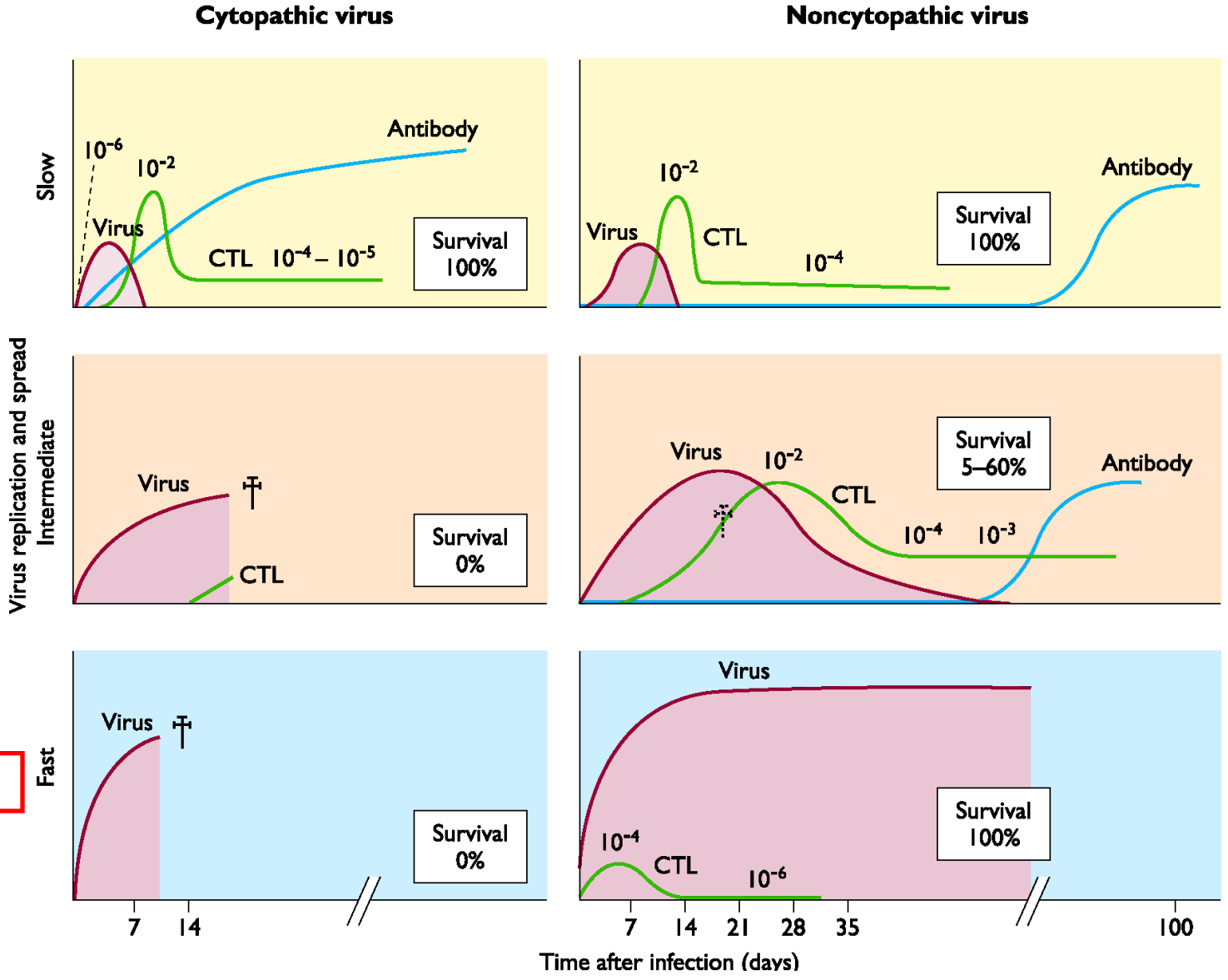
- cytopathogenic attitude of the virus
- immunopathology
- initial inoculum of the virus
- compromised host, competence of the immune system
- host genetic background
- age
- previous exposure to the virus (immunity)

Importance of kinetics of virus replication and immune responses

Slow localized spread

Intermediate spread

Fast disseminate spread



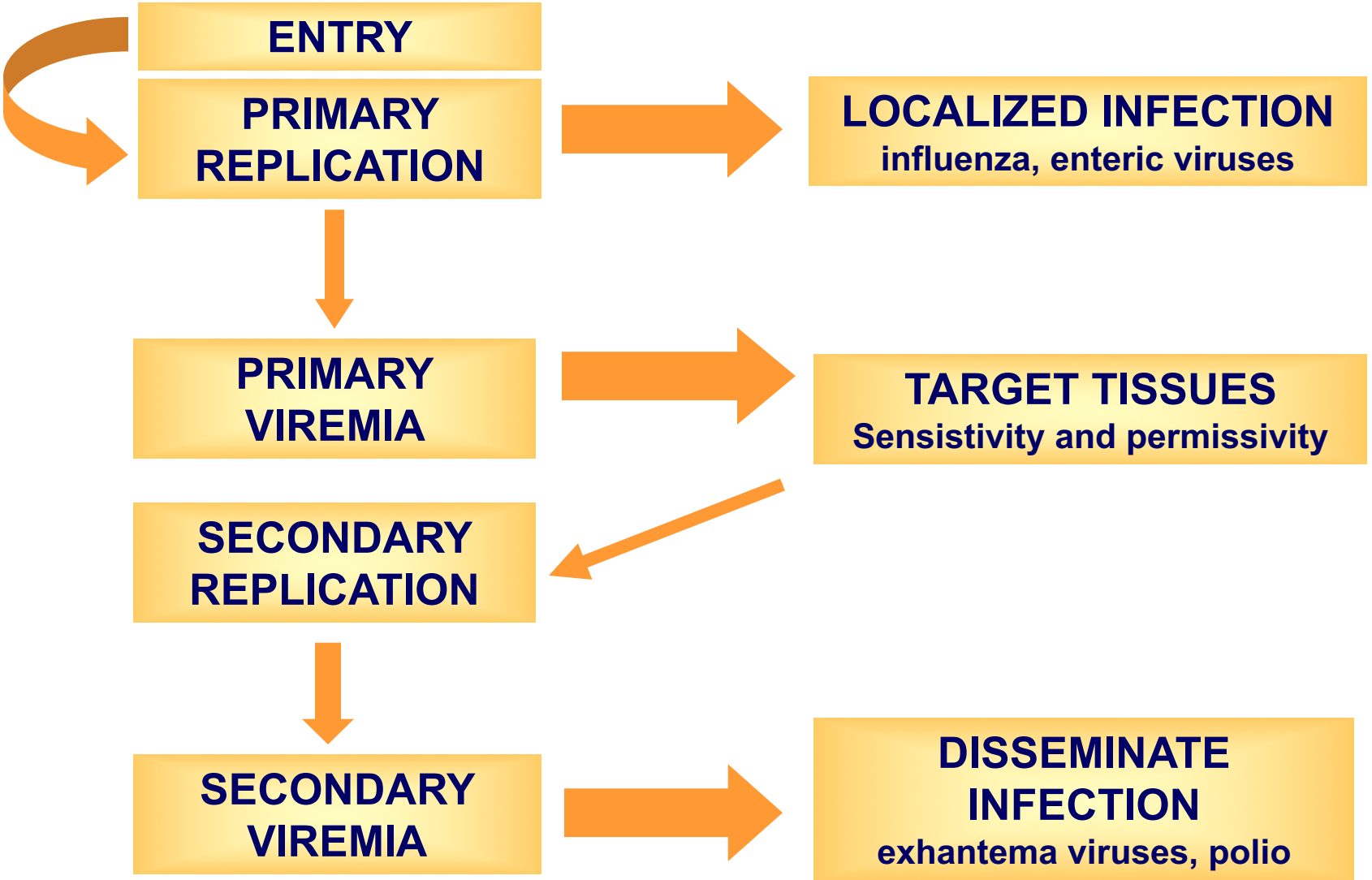
The kinetics of virus replication and the kinetics of the defensive response both affect the outcome of infection

Viral pathogenesis

Fundamental questions about viral pathogenesis:

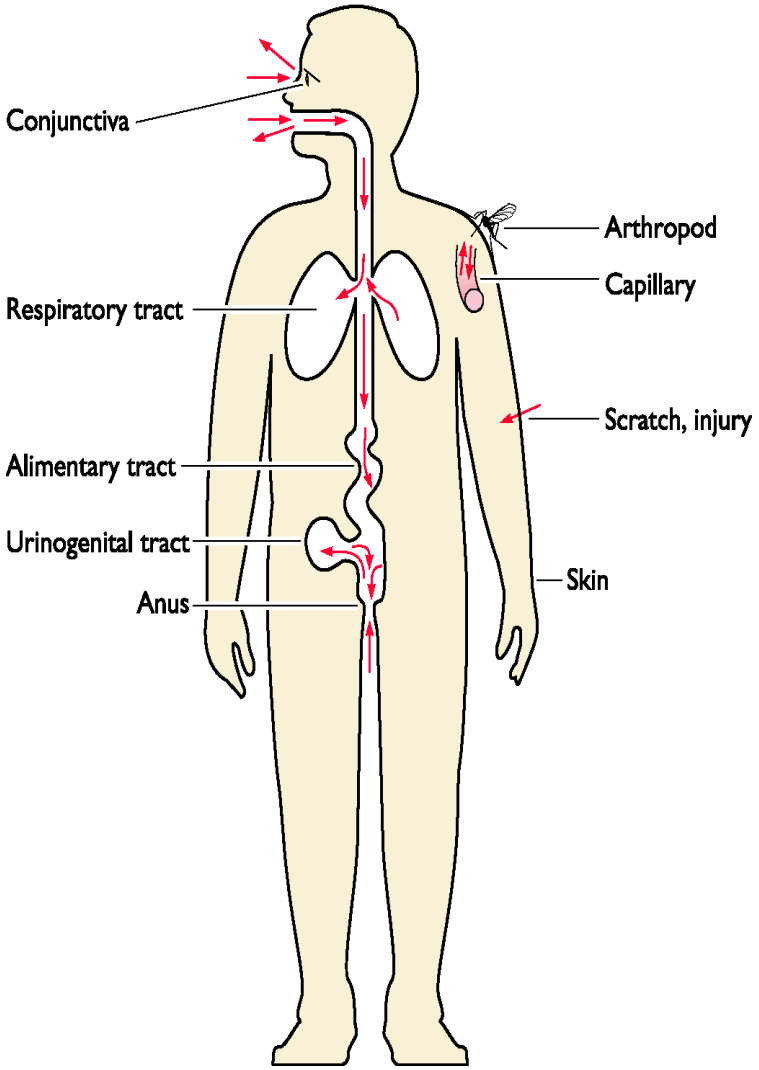
- How does a virus particle enter the host?
- What is the initial host response?
- Where does primary replication occur?
- How does the infection spread in the host?
- What organs and tissues are infected?
- Is the infection cleared from the host or is a persistent infection established?
- How is the virus transmitted to other hosts?

Viral pathogenesis: entry and spread into the host



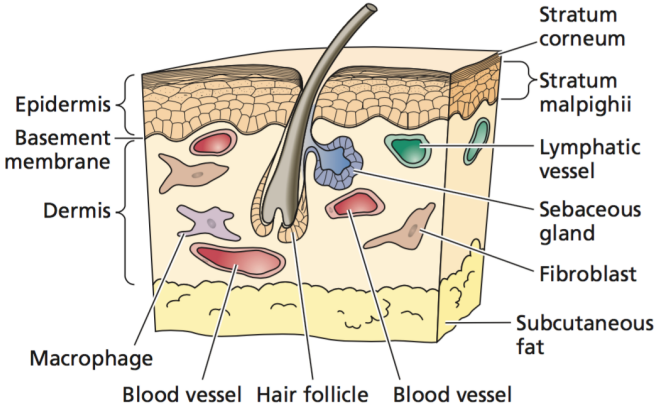
Viral pathogenesis:
three requirements for a successful viral infection

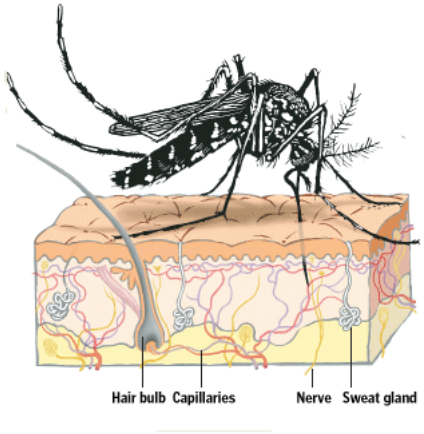
- Enough virus
- Cells accessible, susceptible, permissive
- Local antiviral defense absent or overcome

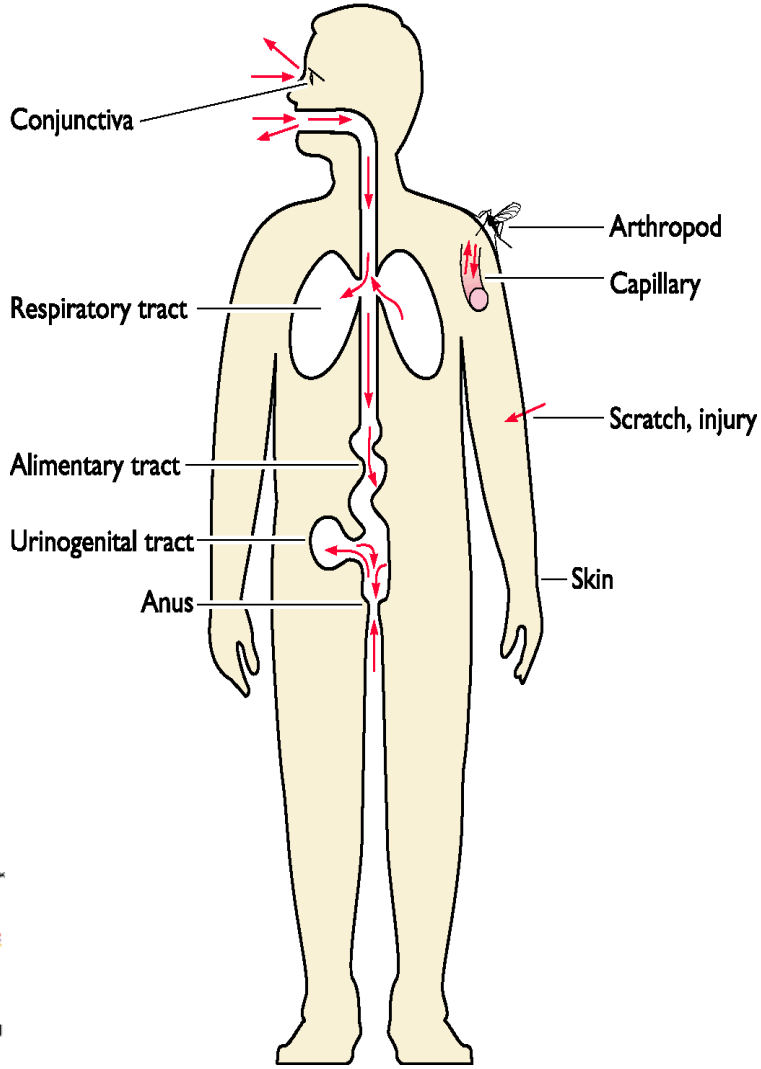


Viral pathogenesis:
gaining access: site of entry is critical

The human body presents only a limited spectrum of entry sites for viral infection.



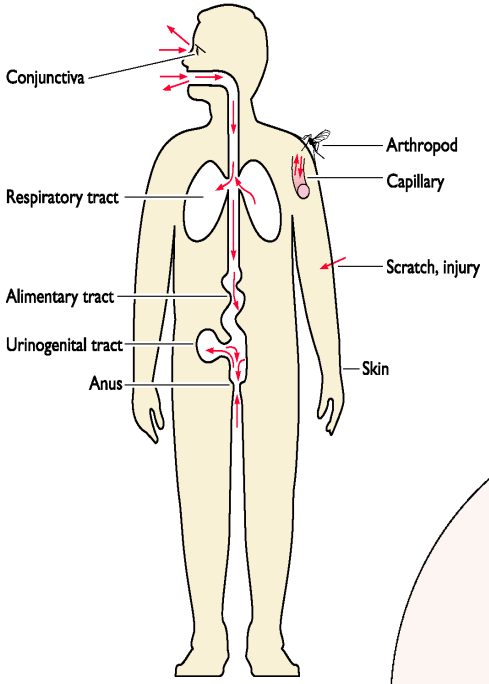


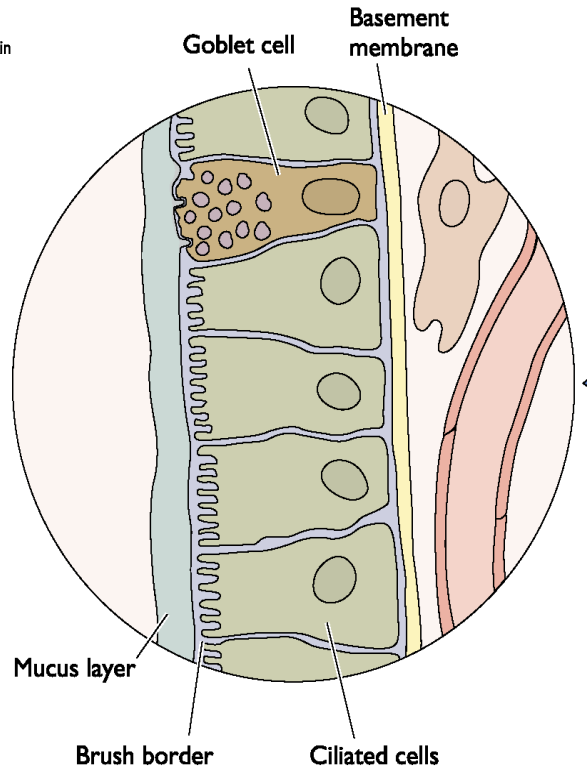


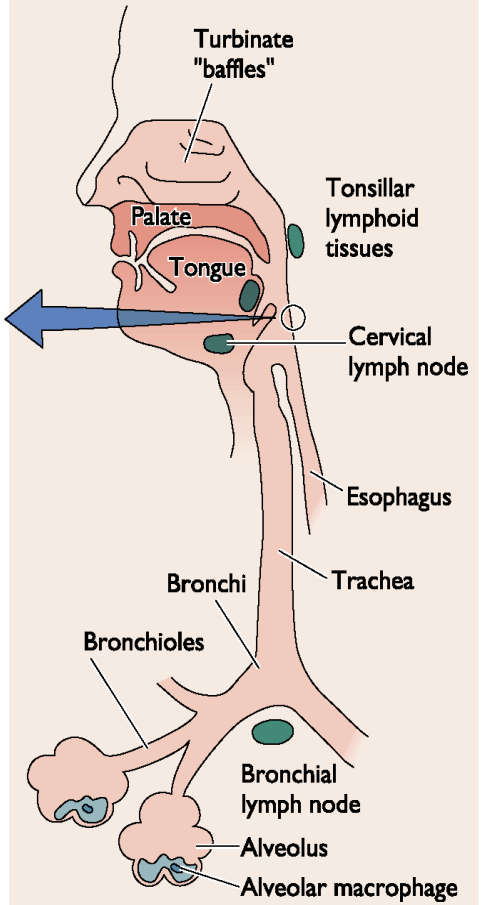
Viral pathogenesis:
different routes of viral entry into the host

Location	Virus(es)
Respiratory tract	
Localized upper tract	Rhinovirus; coxsackievirus; coronavirus; arenaviruses; hantavirus; parainfluenza virus types 1–4; respiratory syncytial virus; influenza A and B viruses; human adenovirus types 1–7, 14, 21
Localized lower tract	Respiratory syncytial virus; parainfluenza virus types 1–3; influenza A and B viruses; human adenovirus types 1–7, 14, 21
Entry via respiratory tract followed by systemic spread	Rubella virus, arenaviruses, hantavirus, mumps virus, measles virus, varicella-zoster virus, poxviruses
Alimentary tract	
Systemic	Enterovirus, reovirus, adenovirus
Localized	Coronavirus, rotavirus
Urogenital tract	
Systemic	Human immunodeficiency virus type 1, hepatitis B virus, herpes simplex virus
Localized	Papillomavirus
Eyes	
Systemic	Enterovirus 70, herpes simplex virus
Localized	Adenovirus types 8, 22
Skin	
Arthropod bite	Bunyavirus, flavivirus, poxvirus, reovirus, togavirus
Needle puncture, sexual contact	Hepatitis C and D viruses, cytomegalovirus, Epstein-Barr virus, hepatitis B virus, human immunodeficiency virus, papillomavirus (localized)
Animal bite	Rhabdovirus

Mucosal surfaces are perfect for viral entry: the respiratory tract

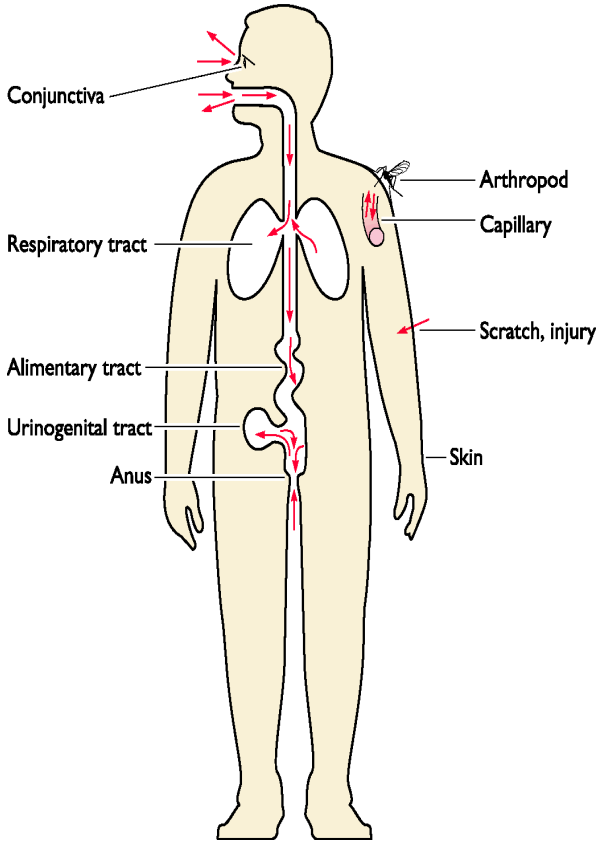


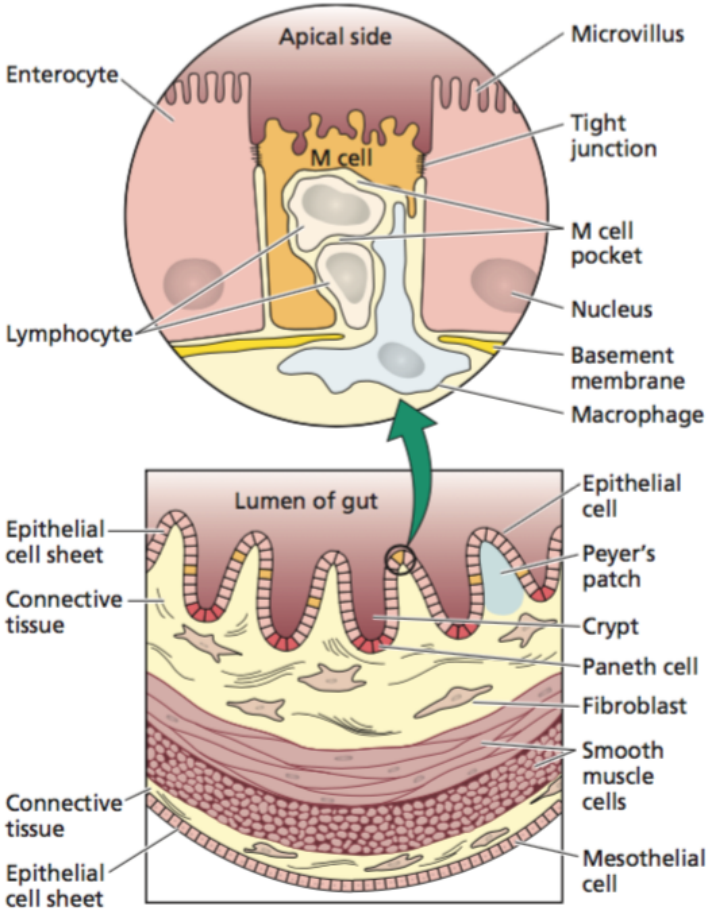




Site of replication	Clinical manifestation	Virus
	Rhinitis (common cold)	Rhinovirus Coronavirus Parainfluenza virus Respiratory syncytial virus Influenza virus Adenovirus Herpes simplex virus Epstein-Barr virus
	Pharyngitis	
	Laryngitis	
	Tracheitis	Parainfluenza virus Respiratory syncytial virus Influenza virus Adenovirus
	Bronchitis	
	Bronchiolitis	
	Bronchopneumonia	

Mucosal surfaces are perfect for viral entry: the alimentary tract

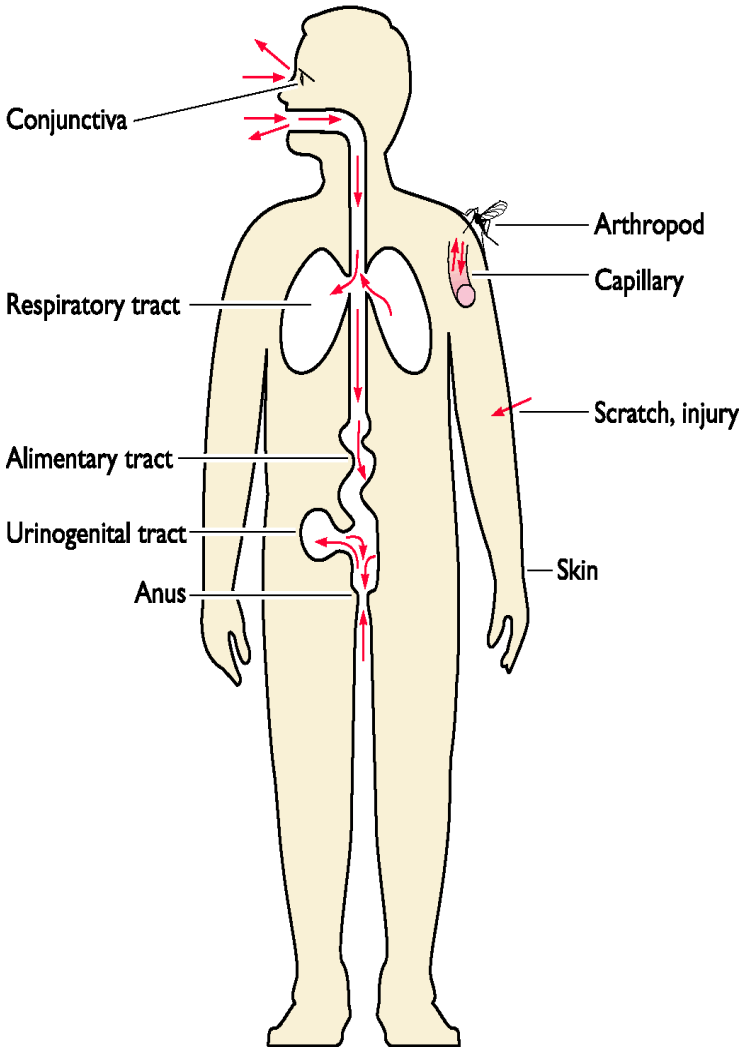




The small intestine

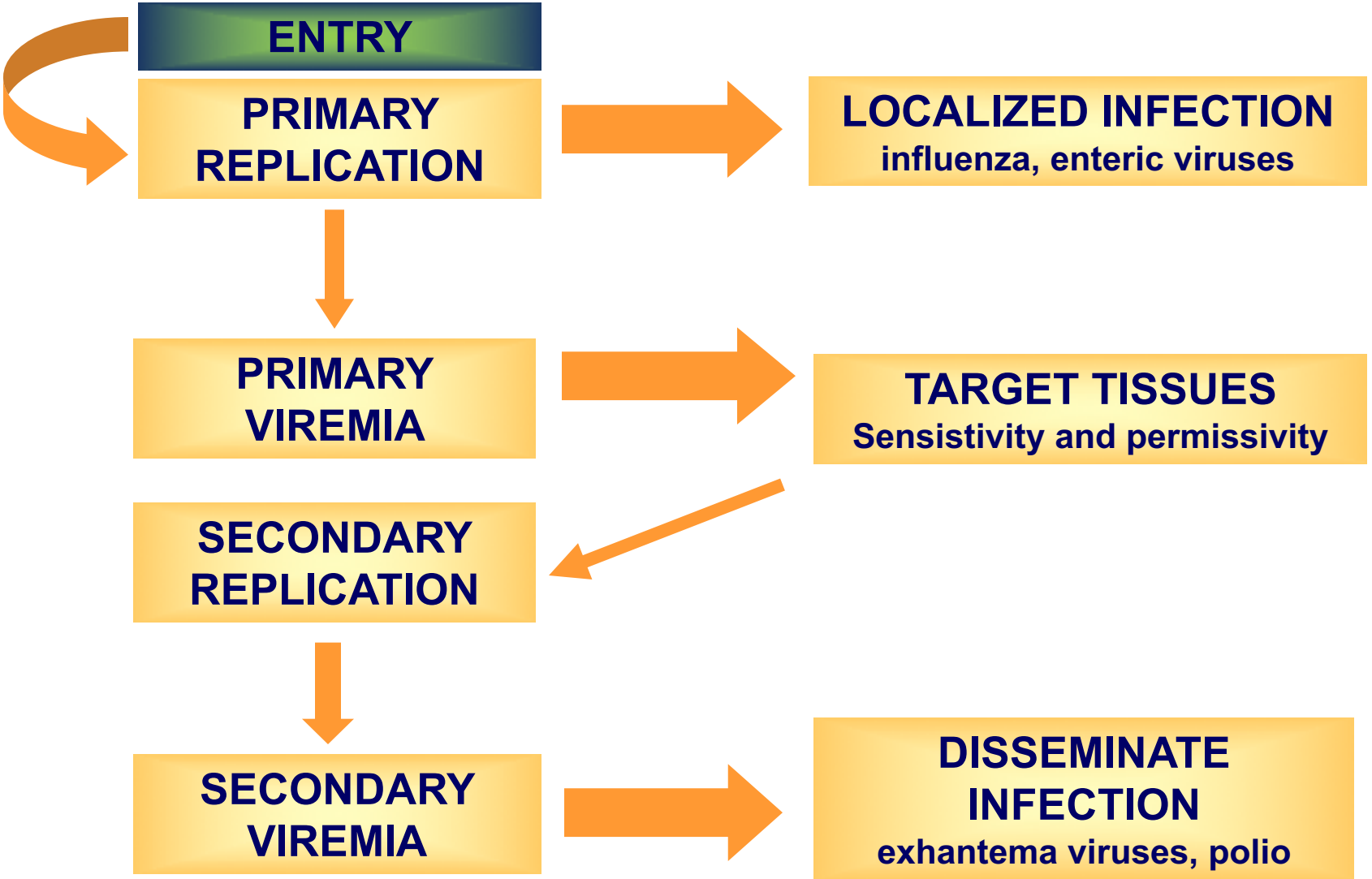
- A selectively permeable barrier endowed with polarized epithelial cells
- Direct contact with outside world
- Direct contact with the immune system and the nervous system

Mucosal surfaces are perfect for viral entry: the 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)

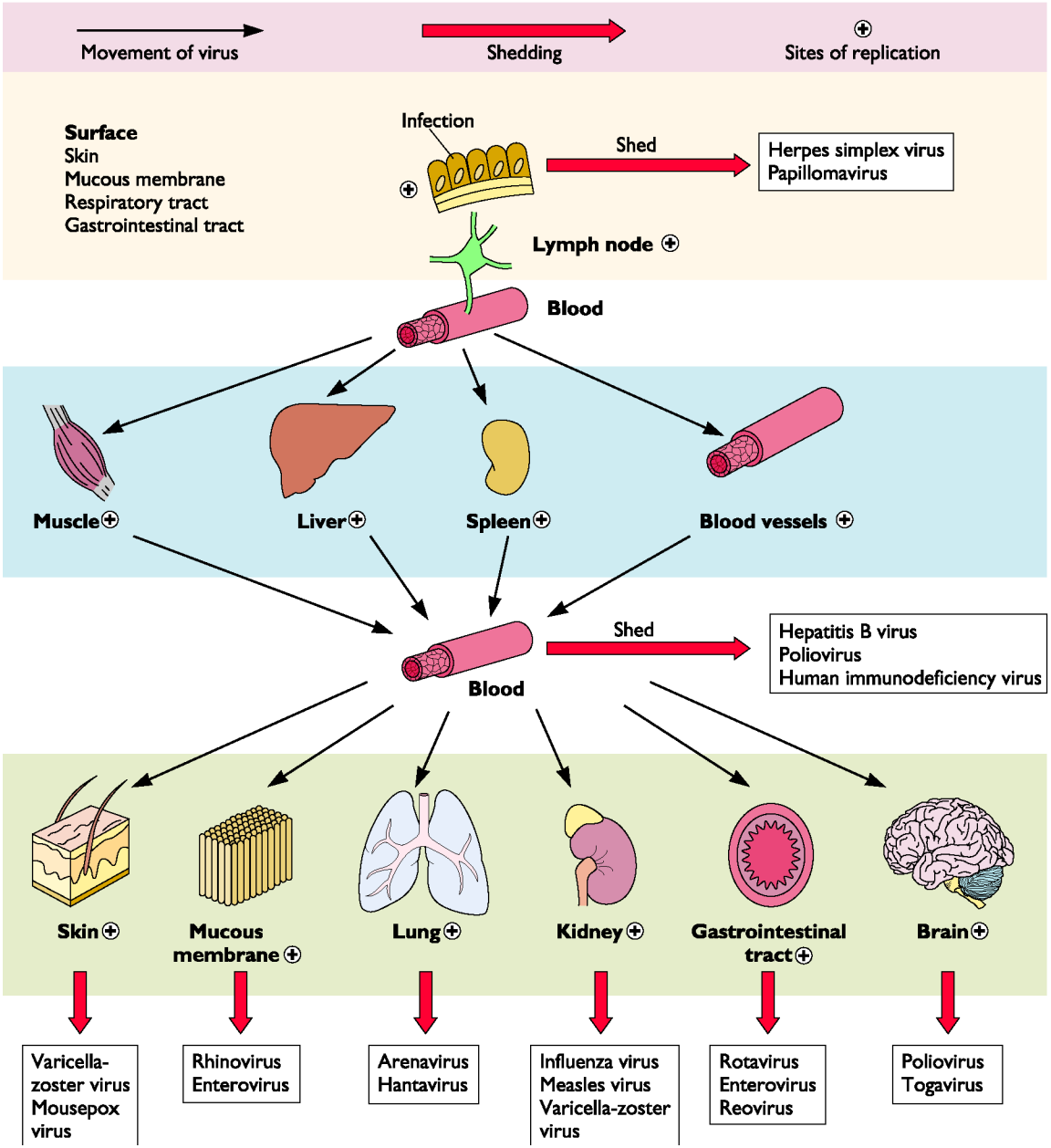
Viral pathogenesis: entry and spread into the host



Entry, dissemination and shedding of blood-borne viruses

Infections can be localized, or can spread beyond the initial site of replication (a disseminate infection)

With many organs involved the infection becomes systemic



Viral pathogenesis: the incubation period

- Initial period before symptoms of disease are obvious
- Viral genomes are replicating
- Host is responding
- Virus may or may not be transmitted

Incubation periods of some common viral infections

Disease	Incubation period (days)[a]
Influenza virus	1–2
Rhinovirus	1–3
Ebola virus	2–21
Acute respiratory disease (adenoviruses)	5–7
Dengue	5–8
Herpes simplex	5–8
Coxsackievirus	6–12
Poliovirus	5–20
Human immunodeficiency virus	8–21
Measles	9–12
Smallpox	12–14
Varicella-zoster virus	13–17
Mumps	16–20
Rubella	17–20
Epstein-Barr virus	30–50
Hepatitis A	15–40
Hepatitis B and C	50–150
Rabies	30–100
Papilloma (warts)	50–150

[a]Until first appearance of prodromal symptoms.

Short - replication at primary site produces symptoms

Long – symptoms beyond primary site

Mechanisms of Viral Pathogenesis:

Examples

Entry, dissemination and shedding of blood-borne viruses

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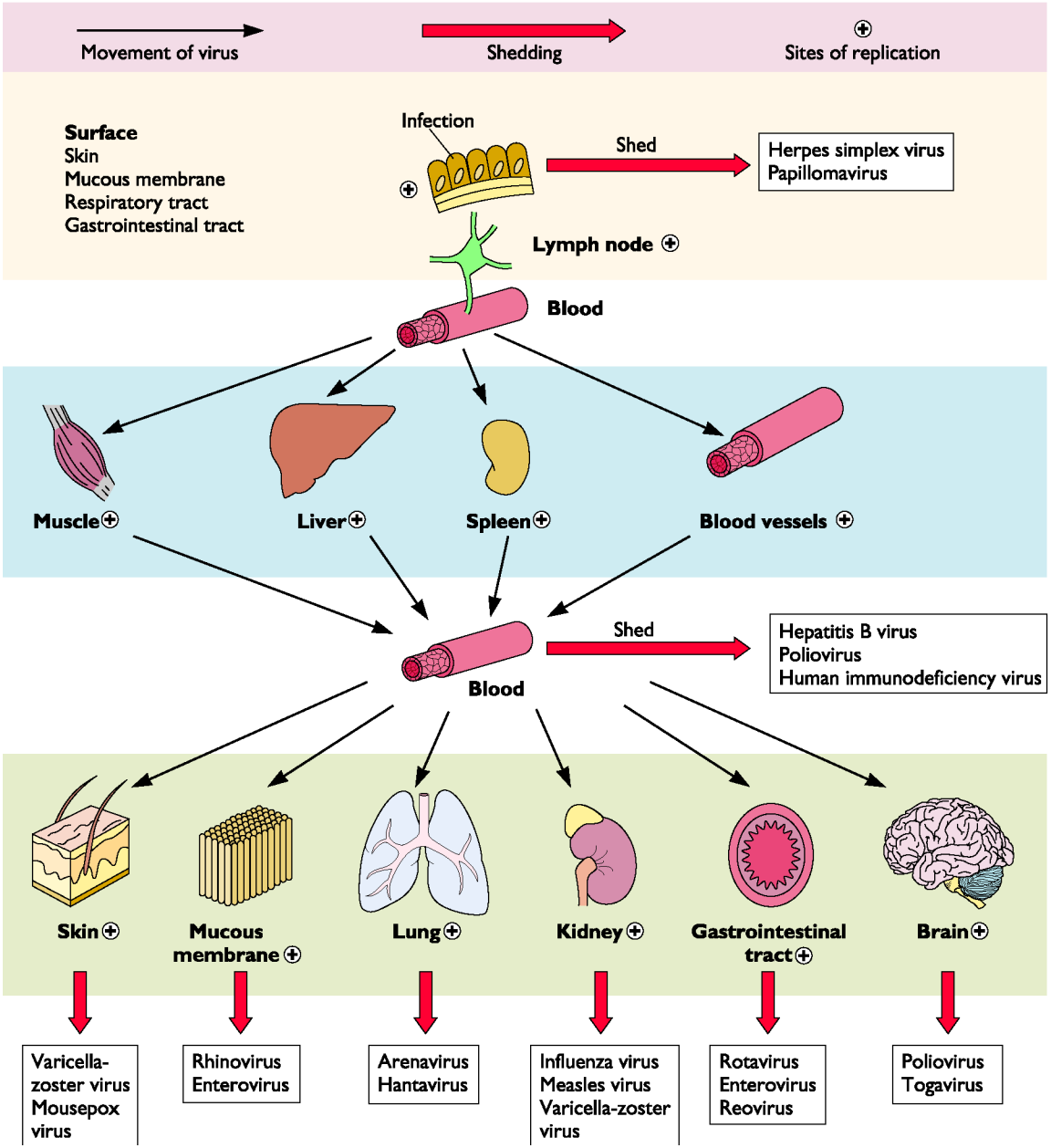
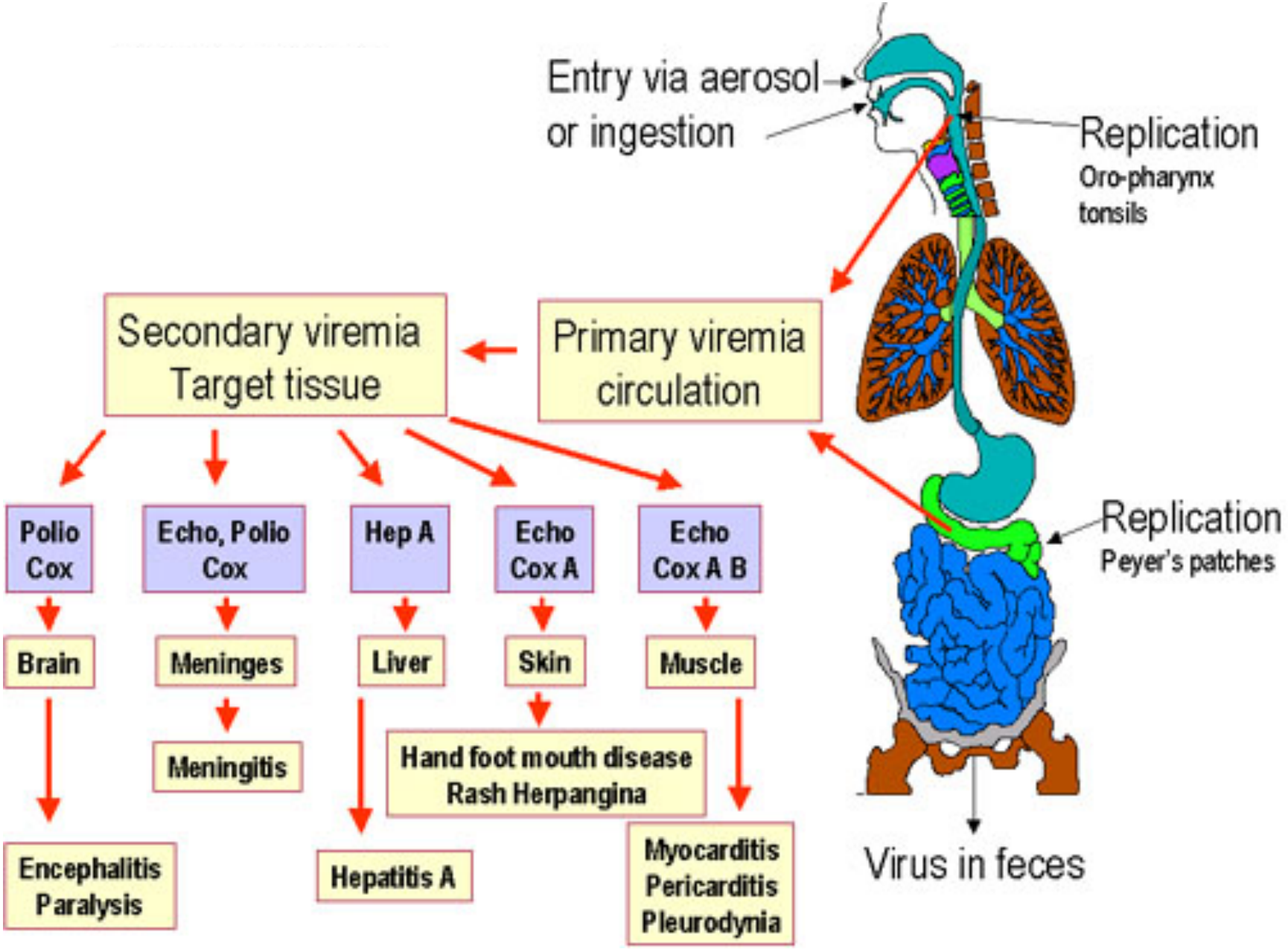
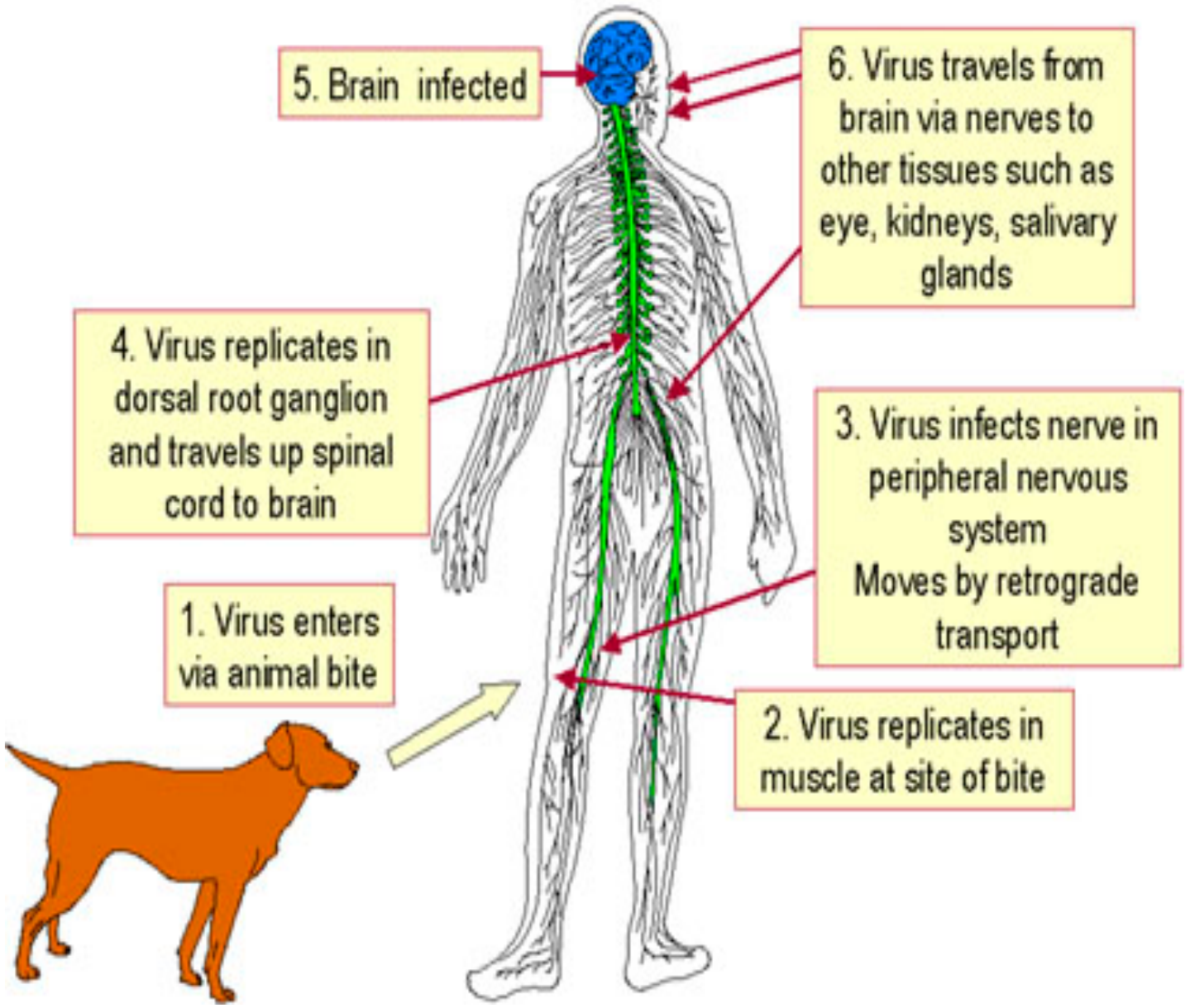


Enterovirus pathogenesis

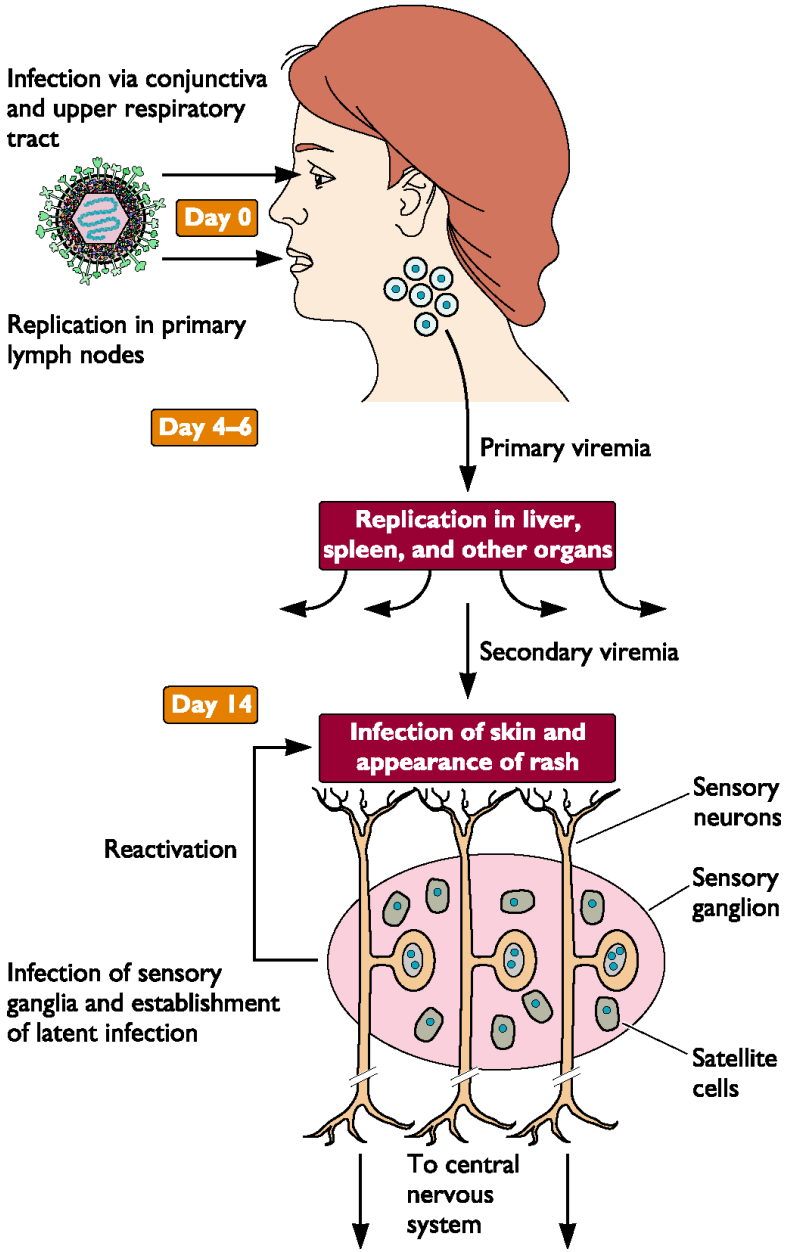


Rabies pathogenesis



Varicella-zoster (VZV):

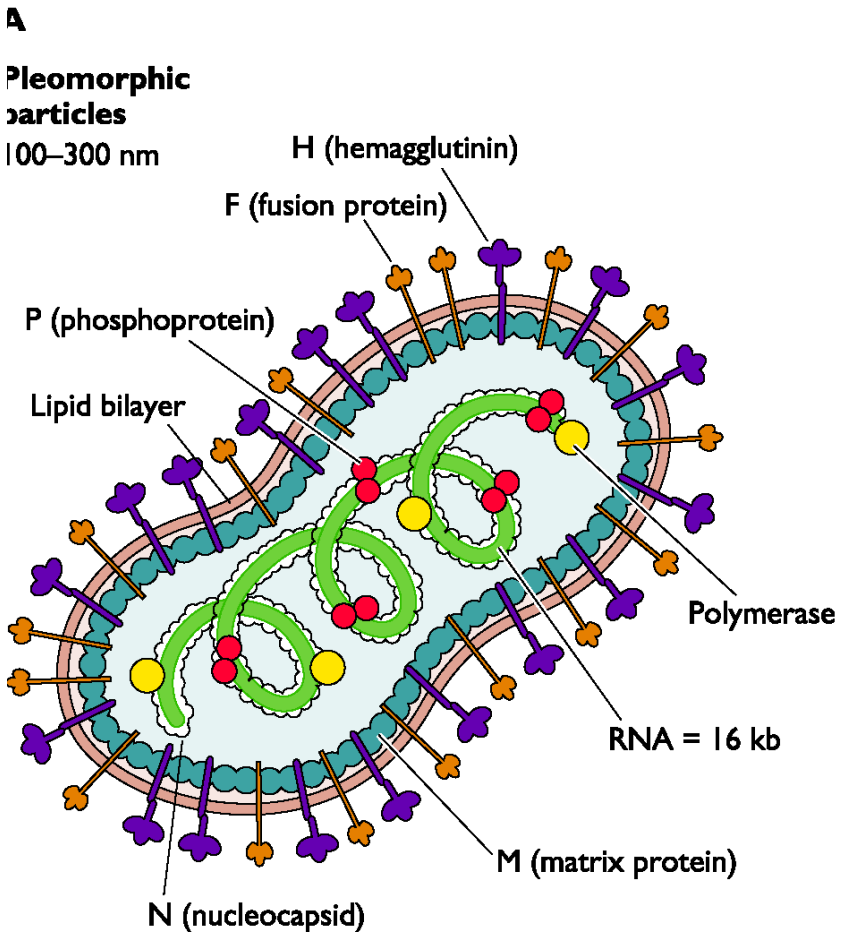
an acute infection with a systemic spread followed by latency

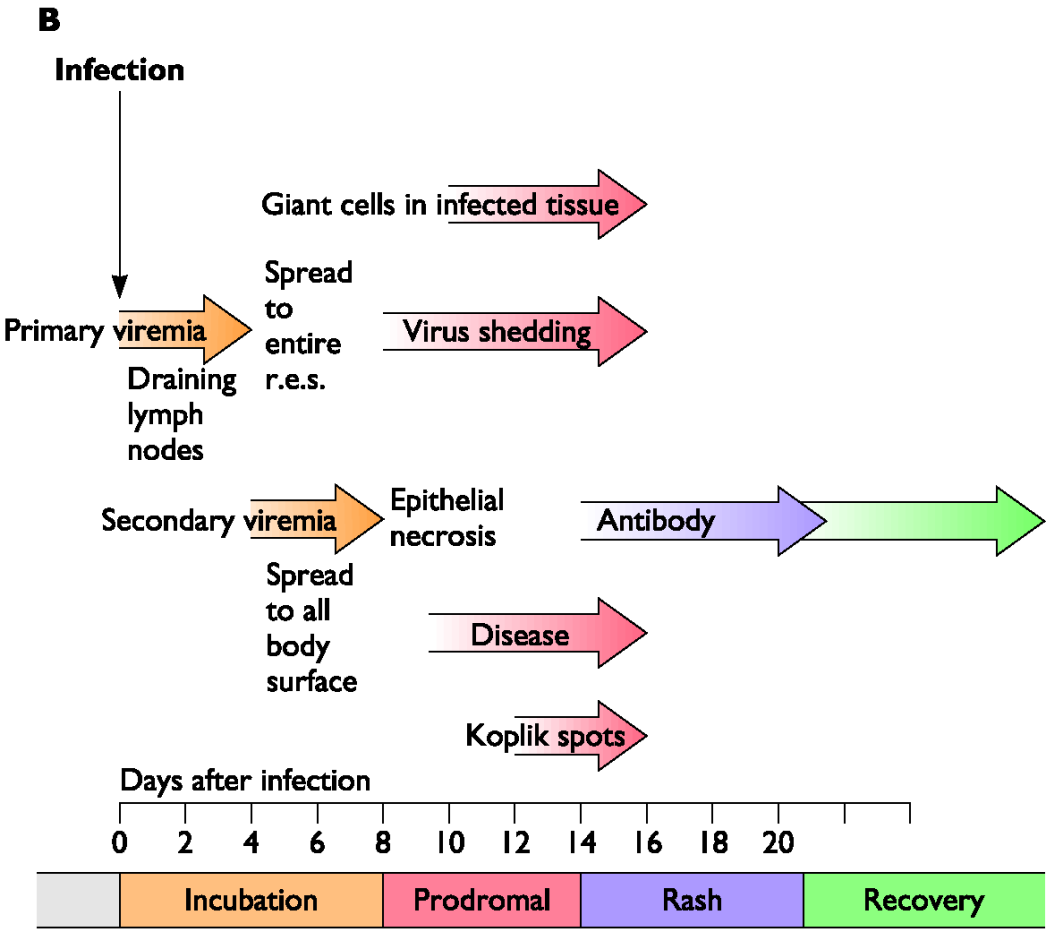


- VZV enters via conjunctiva and upper respiratory tract
- Replication occurs in regional lymph nodes
- Primary viremia via infected T cells
- Replication in visceral organs (liver, spleen, etc.)
- Secondary viremia and subseqeunt acute infection of skin -**"chicken pox"** rash (vesicular lesions with infectious virus)
- Latency establish in sensory ganglia of PNS
- Reactivation results in "shingles"-postherpetic neuralgia

Measles:

an acute viral infection with a systemic spread that can evolve in a non infectious slow progressive disease



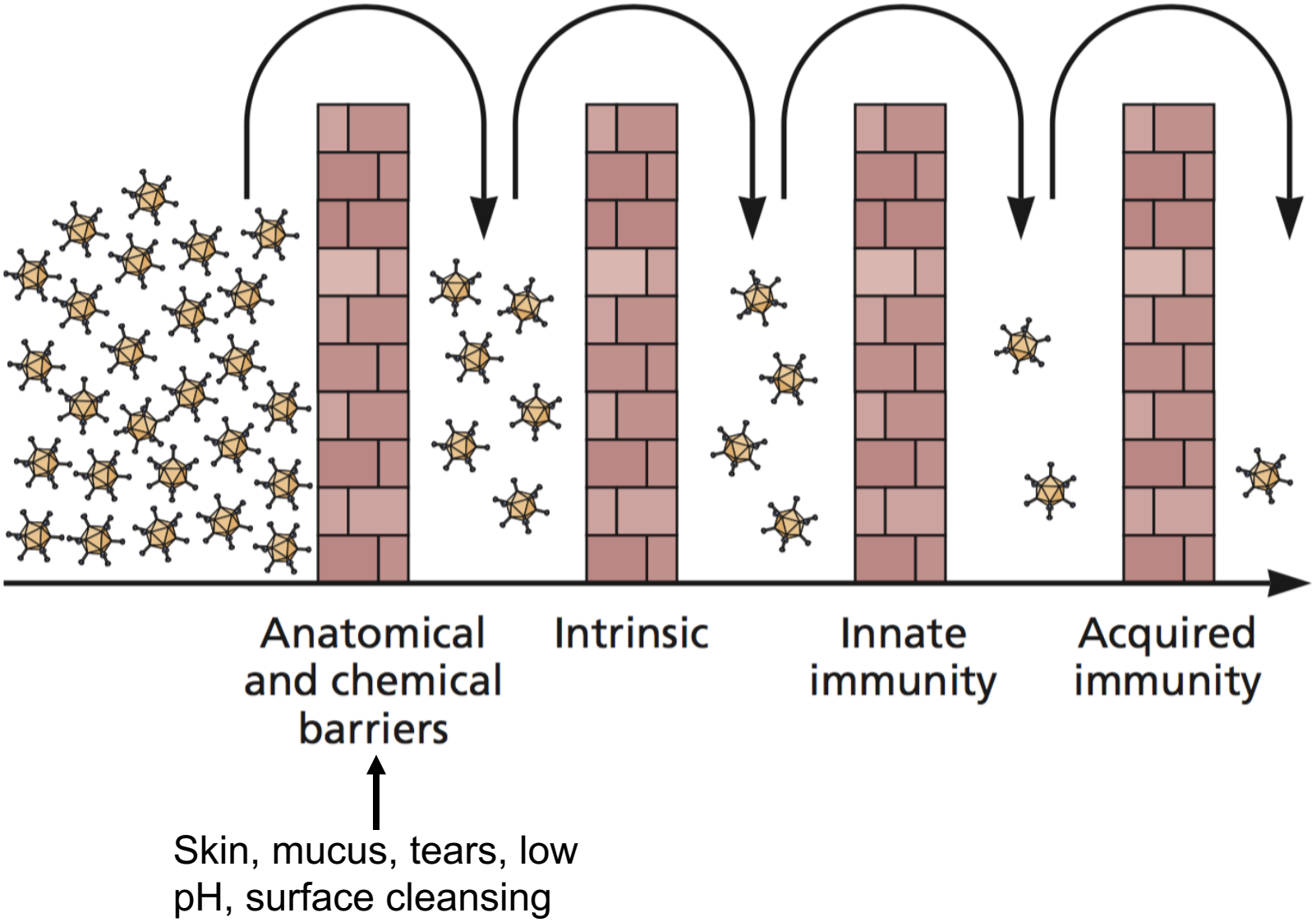


Measles Inclusion Body Encephalitis (MIBE) 1 case over 2 x10^3 acute measles
Subacute Sclerosing Panencephalitis (SSPE) 6/22 cases over 10^6 of acute measles

Mechanisms of Viral Pathogenesis:

The host immune responses

Viral pathogenesis: *host defenses*



Viral pathogenesis:
intrinsic and innate immunity

- Viruses replicate very rapidly and would quickly overwhelm a host organism if it were undefended.
- The adaptive immune response is tailored to pathogen, is restricted to animals, and takes several days to gather momentum.
- In that period, processes of innate and intrinsic immunity slow down and contain a virus so that the host can gain ascendancy over it.
    - *Intrinsic:* Always present in the uninfected cell, sometimes specific against certain viruses: apoptosis, autophagy, antiviral restriction proteins
    - *Innate immune system*: Induced by infection (cytokines and cells)

Viral pathogenesis:
host defenses against viral infections

1. Physical barriers
2. Chemical barriers
3. Intrinsic cellular defenses
4. Innate soluble immune response: interferons, cytokines, inflammation, fever, complement
5. Innate cellular immune response: DC, macrophages
6. Adaptive soluble immune response: antibodies
7. Adaptive cellular immune response: NK, CTL

Viral pathogenesis:
host defenses against viral infections

First: physical and chemical defenses

The skin, surface coatings of tissues such as mucous secretions, tears, acid pH, and surface-cleansing mechanisms

Second: frontline defense

Cell-autonomous, intrinsic defense systems

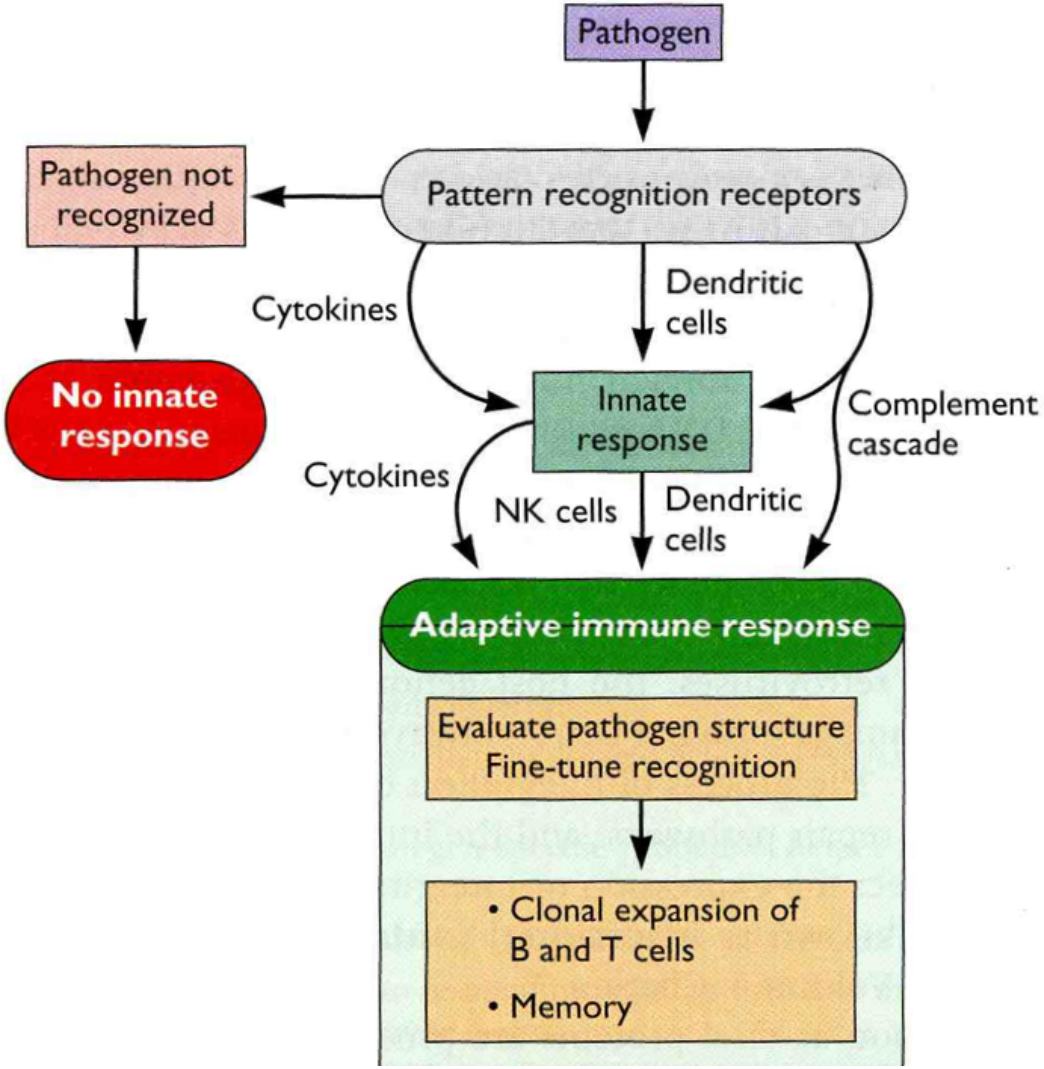
- Detection of altered cell metabolism
- Detection of unusual macromolecules made only by invading parasites
- Production of cytokines, induction of apoptosis, interference with early steps of viral replication

Third: attack and clean up

Innate and adaptive immune defense

- Direct, amplified response by coordinated action of cytokines and lymphocytes.
- Infection cleared by pathogen-specific antibodies, helper T cells, and cytotoxic T cells
- Production and maintenance of B-cell and T-cell "memory" cells
- "Immune" host, ready to respond instantly to the same infection that induced the memory response

Viral pathogenesis:
integration of intrinsic defense with the innate and adaptive immune response

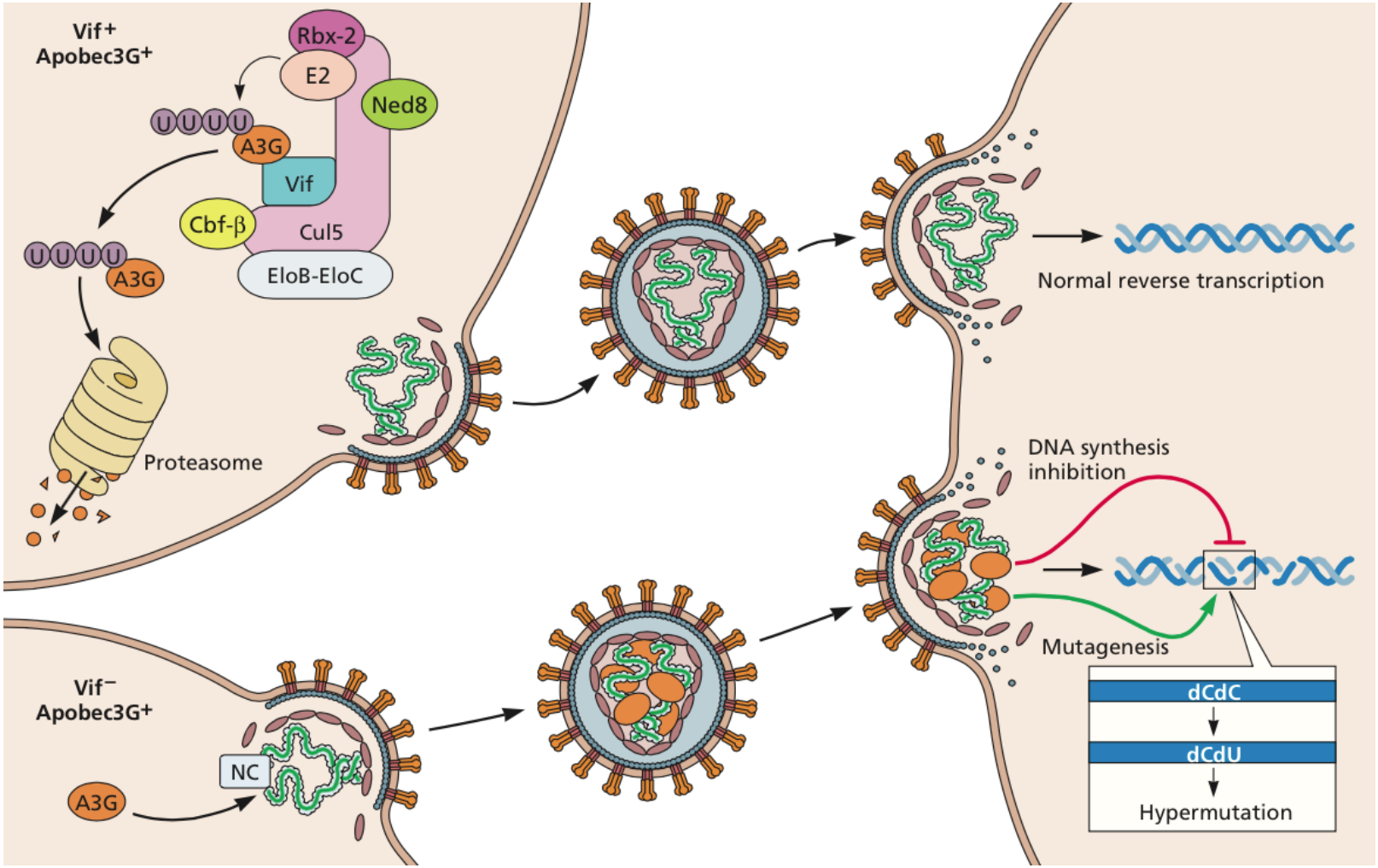


Viral pathogenesis:
Intrinsic resistance to viruses in vertebrates

- Virus specific Intrinsic resistance factors
- Autophagy
- Apoptosis

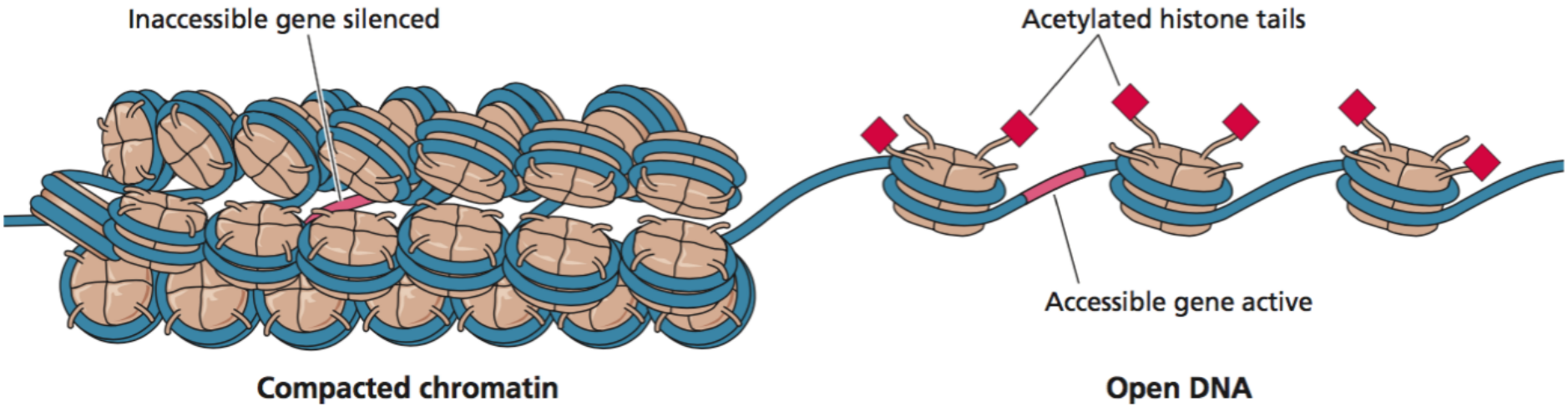
Resistance factor	Virus targeted	Action
APOBEC3G	HIV	Causes genome hypermutation
IFITM3	Influenza A	Interferes with attachment or entry
MxA	Influenza A; other viruses	Binds nucleoprotein complexes
Tetherin	HIV, other retroviruses; paramyxo, filo, rhabdo and arenaviruses; KSHV	Reduces particle release

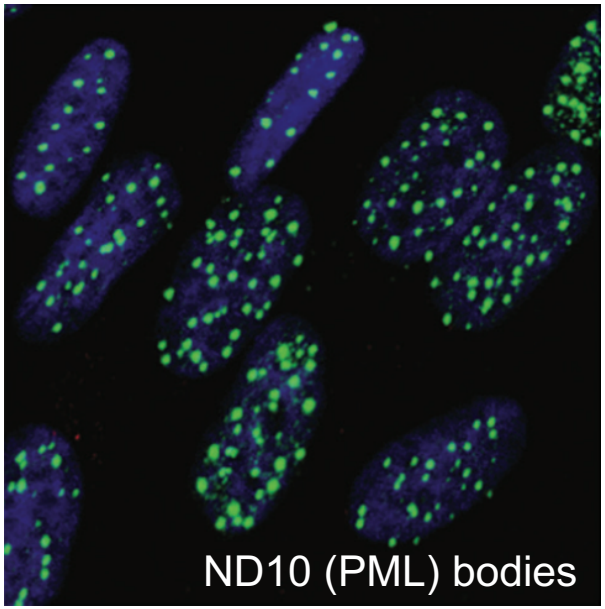
Viral pathogenesis:
APOBEC3G an example of intrinsic immunity



Apolipoprotein B mRNA editing catalytic polypeptide (APOBEC3G) causes HIV genome hypermutation

Viral pathogenesis:
Epigenetic silencing as intrinsic immunity

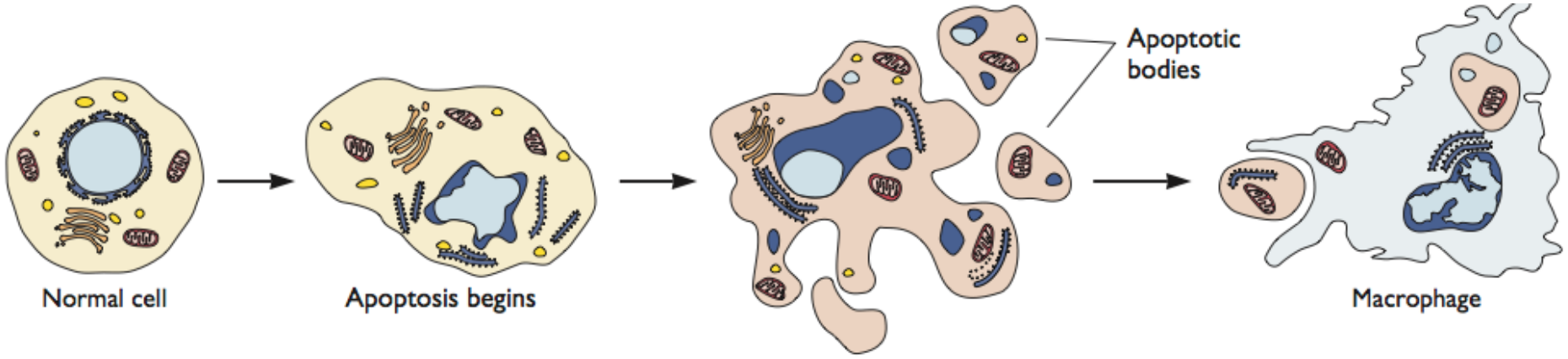




Viral Countermeasures

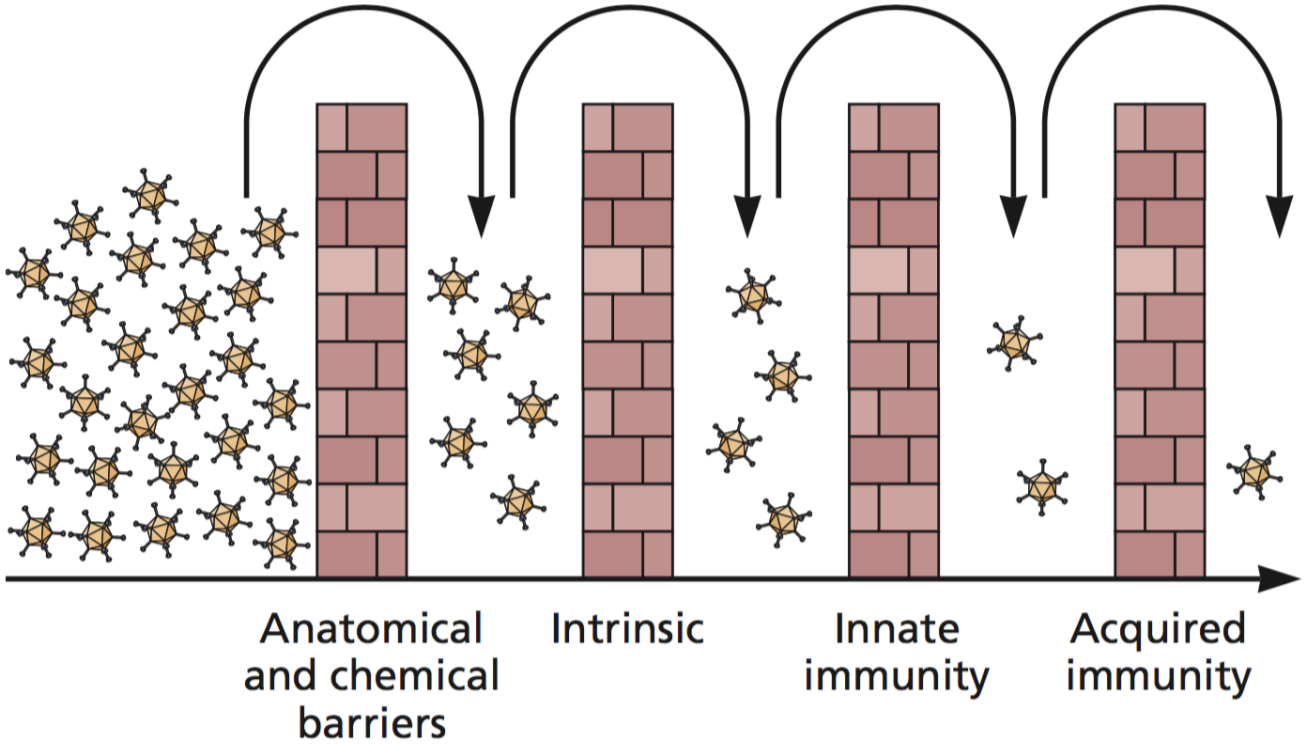
- HCMV tegument pp71 causes degradation of cell Daxx, needed for histone deacetylation
- EBV Ebna5 and AdV E4 ORF3 affect PML protein localization or synthesis

Viral pathogenesis:
Apoptosis as a intrinsic defense against viruses



Cellular Target	Virus	Gene	Function
Bcl-2	Adenovirus	E1B 19K	Bcl-2 homolog
	Epstein-Barr virus	LMP-1	Increases synthesis of Bcl-2; mimics CD40/Tnf receptor signaling
Caspases	Adenovirus	14.7K	Inactivates caspase-8
Cell cycle	Hepatitis B virus	pX	Blocks p53-mediated apoptosis
	Human papillomavirus	E6	Targets p53 degradation
	Simian virus 40	Large T	Binds and inactivates p53
Fas/Tnf receptors	Adenovirus	E3 10.4/14.5K	Internalizes Fas
	Cowpox	CrmB	Neutralizes Tnf and LT-α
	Myxoma virus	MT-2	Secreted Tnf receptor homolog
vFLIPs; DED box-containing proteins	Human herpesvirus 8	K13	Blocks activation of caspases by death receptors
Oxidative stress	Molluscum contagiosum virus	MC066L	Inhibits UV- and peroxide-induced apoptosis; homologous to human glutathione peroxidase
Caspase 8 activation, Bax localization	**Human Cytomegalovirus**	**UL36, UL37x1**	**Inhibit extrinsic and mitochondrial apoptosis**

Viral pathogenesis: *innate immunity*



- Activated within minutes to hours after infection
- Cytokines, sentinel cells (dendritic cells, macrophages, NK cells), complement
- Can inform adaptive response when infection reaches dangerous threshold

Viral Innate Immunity to Viral Infections: *sensing viruses*

- Binding of viral components (PAMPs) by a series of molecular detectors (PRRs) in cells triggers the type I interferon (IFN) response, setting in motion a series of events leading to a gross change in gene expression within the cell.
- The first purpose of this is to produce an environment that is more hostile to pathogen replication
- The second is to signal to neighbouring cells that they might be at risk, so they too initiate production of that hostile environment.

Receptor	Cellular compartment	Ligand(s) detected	Virus infection(s) detected
Rig-I	Cytoplasm	dsRNA; ssRNA with 5′ phosphate	Influenza virus
Mda5	Cytoplasm	dsRNA	Encephalomyocarditis virus, measles virus
Tlr2	Plasma and endosomal membranes	Measles virus HA protein	Human cytomegalovirus
Tlr4	Plasma and endosomal membranes	Mouse mammary tumor virus envelope protein	Respiratory syncytial virus
Tlr3	Plasma and endosomal membranes	dsRNA	Murine cytomegalovirus, reovirus, West Nile virus
Tlr7 and Tlr8	Plasma and endosomal membranes	ssRNA	Human immunodeficiency virus, influenza virus
Tlr9	Plasma and endosomal membranes	dsDNA; synthetic, unmethylated CpG DNA	Herpes simplex virus 1 and 2

Innate Immunity to Viral Infections: *PRRs*

Pattern recognition receptors that respond to virus infection*

Receptor†	Pathogen pattern recognized	Examples of viruses affected‡
TLR2 (PM)	Envelope fusion proteins	Class 1: CMV, HSV, VZV Class 5: LCMV, measles, VSV
TLR3 (endo)	dsRNA	Class 1: EBV Class 3: Reovirus Class 4: EMCV, West Nile virus Class 5: RSV
TLR4 (PM)	Envelope fusion proteins	Class 5: Ebola virus, RSV Class 6: MMTV
TLR7/8 (endo)	GU-rich ssRNA	Class 4: Coxsackie B, Sendai virus Class 5: influenza A virus, VSV Class 6: HIV1
TLR9 (endo)	DNA with unmethylated CpG	Class 1: CMV, HAdV, HSV
RIG-I (RLR; cyto)	RNA with 5′ triphosphate; short dsRNA	Class 1: EBV Class 3: Reovirus Class 4: flaviviruses Class 5: orthomyxo, paramyxo and rhabdoviruses
Mda5 (RLR; cyto)	Long dsRNA	Class 1: vaccinia Class 5: PIV5 (was SV5), other paramyxoviruses
IFI16 (cyto)	dsDNA	Class 1: HSV, KSHV
AIM2 (cyto)	dsDNA	Class 1: vaccinia
DDX41 (cyto)	dsDNA	Class 1: HAdV, HSV
DDX1/3/21 DHX9/36 (cyto)	dsRNA	Class 3: Reovirus Class 5: influenza A virus, VSV
DHX9/36 (cyto)	CpG dsDNA	Class 1: HSV
PolIII (cyto)	AT-rich dsDNA	Class 1: EBV, HAdV, HSV
DAI (cyto)	dsDNA	Class 1: HSV
cGAS (cyto)	dsDNA	Class 1: HSV; vaccinia

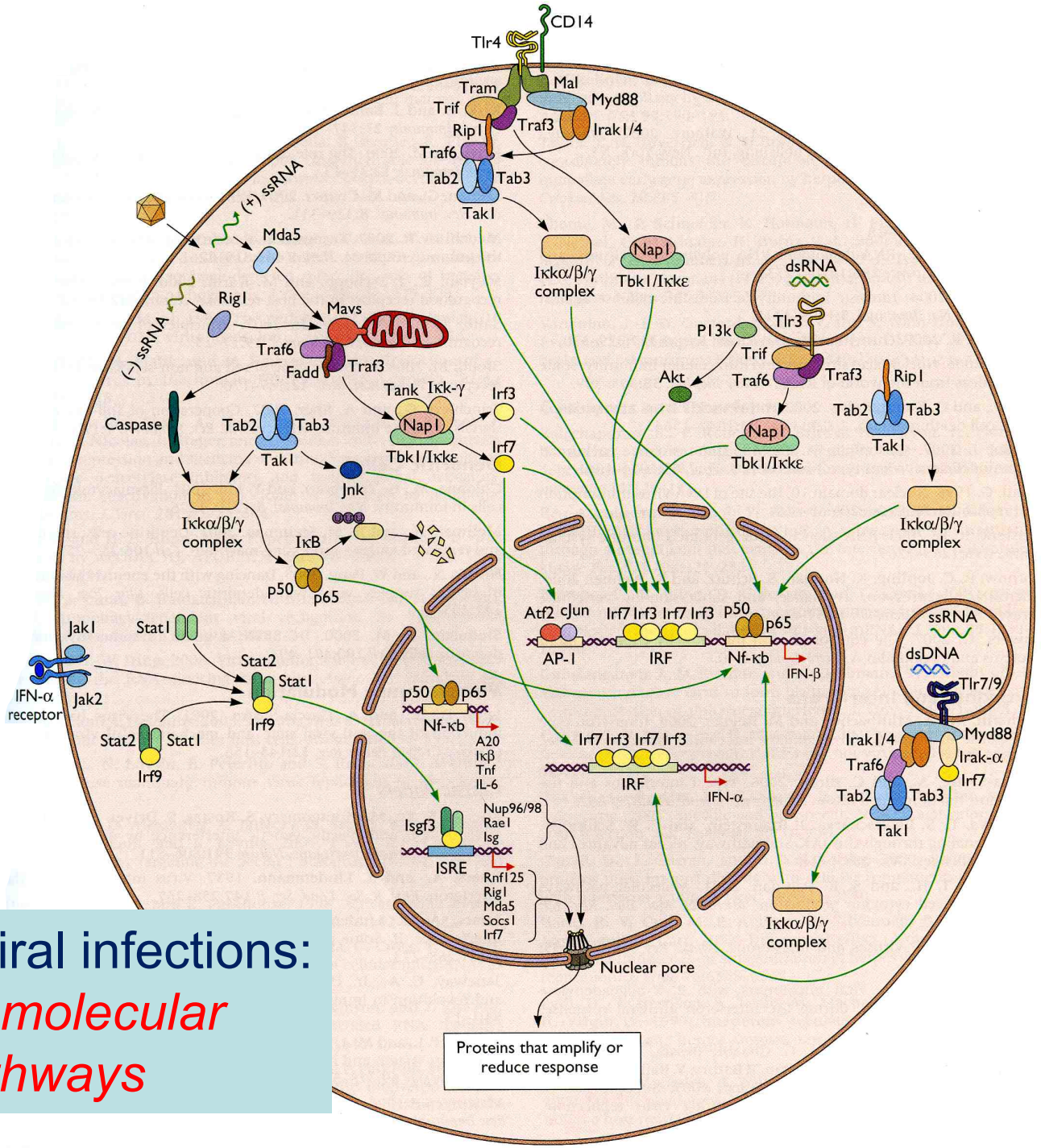
* The fact that a virus is not listed for a given receptor does not mean that the receptor is necessarily irrelevant for that virus.

† Receptor abbreviations: cGAS – cyclic GMP-AMP synthetase; cyto – cytoplasmic; DDX/DHX – DEAD/DEAH box helicase; endo – endocytic compartment; PM – plasma membrane; PolIII – RNA polymerase III; RLR – RIG-I-like receptor; TLR – Toll-like receptor.

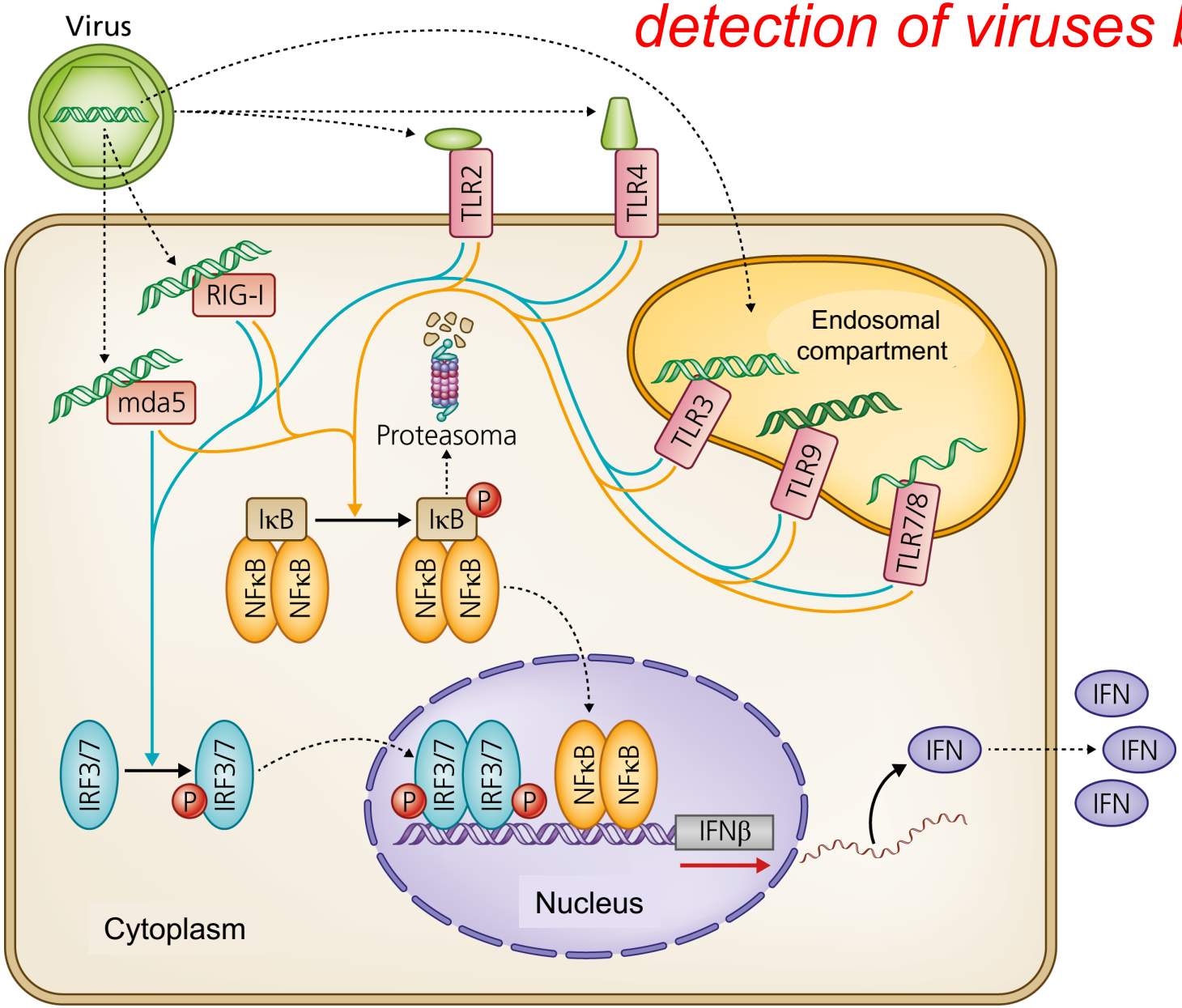
‡ Virus abbreviations: CMV – cytomegalovirus; EBV – Epstein Barr virus; EMCV – encephalomyocarditis virus; HAdV – human adenovirus; HIV1 – human immunodeficiency virus 1; HSV – herpes simplex virus; KSHV – Kaposi's sarcoma herpesvirus; LCMV – lymphocytic choriomeningitis virus; MMTV – mouse mammary tumour virus; PIV5 – parainfluenzavirus 5; RSV – respiratory syncytial virus; SV5 – simian virus 5; VSV – vesicular stomatitis virus; VZV – varicella-zoster virus.

Innate immunity to viral infections:

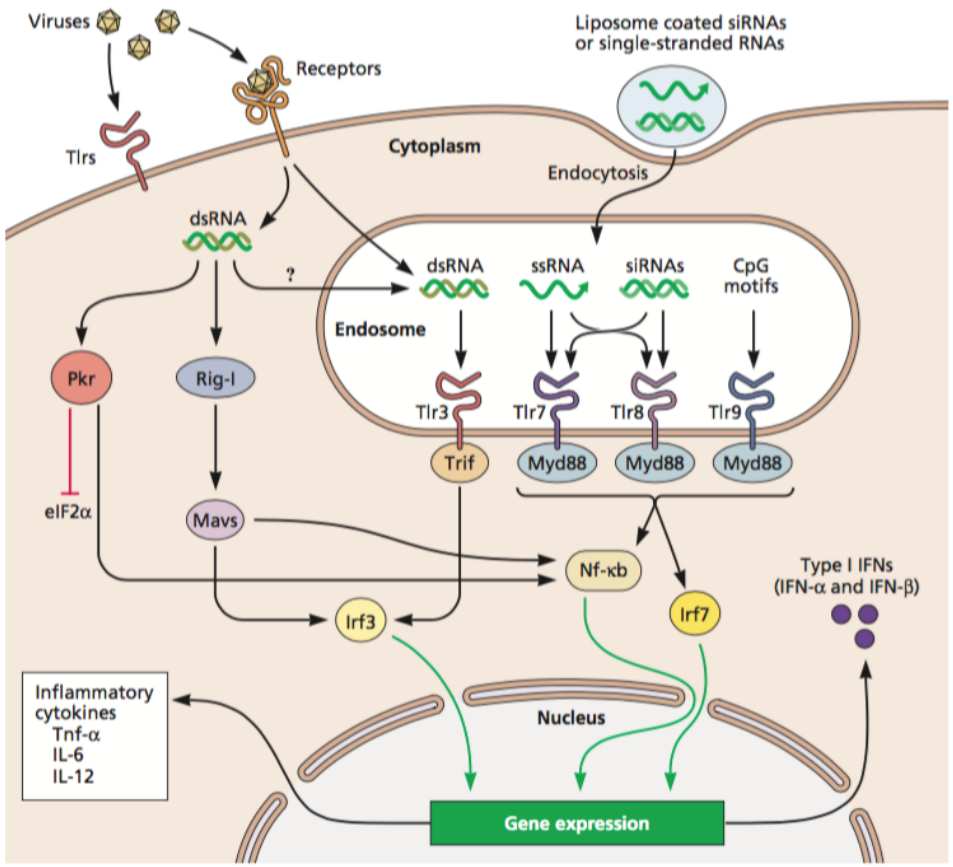
overview of the molecular detection pathways



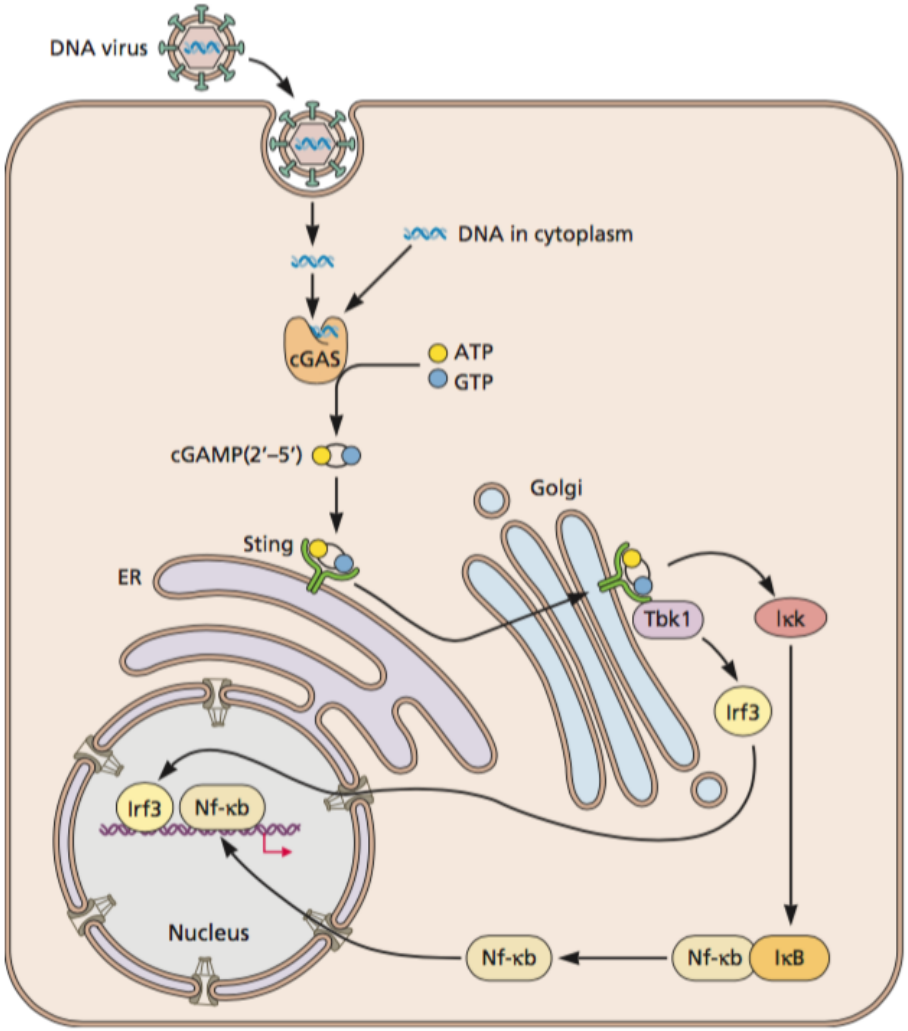
Innate Immunity to Viral Infections:
detection of viruses by PRR



Innate Immunity to Viral Infections:
recognition of viral nucleic acids



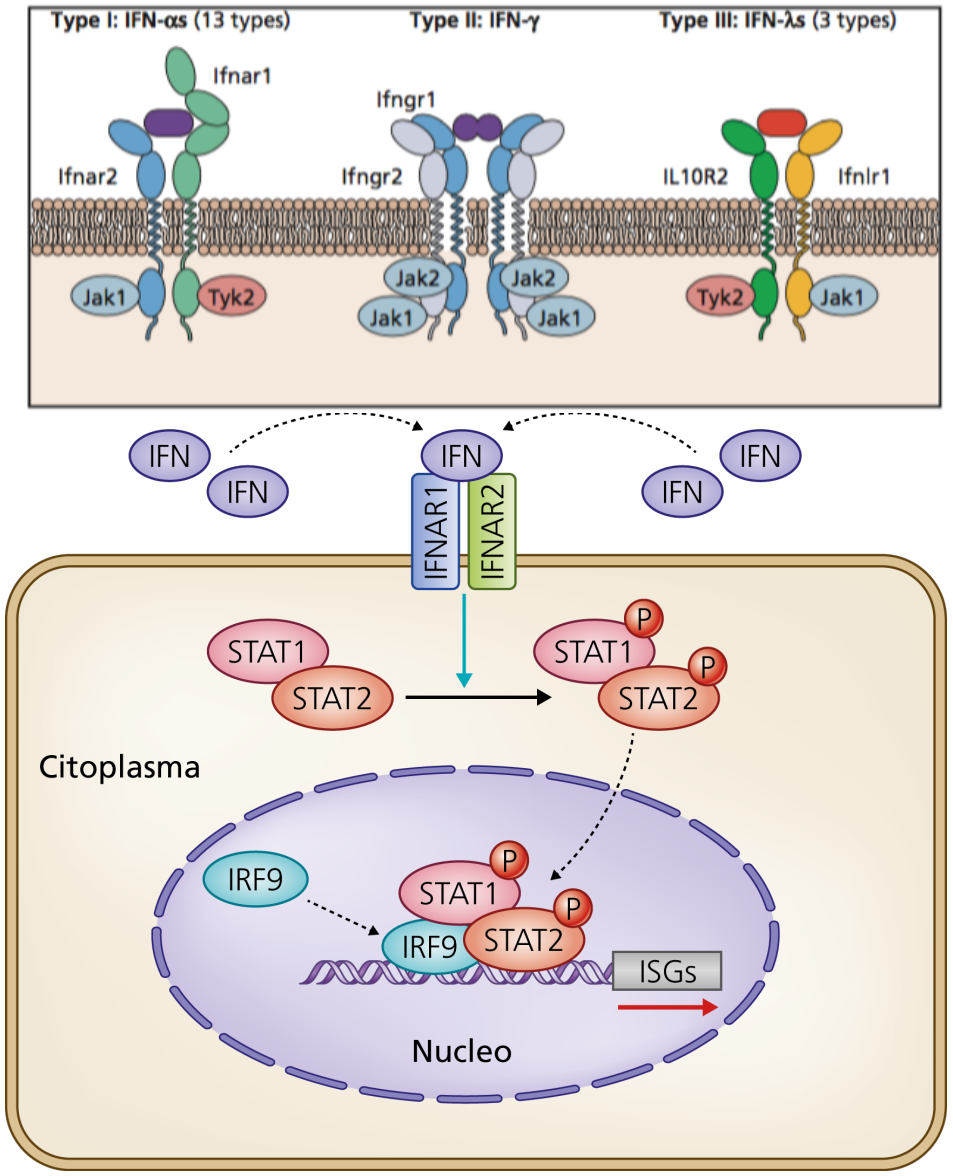
Sensing viral RNA



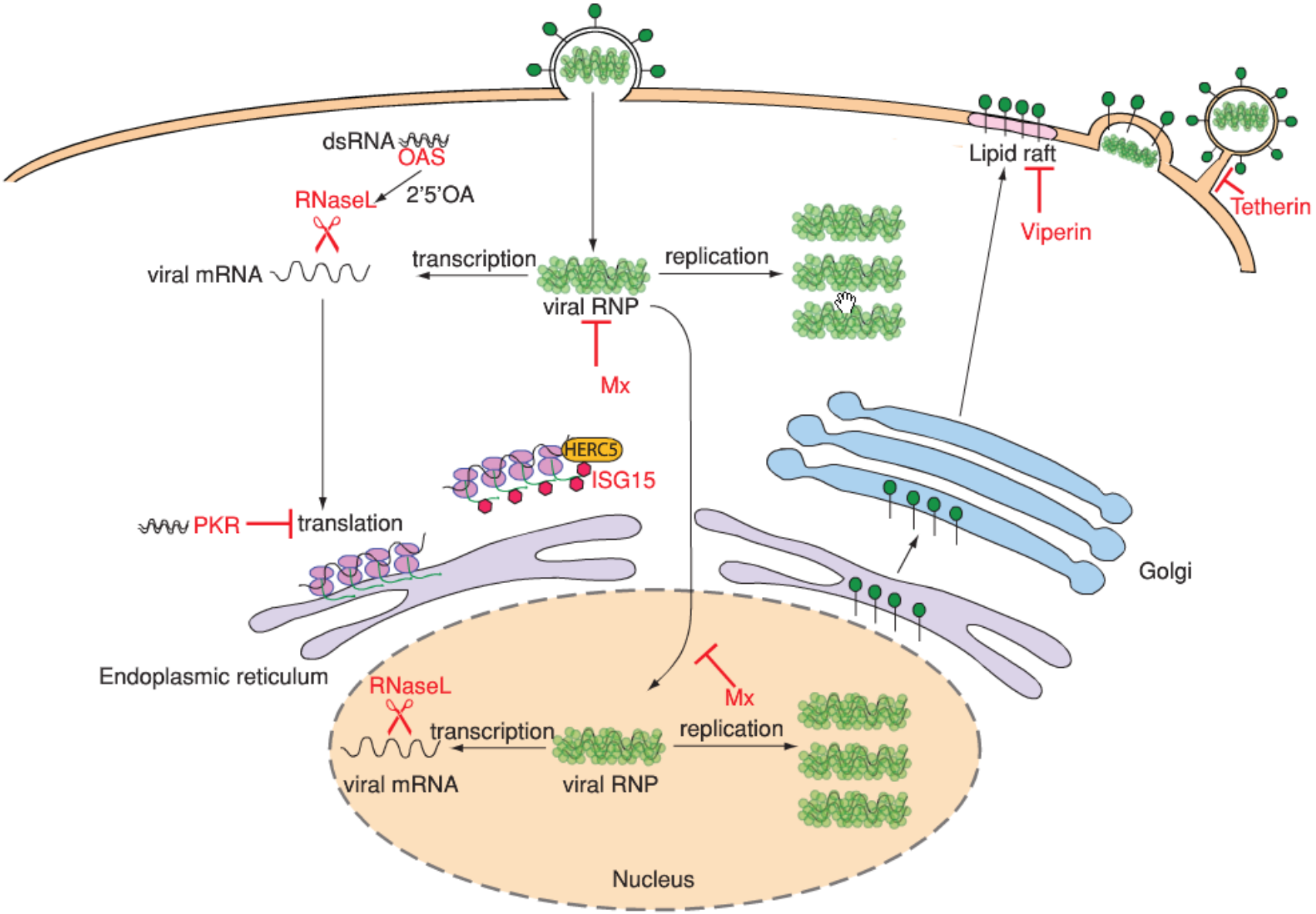
Sensing viral DNA

Innate Immunity to Viral Infections: *interferons (IFNs)*

- 1957: Isaacs and Lindenmann; chicken cells exposed to non-infectious influenza virus produce substance that "interfered" with infection of other cells
- Produced by virus-infected cells and uninfected sentinel cells in response to products released from cells (e.g. viral nucleic acid)
- Type I (IFNα, IFNβ); type II (IFNγ); Type III (IFNλ)
- Production of IFN**α/β** is rapid: within hours of infection, declines by 10 h
- IFN binding to IFN receptors leads to synthesis of >1000 cell proteins (ISGs, IFN stimulated genes)
- Mechanisms of most ISGs not known



Innate Immunity to Viral Infections:
mechanism of the antiviral state mediated by IFNs

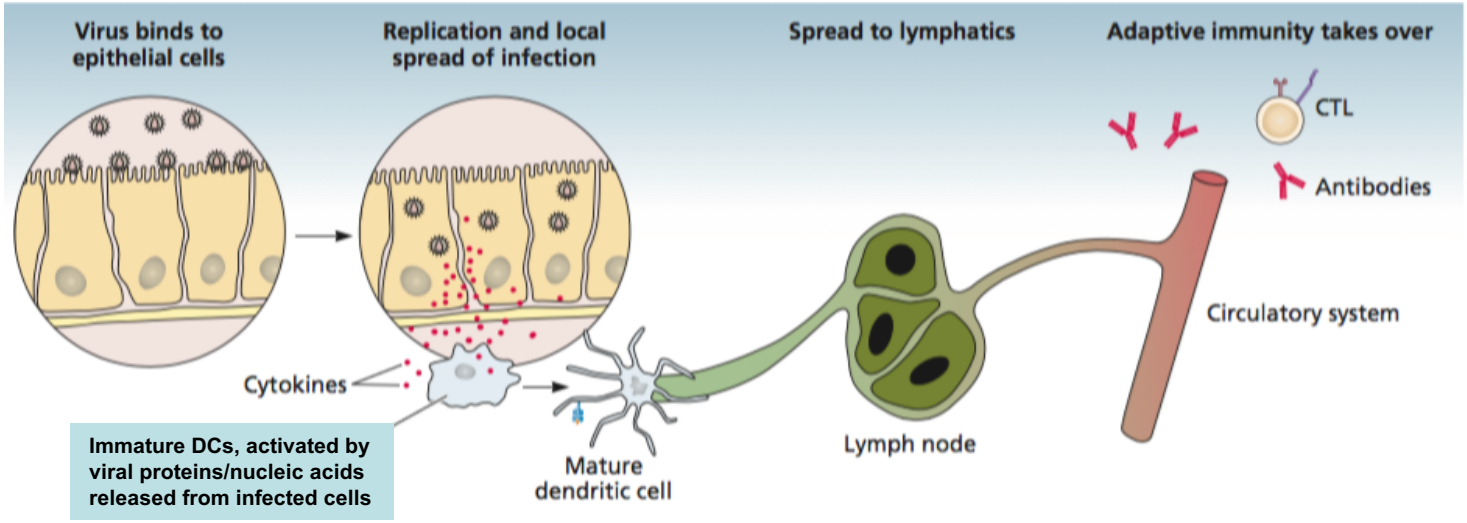


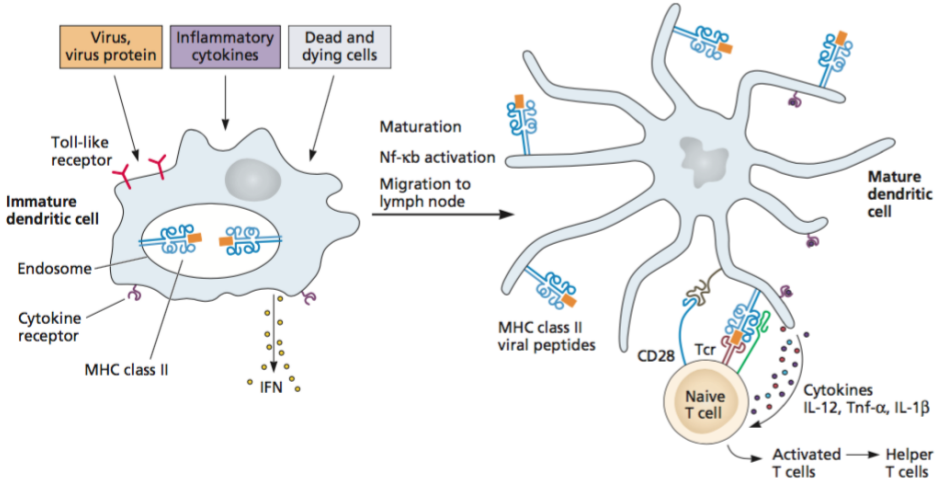
Innate Immunity to Viral Infections:
The IFN system is dangerous

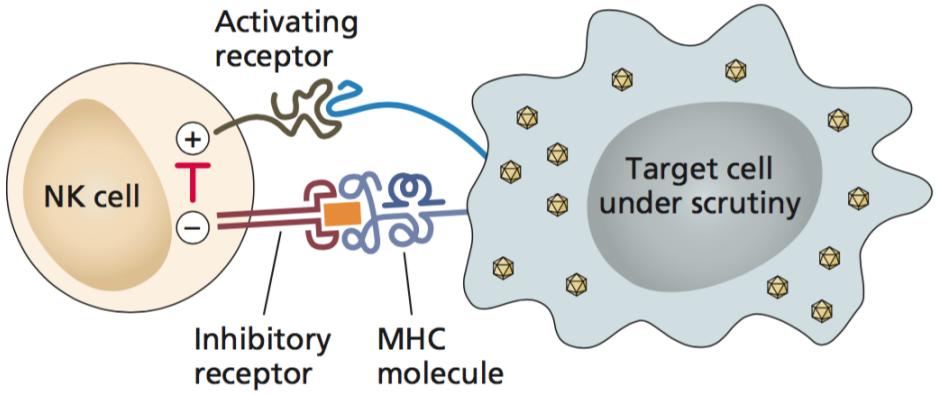
- IFN induces the expression of many deleterious gene products - most of our cells have IFN receptors
- Large quantities of IFN have dramatic physiological consequences: *fever*, *chills*, *nausea*, *malaise* *(the so-called flu-like symptoms)*
- Every viral infection results in IFN production, one reason why 'flu-like' symptoms are so common among different viral diseases

Innate Immunity to Viral Infections:

The sentinel cells of innate immunity



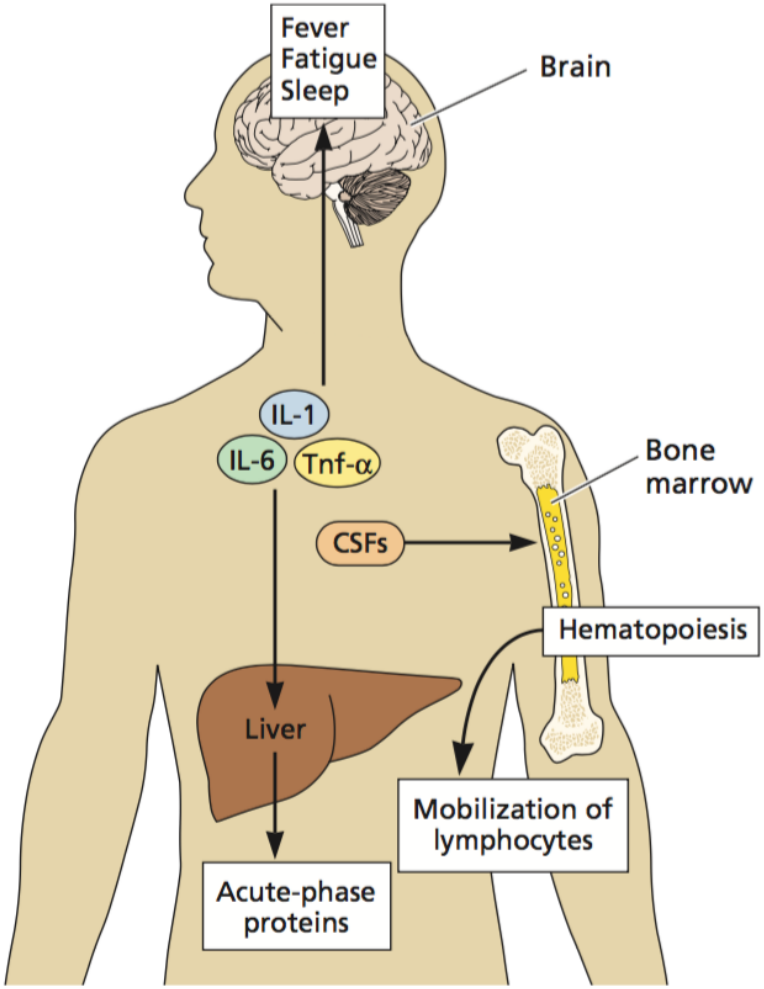




- Dendritic cells (DCs), macrophages, natural killer (NK) cells
- They patrol all our tissues looking for signs of change

Innate Immunity to Viral Infections:
determination of the inflammatory response

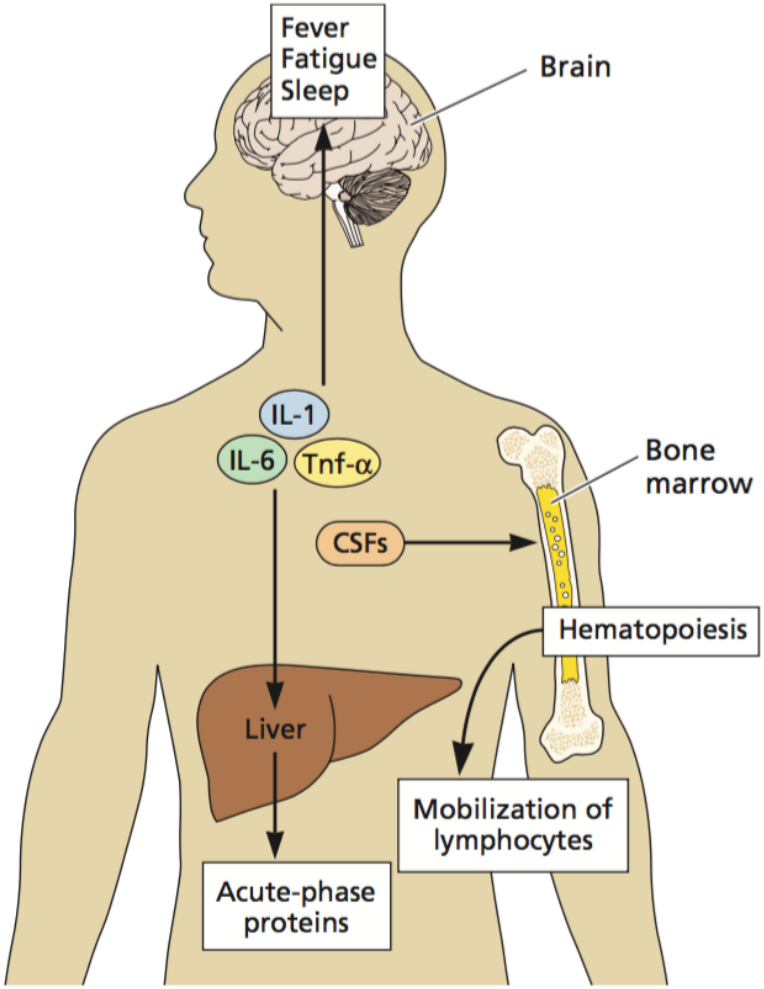
- Infected cells produce cytokines & chemokines
- Initially function locally in antiviral defense. In larger quantities, enter circulation, have global effects (sleepiness, lethargy, muscle pain, no appetite, nausea)
- Redness; pain; heat; swelling, the four classic signs of inflammation (rubor, dolor, calor, tumor)
- Result from increased blood flow, increased capillary permeability, influx of phagocytic cells, tissue damage



Innate Immunity to Viral Infections: *the inflammatory response*

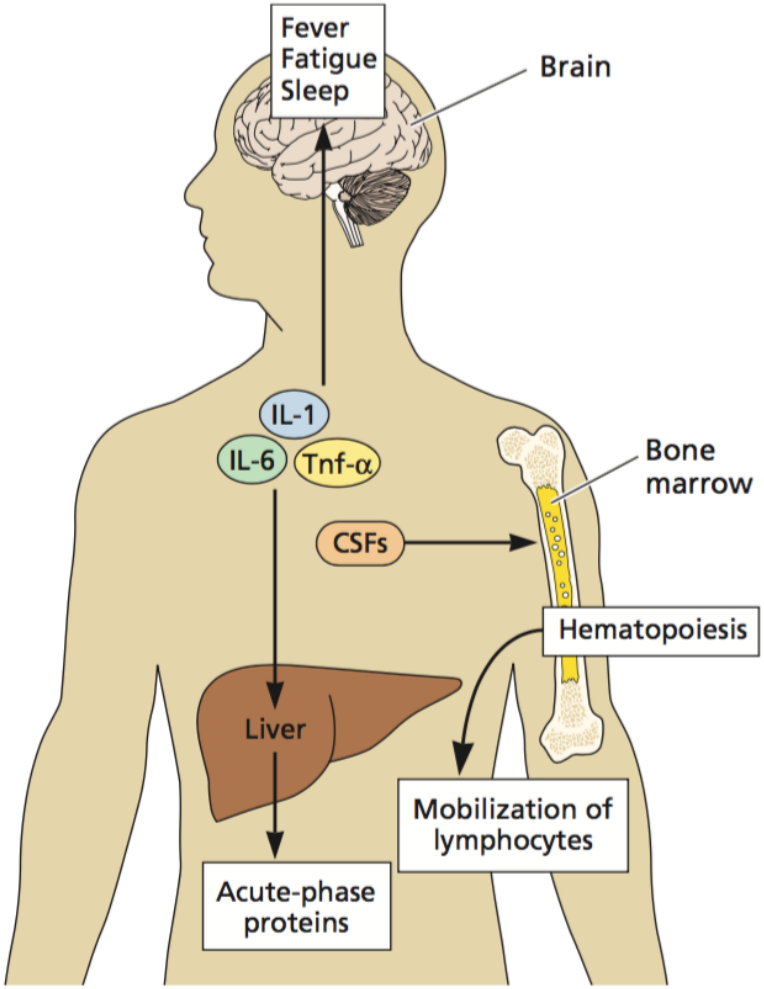
- Inflammation usually stimulates potent immune responses
- Cytopathic viruses cause inflammation because they promote cell and tissue damage, and thus activate the innate response
- Consequently cytopathic viral genomes encode proteins that modulate this immune response

  Adenoviruses, Herpesviruses, Poxviruses



Innate Immunity to Viral Infections: *absence of the inflammatory response*

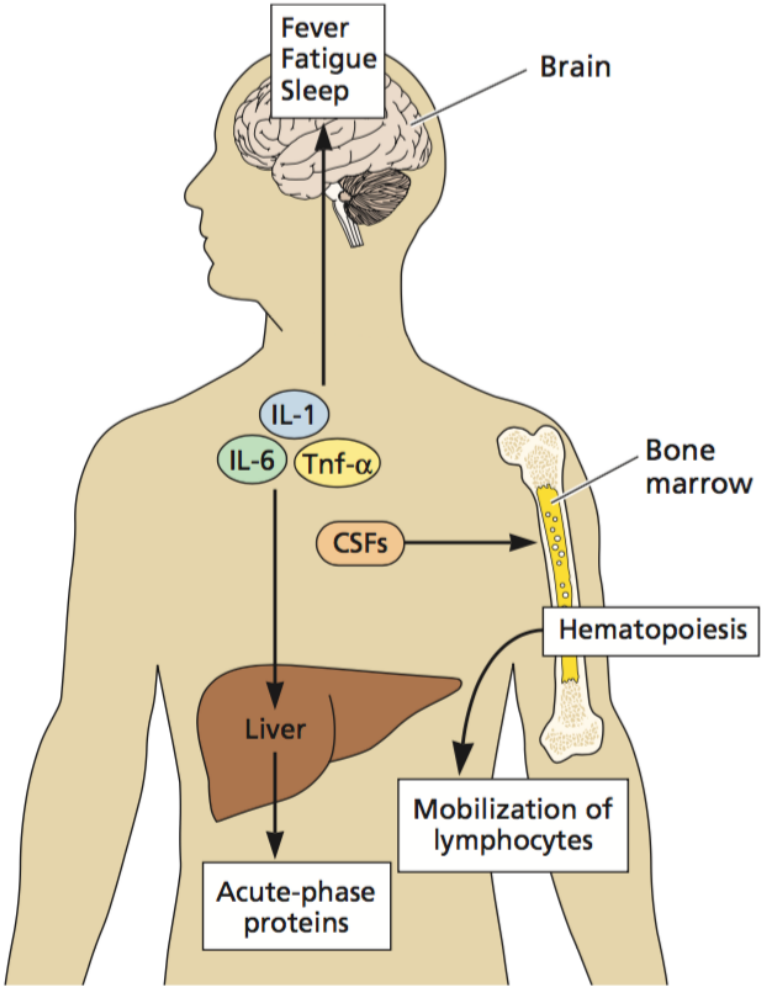
- Some viruses do not stimulate inflammatory responses
- Typically non-cytopathic viruses
  - *Cells are not damaged, no apoptosis/necrosis*
  - *Low or ineffective innate immune response*
  - *Do not effectively activate adaptive immune response*
- Non-cytopathic viruses have dramatically different interactions with the host immune system
- Persistent infections: rarely or inefficiently cleared



Innate Immunity to Viral Infections: *importance of the inflammatory response*

The lesson

- The classic inflammatory response (heat, swelling, redness, pain) reflects the communication of innate and adaptive immune defense
- No inflammatory response = ineffective adaptive response
- One reason for using inflammation-stimulating adjuvants for noninfectious vaccines



Viral pathogenesis: *counteracting the innate immunity*

Examples of viral functions that inhibit innate immunity.

Virus Classification	Virus and viral function	Host target and effect
Class 1: adenovirus	Human adenovirus 5: VA1 RNA	Protein kinase R; blocks its activation by dsRNA
	Human adenovirus 5: E4 Orf3	Promyelocytic leukemia protein; other PML body components; permits virus growth in IFN-treated cells
	Human adenovirus 5: E1A	Inhibits IFN transcription
Class 1: herpesvirus	Herpes simplex type 1: ICP0	As for adenovirus E4 Orf3
	Cytomegalovirus: IE72	
Class 1: poxvirus	Vaccinia virus: E3L, K3L	Block RNaseL, PKR activity
	Vaccinia virus: B18R	Binds type I IFN; acts as decoy receptor
	Vaccinia virus: A46R	Binds the essential adaptors for TLR activation; blocks TLR signalling
Class 3: rotavirus	Group A rotavirus: NSP1	In various isolates, targets RIG-I, IRF3, IRF7 and/or βTrCP for degradation
Class 4: picornavirus	Picornaviruses: 3C protease	Cleaves RLRs and/or their essential activating adaptors
	Enterovirus: 2A protease	Inhibits IFN downstream signalling
Class 4: hepacivirus	Hepatitis C: NS3/4A	Cleaves the essential adaptor for RIG-I/mda5 activation, MAVS
Class 5: paramyxovirus	Paramyxoviruses: V proteins	Blocks mda5 activation and causes STAT1 degradation
	Pneumoviruses: NS1, NS2	Block activation of IRF3
Class 5: orthomyxovirus	Influenza A virus: NS1	Binds dsRNA and PKR; blocks IFN induction at multiple levels
Class 5: filovirus	Ebolavirus: VP35	Binds dsRNA

Viral countermeasures

All viruses must encode at least one regulator intrinsic/innate defenses that can target: *PAMPs sensing, IFN production, IFN signal transduction, cytokines, chemokines, NK cells, DCs, complement.*

Viral pathogenesis is the sum of the effects on the host due to *virus replication* and the *immune response*

- *Direct effects of primary infection by cytolityc viruses*
  (e.g. virus-induced lysis of neurons in CNS by poliovirus)
- *Indirect effects of noncytolytic viruses*
  (e.g. conseguence of the immune response)
  - CD8+ T cell-mediated (HIV, HBV, Coxackievirus B)
  - CD4+ T cell-mediated
    Th1 (measle, HSV)
    Th2 (RSV)
  - Antibody-mediated (HBV, rubella)
  - Immunosuppression (HIV, CMV, measles, influenza)

Immune reactions during the response to viral infections that can cause host cell damage and disease

- *Interferons and lymphokines:* fever, headache, malaise.
- *Delayed-Type Hypersensitivity (DTH), Complement fixation, Immunocomplexes:* cell damage and local inflammatory responses.
- *Inflammation due to the cell-mediated response:* severe tissue damages in adults.
- *Immunocomplexes accumulation in the blood and kidney:* glomerulonephritis.