Viral Replication, Genetics and Evolution

(Antigenic Drift and Antigenic Drift)

Medical Microbiology (BMS 4510)

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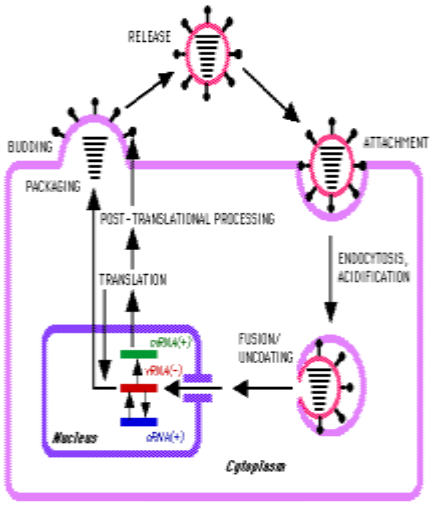
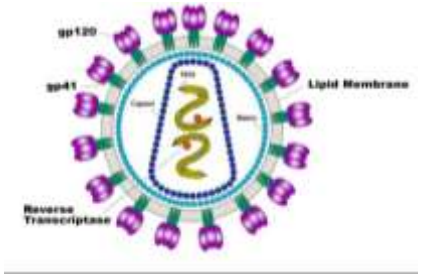
Viral Replication

- General features of viral life cycle
  - Attachment
  - Penetration
  - Uncoating
  - Transcription of early mRNA
  - Translation of early proteins (non-structural proteins)
  - Viral DNA/RNA replication
  - Transcription of late mRNA
  - Translation of late proteins (structural proteins)
  - Assembly of virions
  - Release



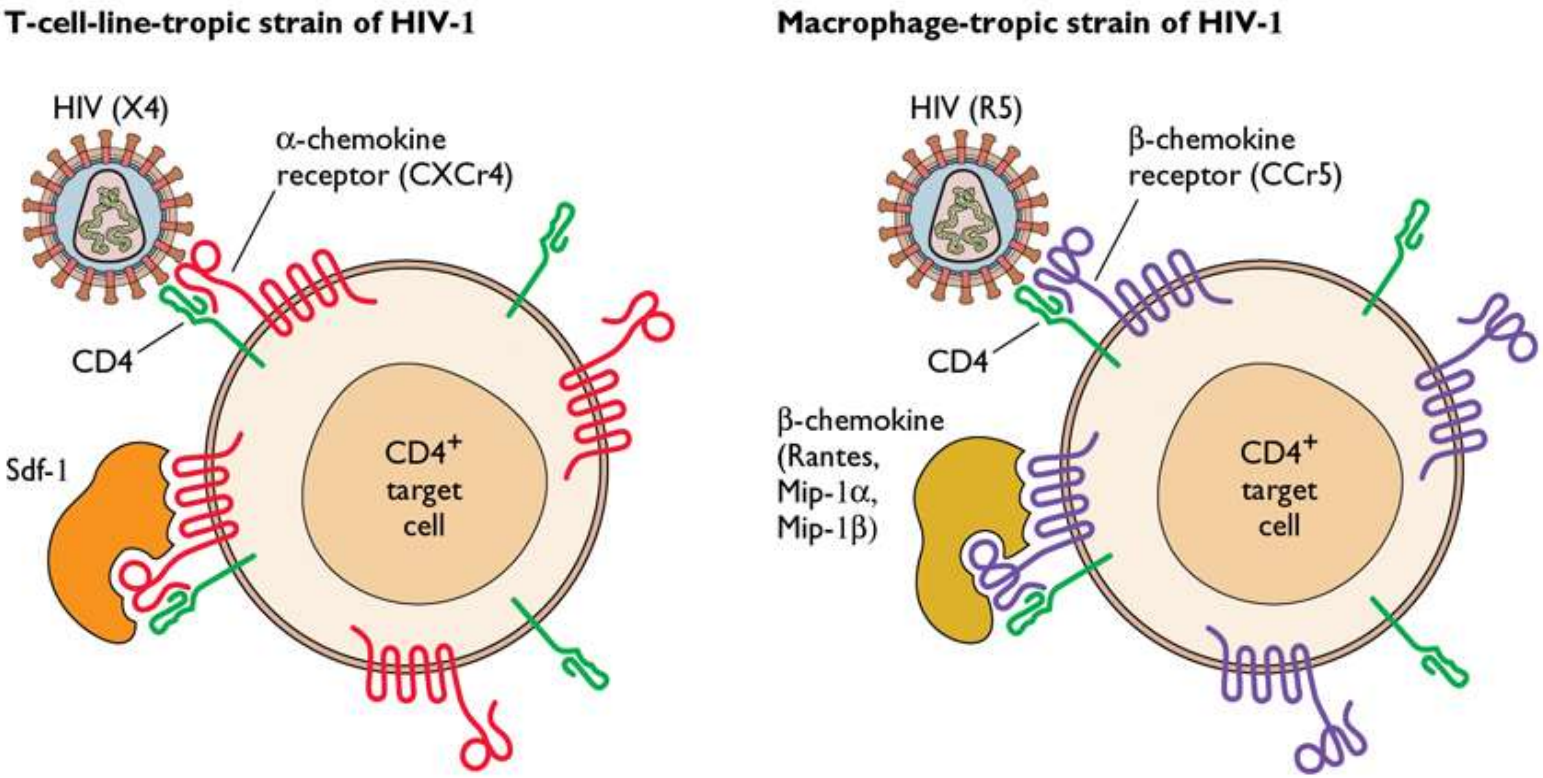
Attachment

- To infect cells viruses need to bind to receptors on cells
- Ligands on viruses aid this process



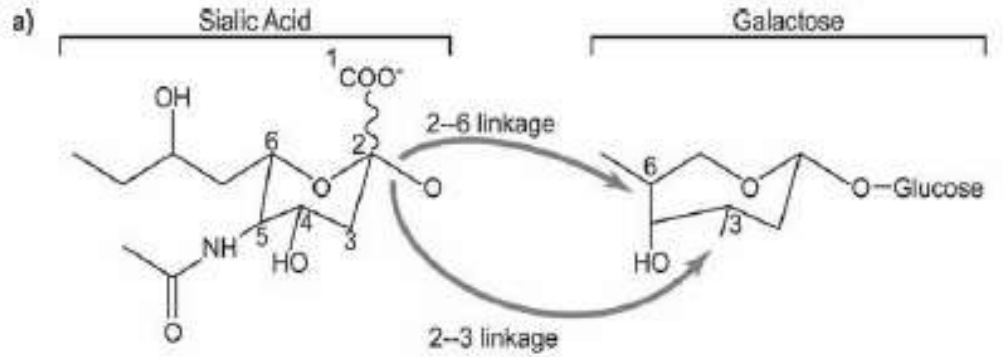
- Examples
  - Orthomyxoviruses use hemaglutinin glycoprotein to bind to sialic acid on cells
  - Other viruses (eg Rhinoviruses use intracellular adhesion molecules (ICAM-1)
  - HIV uses gp120 glycoprotein to bind to CD4 and other chemokine co-receptors (CCR5 and CXCR4) on leukocytes
  - Others use hormone receptors and permeases
- Viral tropism is dependent on receptor binding
  - Viral evolution has given rise to viruses that can adapt and use other receptors
  - Attachment sites are targets for therapeutics

HIV-1 attaches via gp120/gp41 to the CD4 surface receptor and chemokine co-receptors CXCR4 and/or CCR5

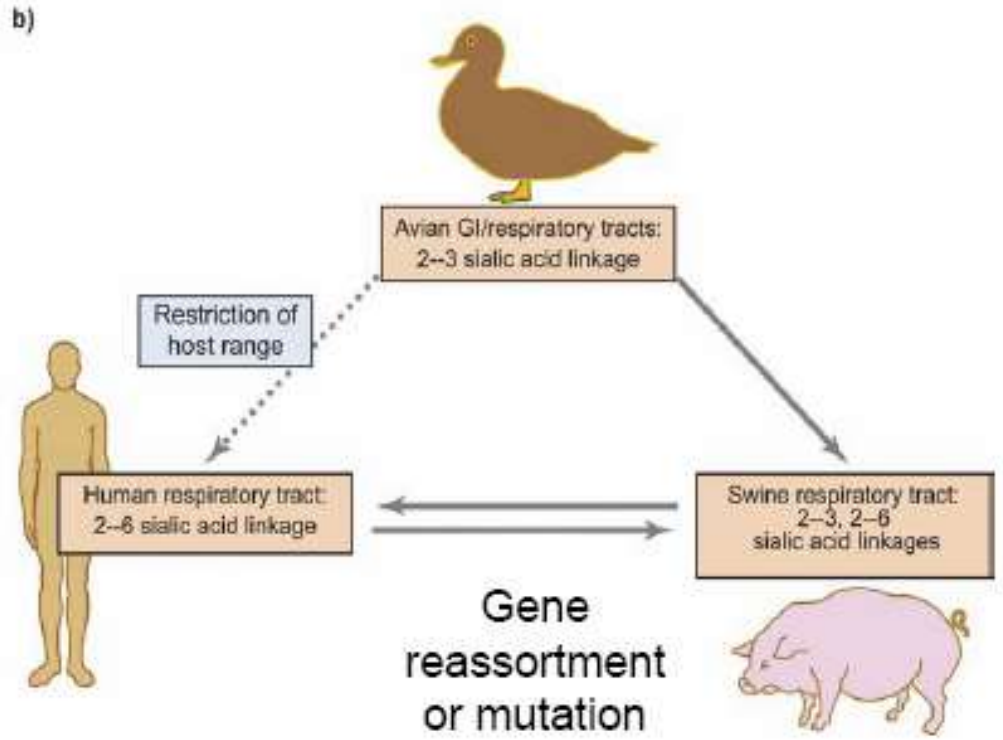


Adapted from Fig. 3 of A. S. Fauci, *Nature* **384:**529–533, 1996, with permission.

Sialic acid residues can be covalently attached to galactose residues of integral glycoproteins and glycolipids via either 2–3 or 2–6 α linkages.



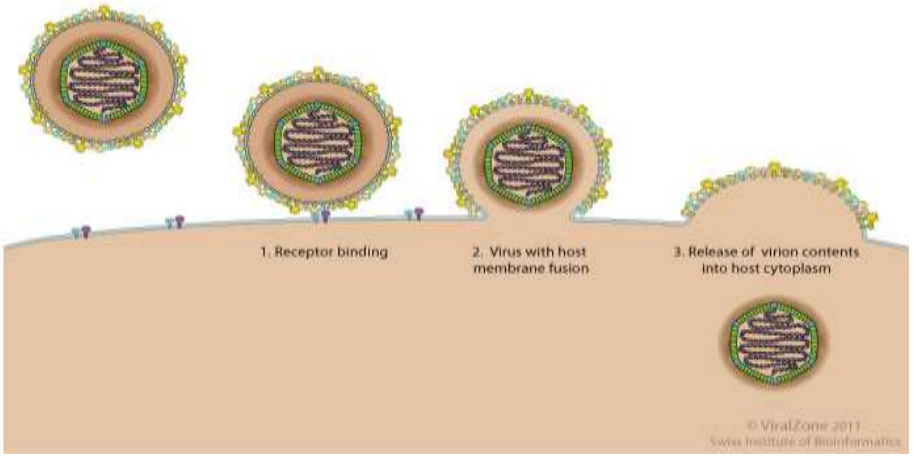
The avian, human, and swine upper respiratory tract epitheliae preferentially express 2–3 linkages, 2–6 linkages, and both 2–3 and 2–6 linkages respectively



Penetration (Uptake)

- Endocytosis
  - Receptor mediated endocytosis forming clathrin-coated pits
  - Formation of clathrin-coated vessicles that enter the cytoplasm and later fuse with endosomes
  - Acidification of endosomes triggers changes in capsid proteins and release of RNA e.g. polioviruses
  - In influenza viruses acidification causes conformational changes to hemagglutinin enabling fusion of viral envelop and endosome membrane and release of viral nucleocapsid into the cytoplasm.

- Fusion with plasma membrane
  - Fusion glycoprotein of paramyxoviruses causes the envelop to fuse directly with the host cell membrane even at neutral pH
  - Nuncleocapsid is then released into the cytoplasm.
  - Fusion proteins are involved e.g. gp41 in HIV
  - Also target for therapies e.g. CCR5 fusion inhibitor Miraviroc


1. Receptor binding
2. Virus with host membrane fusion
3. Release of virion contents into host cytoplasm
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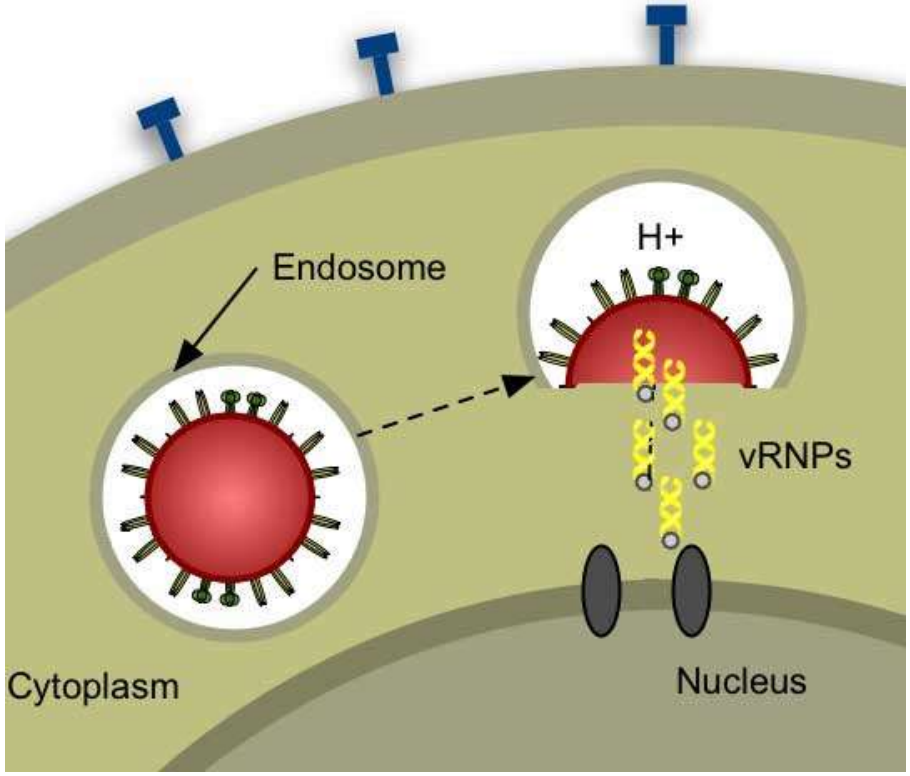
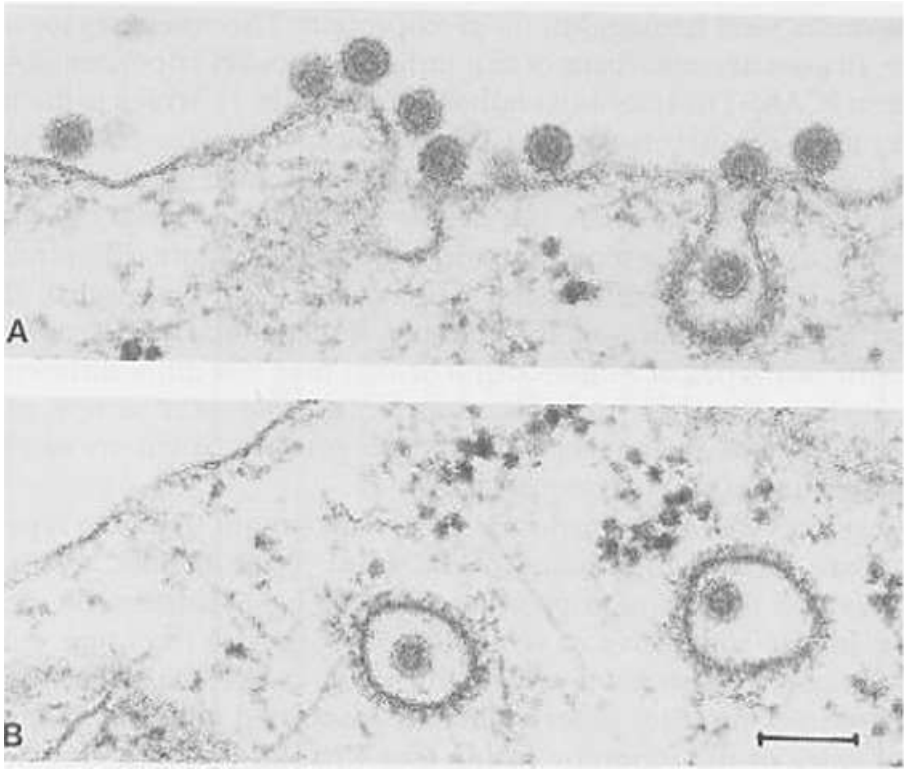

Endosome
H+
vRNPs
Cytoplasm
Nucleus


A
B

Uncoating

- For enveloped RNA viruses that enter via membrane fusion or endocytosis transcription commences immediately the nucleocapsid is in the cytoplasm
- With non-enveloped viruses eg Reoviruses, certain capsid proteins are removed and the genome is expressed without being fully removed from the core (nucleocapsid)
- Most viruses, however, the core is completely uncoated
- For viruses that replicate in the nucleus uncoating is completed in the nucleus

Transcription of the viral genome

- DNA viruses
  - Transcription of mRNA from dsDNA and replication of DNA
  - Similar to mammalian cells
    - papoviruses, adenoviruses, herpesviruses
      - Transcription by cellular DNA-dependent RNA polymerase II
      - Cleavage and splicing to produce monocistronic mRNAs
    - Poxviruses
      - Replicate in the cytoplasm
      - Carry their own transcriptase (DNA-dependent RNA polymerase)
      - Produce other proteins that make them replicate outside the nucleus
    - Parvoviruses
      - Use host cellular DNA polymerase to synthesize dsDNA from viral ssRNA genome.
      - dsDNA is then transcribed by cellular DNA-dependent RNA polymerase II
    - Hepadnaviruses
      - Reverse transcription of RNA intermediate for DNA replication
      - Retroviruses use a similar mechanism
      - Uses host cellular DNA-dependent RNA polymerase
      - Also uses viral DNA polymerase to synthesize dsDNA

Transcription of the viral genome

- RNA viruses
  - + sense ssRNA viruses (Picronaviruses, Togaviruses, Flaviviruses and Caliciviruses) require no trancriptase since genomic RNA serves as mRNA
  - The genome is translated directly into a polyprotein which is cleaved to give individual viral proteins including an RNA-depended RNA polymerase which replicates the viral RNA
    - Synthesizes – sense RNA copies which are used as templates to form + sense genomic RNA
  - - sense ssRNA viruses (paramyxoviruses, filoviruses and Rhabdiviruses carry an RNA-depended RNA polymerase (trancriptase) which transcribes + sense RNAs that serve as mRNA. For segmented viruses (Orthomyxoviruses each segment is transcribed by a trancriptase carried in the virion.

Transcription of the viral genome

- RNA viruses
  - **dsRNA viruses** (Reoviruses) the minus strand is transcribed by a virion associated transcriptase in the cytoplasm to yield mRNA
  - The plus strand serves as a template for replication

  - In retroviruses, the **plus sense RNA** is transcribed by the viral associated RNA-dependent DNA polymerase (reverse transcriptase) to produce an RNA-DNA hybrid which is converted into a dsDNA (proviral DNA or *provirus*) which is integrated into host genomic DNA .
  - Proviral DNA can remain latent for a long time
  - Proviral DNA is transcribed by cellular RNA polymerase II.

Transcription Summary

- Viral RNA of most + sense ssRNA viruses bind directly to ribosomes and is translated in full or in part without requiring transcription
- For other RNA viruses, mRNA must be transcribed
- For DNA viruses that replicate in the nucleus, cellular DNA-dependent RNA polymerase II performs transcription
- Other viruses require an in-house transcriptase
- Cytoplasmic RNA viruses carry a DNA-dependent RNA polymerase (ds or ss RNA-dependent RNA polymerase.

Translation

- Capped, polyadenylated and processed (Methylated) monocistronic or polycistronic viral mRNA bind to ribosomes and are translated just like cellular mRNAs from the 5' to the 3' end

- Produced proteins undergo post translational modifications
  - Phosphorylation for nucleotide binding
  - Fatty acid acylation form membrane insertion
  - Glycosylation (membrane proteins) or proteolytic cleavage (polyproteins)

- Proteins are also transported to various parts of the cells

Replication of Viral Nucleic acid

- Replication of viral DNA
  - Requires a helicase (ATPase) to unwind the dsDNA
  - A helix destabilizing protein to keep the duplex apart
  - A DNA polymerase to replicate the strands from 5" to 3" ends
  - An RNAse to degrade the RNA primers
  - A DNA ligase to join the Okazaki fragments together

    - Eg Papovirus genomes have histones and resemble cellular genome, utilizes cellular DNA polymerase α for replication
    - Adenoviruses have linear DNA which is replicated by a virus encoded DNA polymerase from both ends, no Okazaki fragments are generated.
    - Herpesviruses came with all the proteins/enzymes required for replication

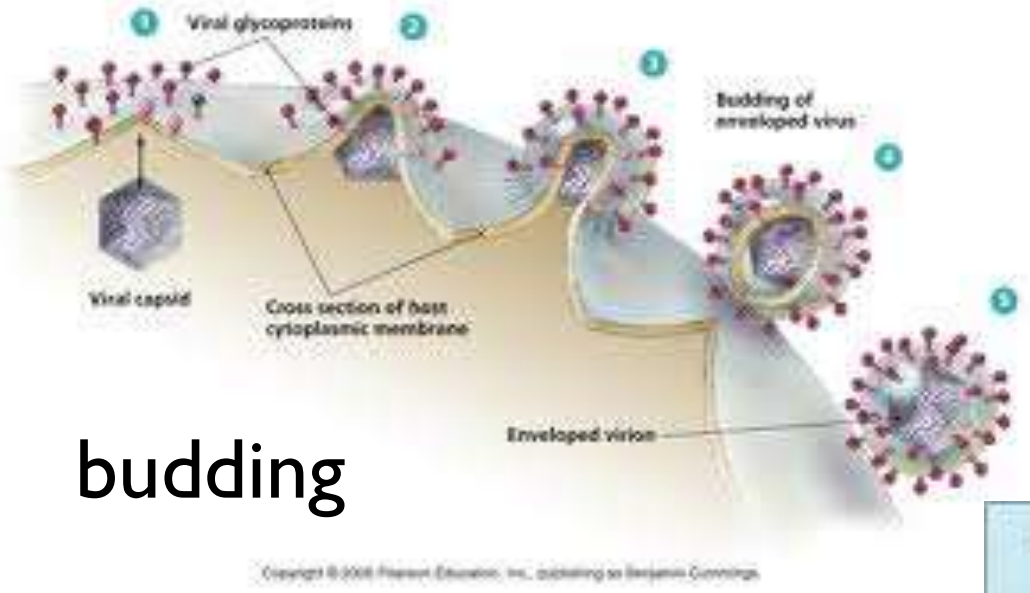
Replication of Viral Nucleic acid

- Replication of viral RNA
  - A phenomenon unique to viruses
  - Requires an RNA-depended RNA polymerase (not found in mammalian cells)
  - Requires synthesis of a complementary strand which serves as a template for replication
  - For retroviruses, replication proceeds via a DNA intermediate which is integrated into host cellular DNA.
    - Replication and transcription of viral RNA occurs from integrated proviral DNA.

Assembly and Release

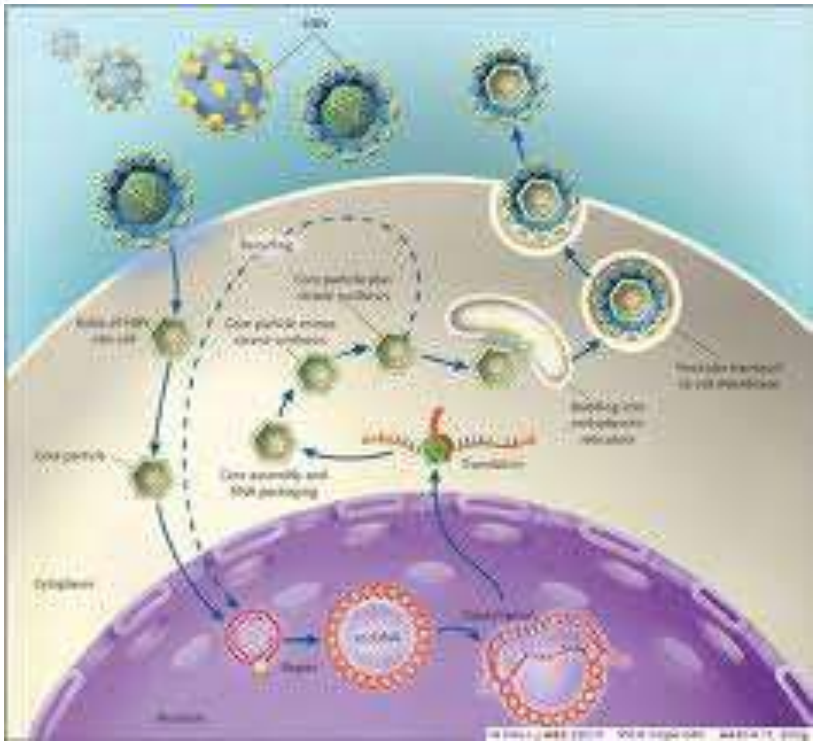
- Non-enveloped (naked) viruses
  - All have icosahedral structures
  - Structural proteins form capsomeres which self-assembles into capsid where viral nucleic acid is packaged
  - Most naked viruses accumulate in the cytoplasm or nucleus until the cell lyses

- Enveloped viruses
  - Mature by acquiring an envelop by budding through cellular membranes
    - Budding from cell membranes
      - Insertion of viral glycoproteins into cell membrane by displacement
      - Eg herpesviruses, togaviruses, retroviruses
    - Exocytosis
      - Bud through Golgi complex or ER into vesicles that migrate to the cell membrane where they fuse and expel viruses by exocytosis
        - Eg Flaviviruses, coronaviruses, bunyaviruses



budding

exocytosis



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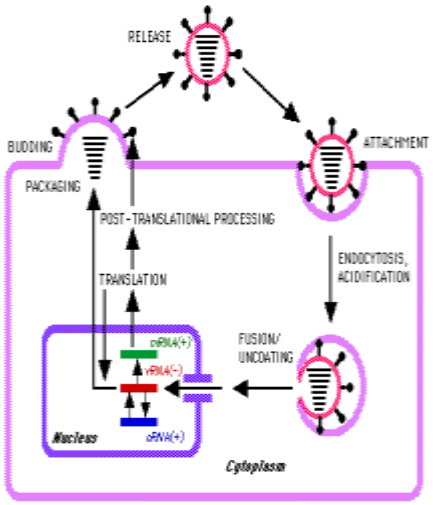


Mechanisms of Infection and Spread of Viruses

- The skin
  - Surface contains keratinized cells provides an impermeable layer to viruses
  - Small cuts and abrasions can cause viruses to enter and replicate (papillomaviruses, poxviruses)
  - Arboviruses are introduced through bites (eg mosquitoes, ticks, sand flies)
  - Zoonotic viruses are introduced by animal bites e.g. Rabies
  - Blood borne viruses are introduced by punctures e.g. HIV, Hep B, C

Mechanisms of Infection and Spread of Viruses

- The Gastrointestinal tract
  - Many viruses are acquired by ingestion
  - Protected by squamous epithelium, mucus, acids, bile, proteolysis enzymes ,IgA
  - Viruses are taken up by M cells and are transported to local lymph nodes (Peyer's patches) where they replicate in mononuclear phagocytes
  - Eg enteroviruses, coronaviruses, caliciviruses, rotaviruses

Mechanisms of Infection and Spread of Viruses

- The respiratory tract
  - Protected cleansing mechanisms (mucus, ciliated cells)
  - Viruses attach to specific receptors on epithelial cells
  - E.g. rhinoviruses, orthomyxoviruses, systemic: measles, rubella, chicken pox)

Mechanisms of spread

- Local spread on epithelial surfaces
- Subepithelial invasion and lymphatic spread
- Spread by the blood stream: viremia

Local spread on epithelial surfaces

- Many viruses can replicate in epithelial cells and can be shed into the environment
  - Papillomaviruses infect basal layers of the skin
  - Poxviruses also infect via the skin
- Viruses that infect via the respiratory or GI tract enter via epithelial cell linings
  - eg Paramyxoviruses, influenza viruses, rotaviruses
- Restriction of viral infection to epithelial cells cannot be equated to lack of severity of clinical disease

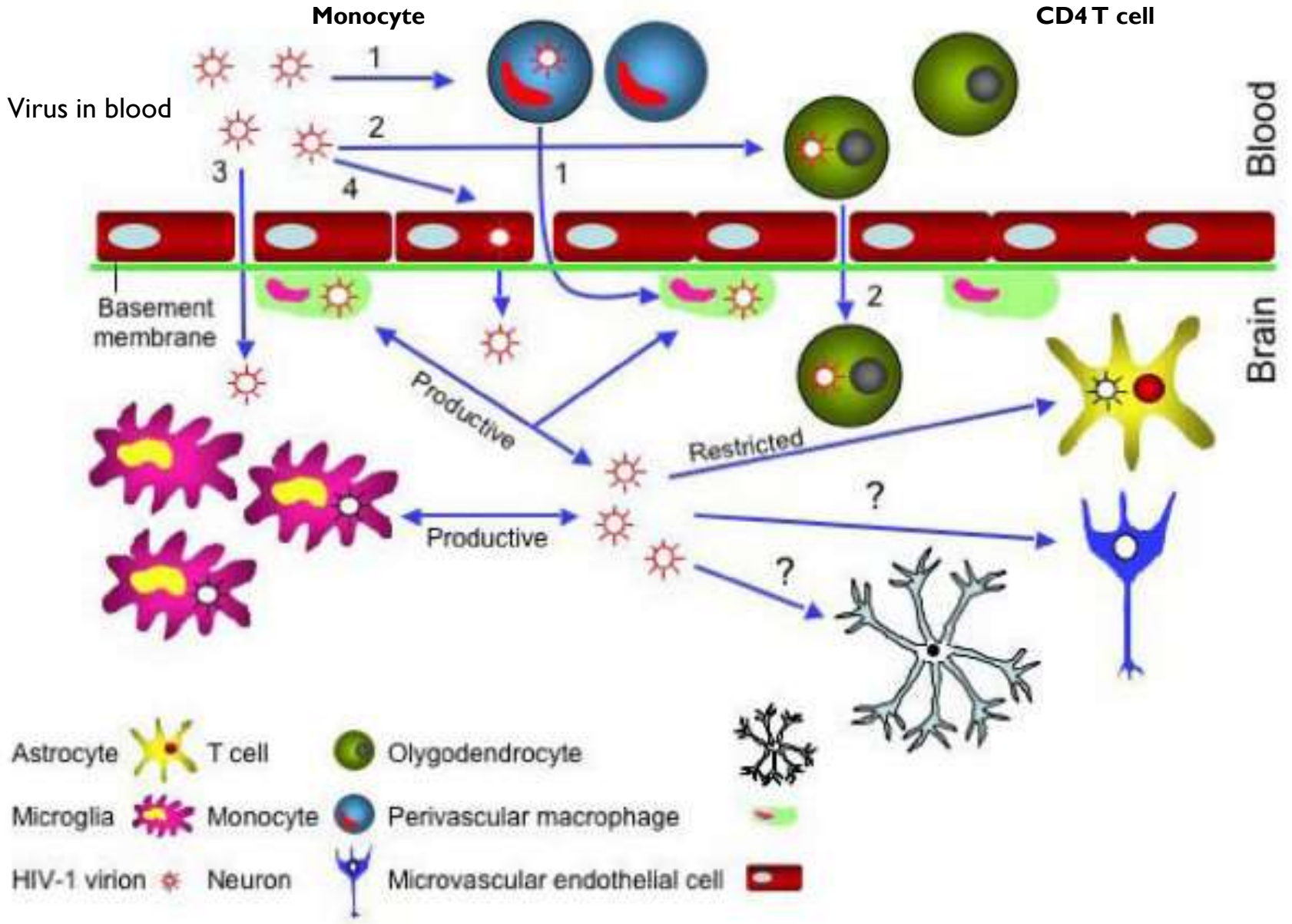
Subepithelial invasion and lymphatic spread

- Viruses via the epithelial surfaces can reach subepithelial tissues and be taken by dendritic cells and tissue macrophages or enter lymphatics to local lymph nodes
- The mononuclear cells process viruses (innate immunity) or present viral antigens to lymphocytes (induction of adaptive immunity)
- However, some virus escape immune defenses and replicate in mononuclear phagocytes
- Some viruses escape lymphatic and enter the blood stream

Spread by the blood stream: viremia

- Most effective and rapid vehicle for viral spread
- Viruses are spread as free virions or associated with lymphocytes/phagocytes ('Trojan horses')
- Vascular endothelial cells (with tight junctions) restrict viral spread to tissues including the brain
  - Some viruses are able to infect endothelial cells and enter tissues/organs from the blood (e.g. WNV)
  - Cell free virus had been shown to pass though tight junctions
  - Lymphocyte/phagocyte associated virus traverse tissues by virtual of circulating lymphocytes/phagocytes trafficking across endothelial cells (Trojan horse hypothesis for viral entry into the CNS e.g. HIV, JC virus, WNV etc
  - Retrograde axonal transport: neural tropic virus e.g. polio viruses can infect peripheral nerves and traffic into the CNS via axons

Entry of viruses into the CNS



Virus Shedding

- Respiratory or oropharyngeal secretion
  - Measles, chickenpox, rubella,
- Feaces
  - Enteric viruses
- Skin
  - Poxviruses, herpesviruses
- Urine (viruria)
  - Mumps, CMV, JC virus
- Milk
  - CMV
- Blood
  - HIV, Hepatitis, B, C, D, HTLV
- Genital secretions
  - Hepersviruses, HIV, HTLV, Papillomaviruses, Hepatitis B, C,

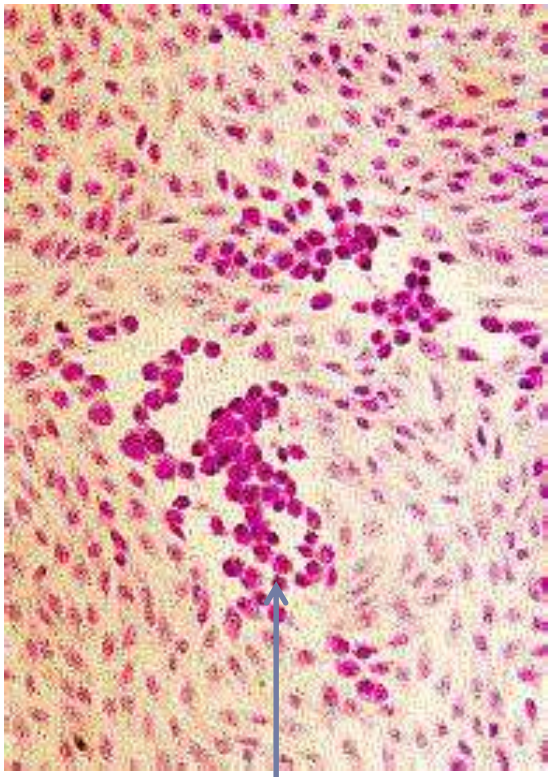
Viral pathogenesis

Types of virus-induced changes in cells

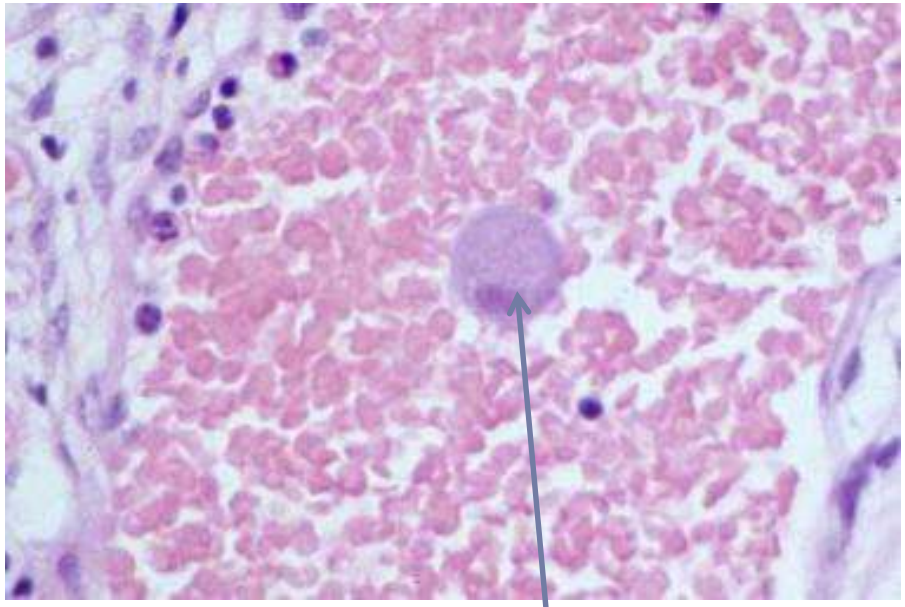
Type of infection	Effects on cells	Production of infectious virions	Examples
Lytic (cytocidal)	Morphological changes (CPE), inhibition of protein, RNA, DNA synthesis and cell death	Yes	Alphaherpesviruses, enteroviruses, reoviruses
Persistent productive	No CPE, little metabolic disturbance, cells continue to divide, some loss of function	Yes	Arenaviruses, rabies virus, most retroviruses
Persistent, nonproductive transformation	Usually nil, Alteration of morphology, cells can be passaged indefinitely, produce tumors when transplanted	No, No, oncogenic DNA viruses Yes, oncogenic retroviruses	Measles in the brain Polyomaviruses, adenoviruses Sarcomaviruses

Cytopathic effects (CPE) of viral infections

- 1. Inclusion bodies
  - Recognized after staining and fixation
  - Single or multiple
  - E.g. poxviruses, paramyxoviruses, reoviruses



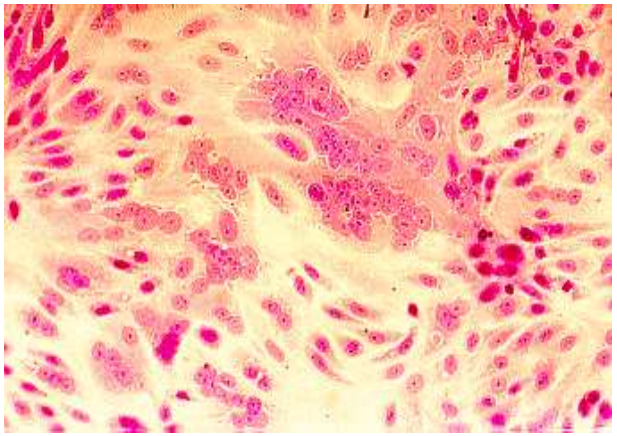
Inclusion bodies in brain: rabies virus



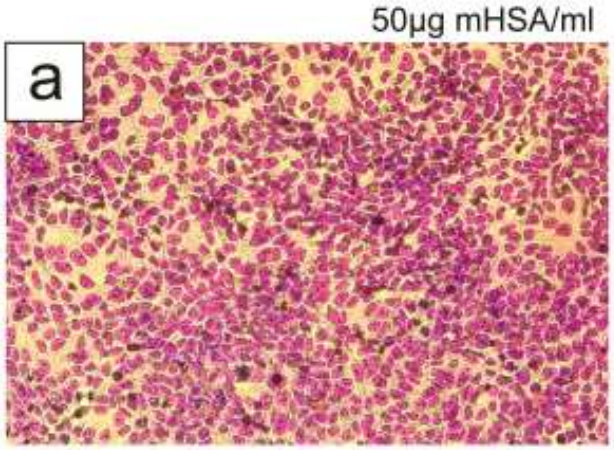
Giant cell inclusion bodies: Cytomegalovirus

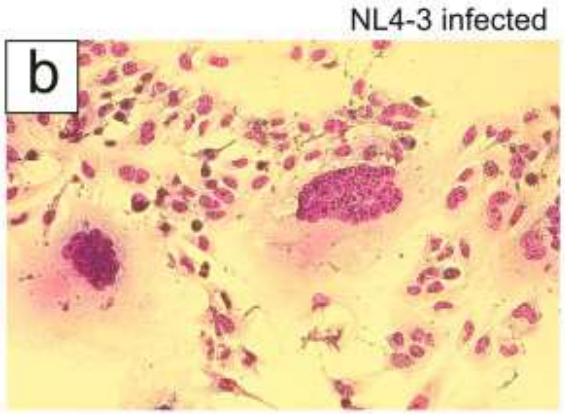
Cytopathic effects (CPE) of viral infections

- 2. Cell fusion (Syncytia formation)
  - Fusion of cells
    - Mechanism of spread and immune evasion (antibody responses)
  - Lentiviruses, paramyxoviruses and some herpesviruses



Syncytia formation in RSV culture





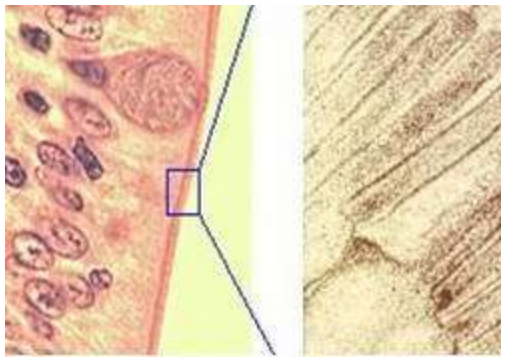
Syncytia formation in HIV culture

Relationship between CPE and disease

- Not direct
  - Lytic viruses like enterovirus may cause mild disease where as non-lytic virus like rabies may cause lethal disease
  - In some organs, a great deal of cellular damage may occur without causing apparent illness (e.g. Liver)
  - Edema may not be important in some organs but can be serious in the brain

Viral damage to tissues and organs

- Direct damage by lytic viruses
  - Paralysis in a polio patient is a direct consequence of death of motor neurons in the anterior horn of the spinal code leaving the muscles nonfunctional
- Damage to epithelium of the respiratory tract
  - Influenza viruses
    - Inflammation and necrosis of epithelial debris
    - Accumulation of fluid and necrotic debris causing obstruction/blockage (hypoxia)
- Damage to epithelium of the intestinal tract
  - Rotaviruses
    - Shortening and fusion of microvilli
      - Fluid accumulation in the gut lumen and diarrhoea
      - Impaired absorption, osmotic loss, electrolyte loss and development of acidosis



Viral damage to tissues and organs

- Bacterial superinfection
  - Epithelial damage predisposes to secondary bacterial infection
  - Pneumococcal infection during influenza infection
  - RSV infection predisposes patients to rhinitis, pharyngitis, sinusitis, and otitis media.
  - Rotavirus infection can increase susceptibility to *E coli* diarrhoea

- Physiological changes without causing cell death
  - Viral infection of islets of the pancreas
  - Alter expression of MHC class I
  - Over expression of MHC class II

Viral damage to tissues and organs

- Immunopathology
  - Type I (Anaphylactic hypersensitivity)
    - IgE on mast cells and basophils
    - Release of histamines, leukotrienes and heparin
    - Rushes, acute respiratory syndrome, anaphylaxis
    - Not very important in viral infections but important in helminth infections and allergy
  - Type II (Antibody dependent cototoxic hypersensitivity)
    - ADCC
    - Herpesviruses, unclear
  - Type III (Immune complex mediated hypersensitivity)
    - Common cause in mild inflammation
    - Filoviruses, flaviviruses
  - Type IV (delayed cell-mediated hypersensitivity)
    - E.g. lymphocytic choriomeningitis (LCM)
    - Severe meningitis, cerebral edema, and death

Viral damage to tissues and organs

- Autoimmunity
  - Molecular mimicry

Molecular mimicry between proteins of infectious organisms and human host proteins

Protein*	Residue†	Sequence‡
Human cytomegalovirus IE2	79	P D P L G R P D E D
HLA-DR molecule	60	V T E L G R P D A E
Poliovirus VP2	70	S T T K E S R G T T
Acetylcholine receptor	176	T V I K E S R G T K
Papilloma virus E2	76	S L H L E S L K D S
Insulin receptor	66	V Y G L E S L K D L
Rabies virus glycoprotein	147	T K E S L V I I S
Insulin receptor	764	N K E S L V I S E
Adenovirus 12 E1B	384	L R R G M F R P S Q C N
α-Gliadin	206	L G Q G S F R P S Q Q N
Human immunodeficiency virus p24	160	G V E T T T P S
Human IgG constant region	466	G V E T T T P S
Measles virus P3	13	L E C I R A L K
Corticotropin	18	L E C I R A C K
Measles virus P3	31	E I S D N L G Q E
Myelin basic protein	61	E I S F K L G Q E

Viral damage to tissues and organs

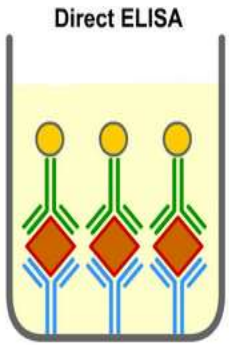
- Autoimmunity
  - Molecular mimicry
  - Polyclonal B cell activation
    - E.g. EBV induced polyclonal B cell activation and antibody production to various tissues/organs
  - Cytokine production of MHC antigens
    - Induction of interferon gamma and tumor necrosis alpha which induce MHC class II on brain cells which start to present antigens (egg myelin) to T cells
      - Multiple sclerosis demyelization
  - Exposure of sequestered cellular proteins
    - Incorporation of cellular proteins into viral envelop
  - T cell dysfunction
    - Down regulation of T cell function

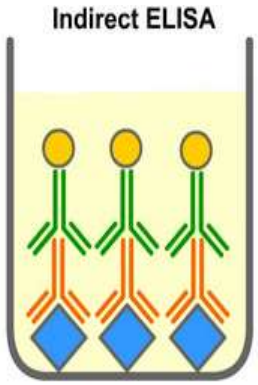
Immunosuppression

- Destruction of T cells
  - CD4+T cell destruction by HIV
    - Impaired antigen processing and presentation, and cytokine production
    - Death by apoptosis, fusion (syncytia formation), lysis by CD8+ T cells
- Abortive infection of monocytes/macrophages and T/B cells
  - CMV, EBV, measles virus

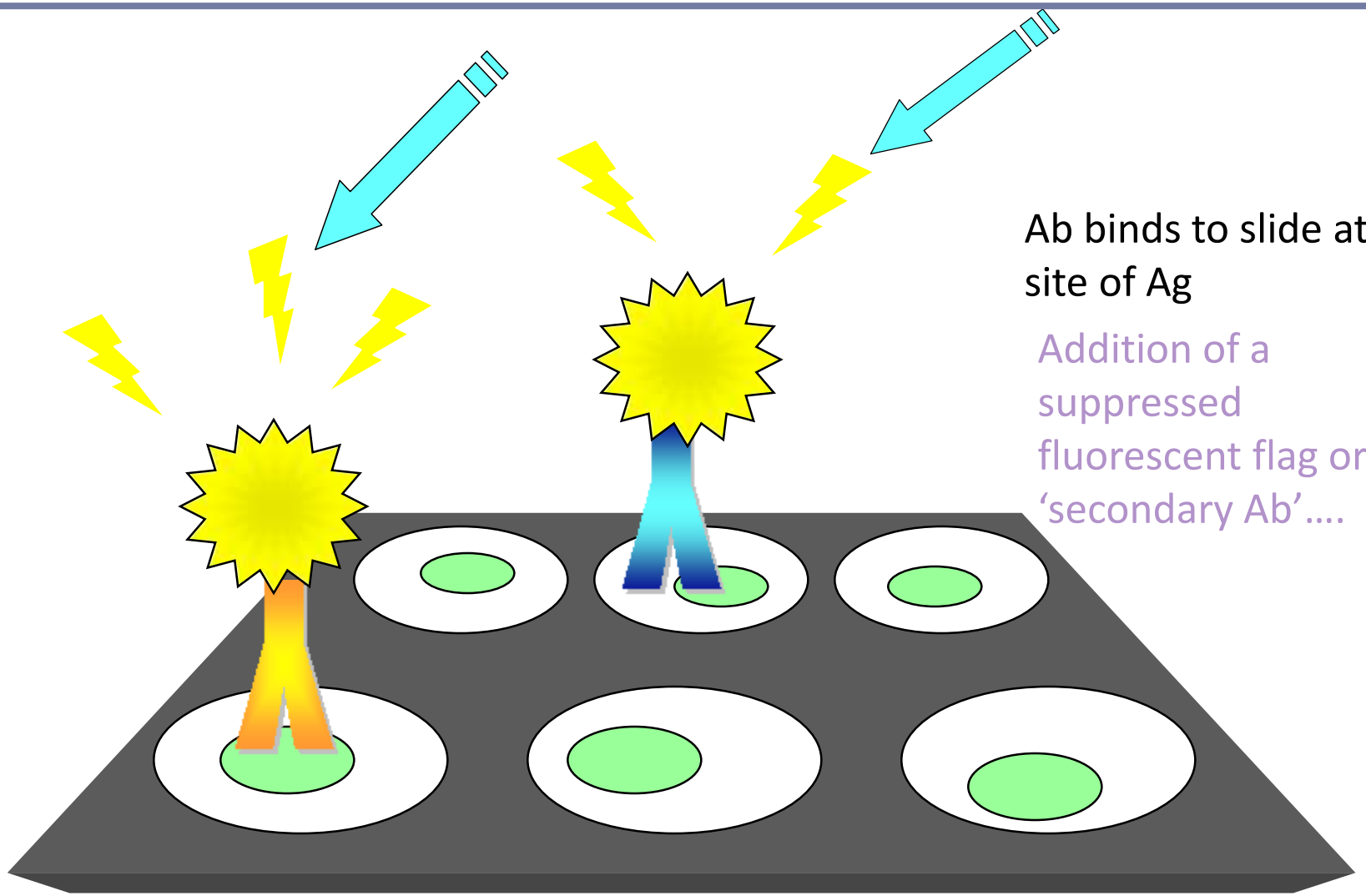
Laboratory diagnosis of Viral Diseases

- Culture
  - Gold standard in many viral diseases
- Serology (Antigen detection)
  - Indirect Immunoflourescence to detect viral proteins
  - Direct ELISA
  - Western blotting
- Serology (antibody detection)
  - Detection of serum antibodies
  - Not very important in clinical decision making
- Nucleic acid detection
  - RT-PCR to detect viral RNA, PCR to detect viral DNA
  - Not available in all settings
  - Nucleic acid sequencing is important in typing and epidemiological investigations



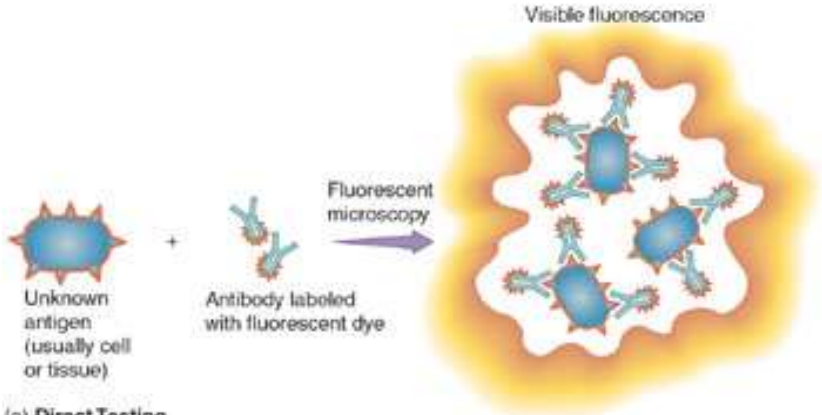


Immunohistochemistry

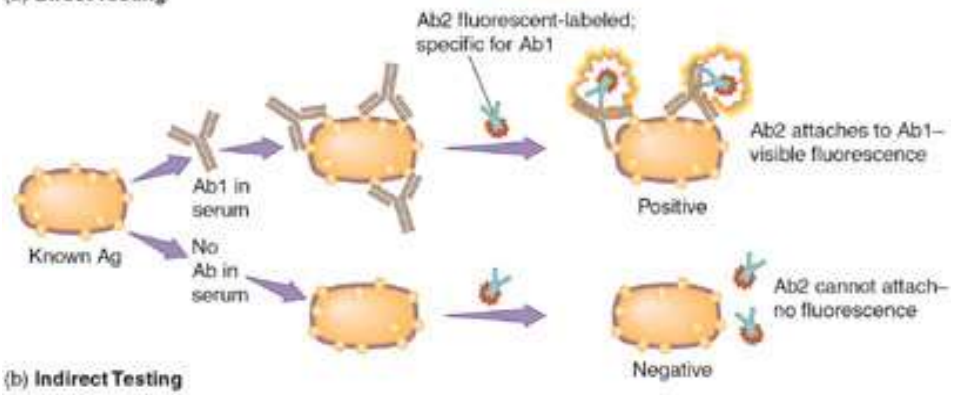


Ab binds to slide at site of Ag

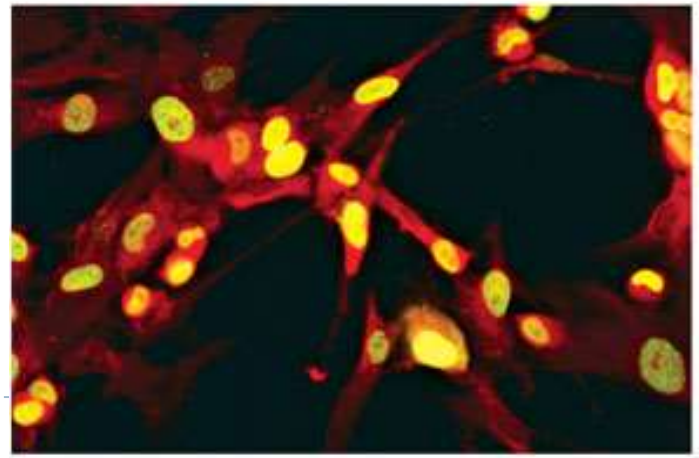
Addition of a suppressed fluorescent flag or ‘secondary Ab’….



(a) **Direct Testing**



(b) **Indirect Testing**



(c) **Indirect Immunofluorescence Testing**

Viral Genetics and Evolution

- Viruses have the greatest genetic diversity
  - Natural selection
    - Mutation (antigenic drift)
    - Recombination
    - Reassortment (antigenic shift)
      - Segmented genomes

- Mutations
  - Caused by errors in replicating (copying) genomes
  - Many are lethal (however, non-lethal mutations result in change of phenotype that may confer selective advantage)
    - Non-functional gene product
    - Stop codon that terminates translations
  - Some mutations are neutral

Mutations

- Classified according to kind of nucleic acid change (genotypic) or phenotype change (phenotypic)

- Genotypic mutations
  - **Point mutations- single nucleotide substitutions**
  - Deletions
  - Insertions
  - Duplications
  - Inversions
  - **Incorporation of foreign nucleic acid (Reassortment)**

Mutations

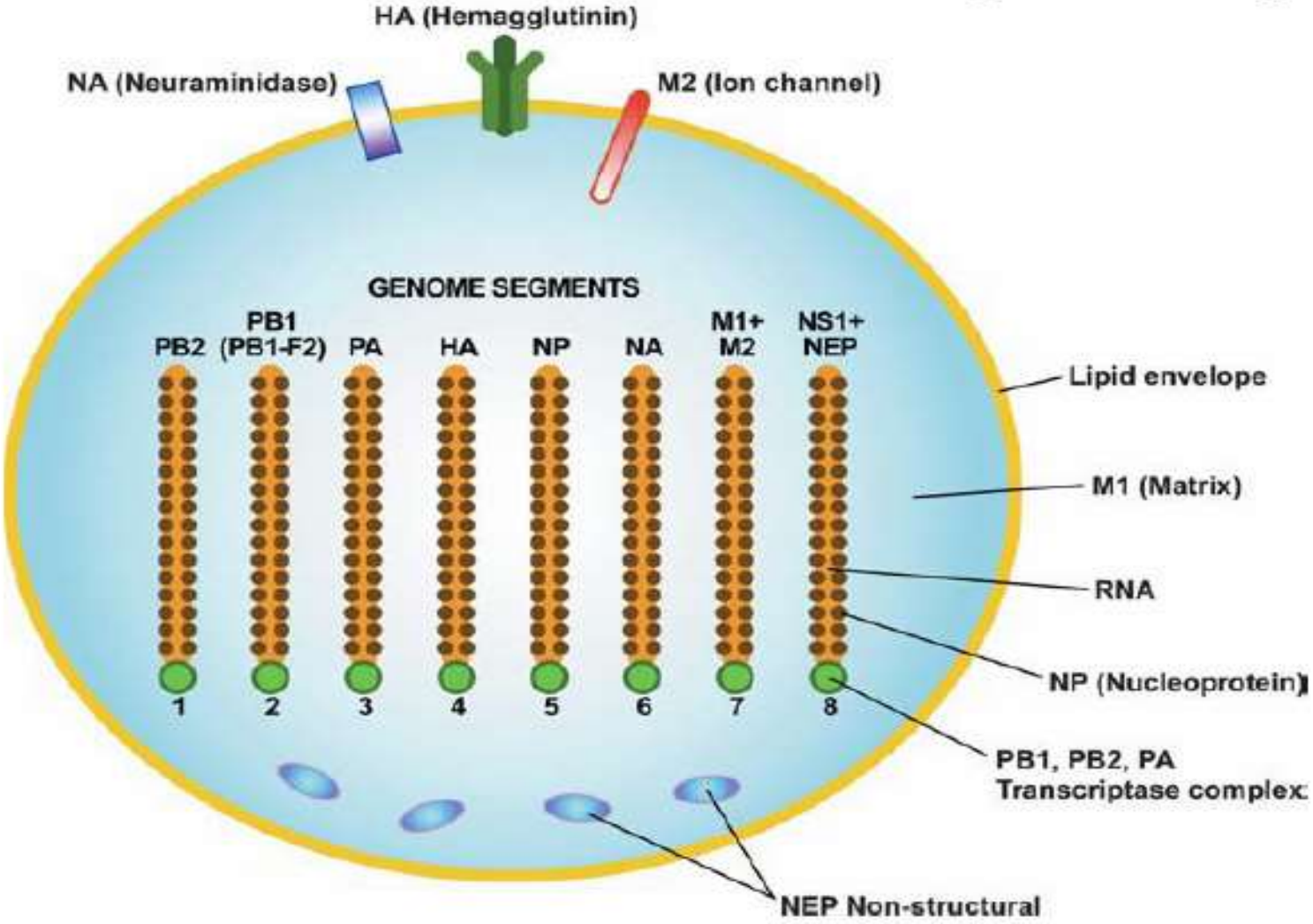
- Classified according to kind of nucleic acid change (genotypic) or phenotype change (phenotypic)

- Phenotypic
  - Type of plaque (plaque mutations)
  - **Resistance mutation (escape mutations)**
  - Conditional lethal mutations
  - **Host range mutations**
  - Temperature sensitive mutations

Mutation Rates

- DNA viruses
  - Rates similar to eukaryotic DNA
    - Viral DNA is subject to same cellular 'proofreading' exonuclease correction
    - Rates occur at 10^{-8} to 10^{-11} per incorporated nucleotide
    - Point mutations in the third nucleotide of a codon are often silent (wobble hypothesis/redundancy of the genetic code)
  - Rates are higher for RNA viruses
    - No cellular proofreading for RNA
    - E.g. 10^{-3} to 10^{-4} nearly every progeny virion is different from parent and from each other
    - Most mutations are lethal but most non-lethal mutations accumulate quickly

Structure of Influenza Viruses



Influenza Genome

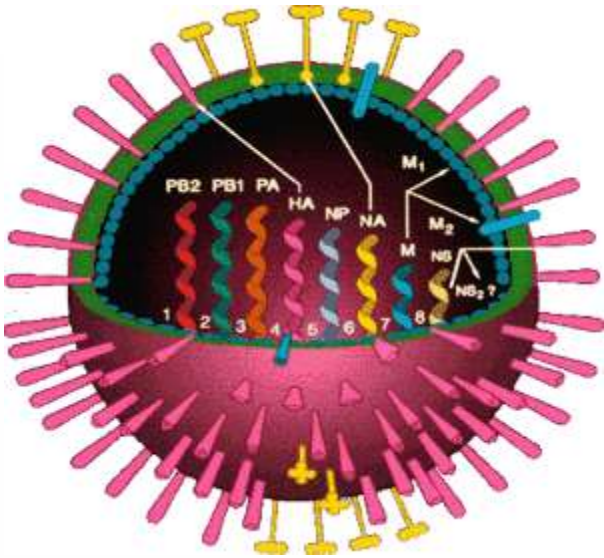
- Consists of s/s (-)sense RNA in 8 segments (7 in Influenza C).
- The structure of the influenza virus genome is known in great detail because of the tremendous amount of genetic investigation (conventional and molecular) which has been done.
- The 5' and 3' terminal sequences of all the genome segments are highly conserved

Influenza Genome

Segment:	Size (nt)	Polypeptide (s)	Function
1	2341	PB2	Transcriptase: cap binding
2	2341	PB1	Transcriptase: elongation
3	2233	PA	Transcriptase: protease activity (?)
4	1778	HA	Haemagglutinin
5	1565	NP	Nucleoprotein: RNA binding; part of transcriptase complex; nuclear/cytoplasmic transport of vRNA
6	1413	NA	Neuraminidase: release of virus
7	1027	M1	Matrix protein: major component of virion
		M2	Integral membrane protein - ion channel
8	890	NS1	Non-structural: nucleus; effects on cellular RNA transport, splicing, translation. Anti-interferon protein.
		NS2	Non-structural: nucleus+cytoplasm, function unknown

Membrane proteins

- Hemagglutinin (HA)
  - Attach to cell surface sialic acid receptors
  - Facilitate entry if the virus into the cell
  - Crucial component of current vaccine
- Neuraminidase (NA)
  - Catalyze the cleavage if glycosidic linkages to sialic acid on host cell and the virion surfaces
  - Inhibition of NA— the most effective antiviral treatment
- M2 protein — small amount in influenza A
  - Ion channel
  - Regulate the internal PH of the virus
  - Blocked by antiviral drug



Influenza Virus

Influenza A

- Clinical and epidemiological importance
- Mutability of virus produces antigenic changes
  - Mutation and whole gene 'swapping' (reassortment) between different strains
  - Recombination
    - Results: antigenic drifts and antigenic shifts
- Subtypes based on H and N antigens
  - H antigens: 15
  - N antigens: 9

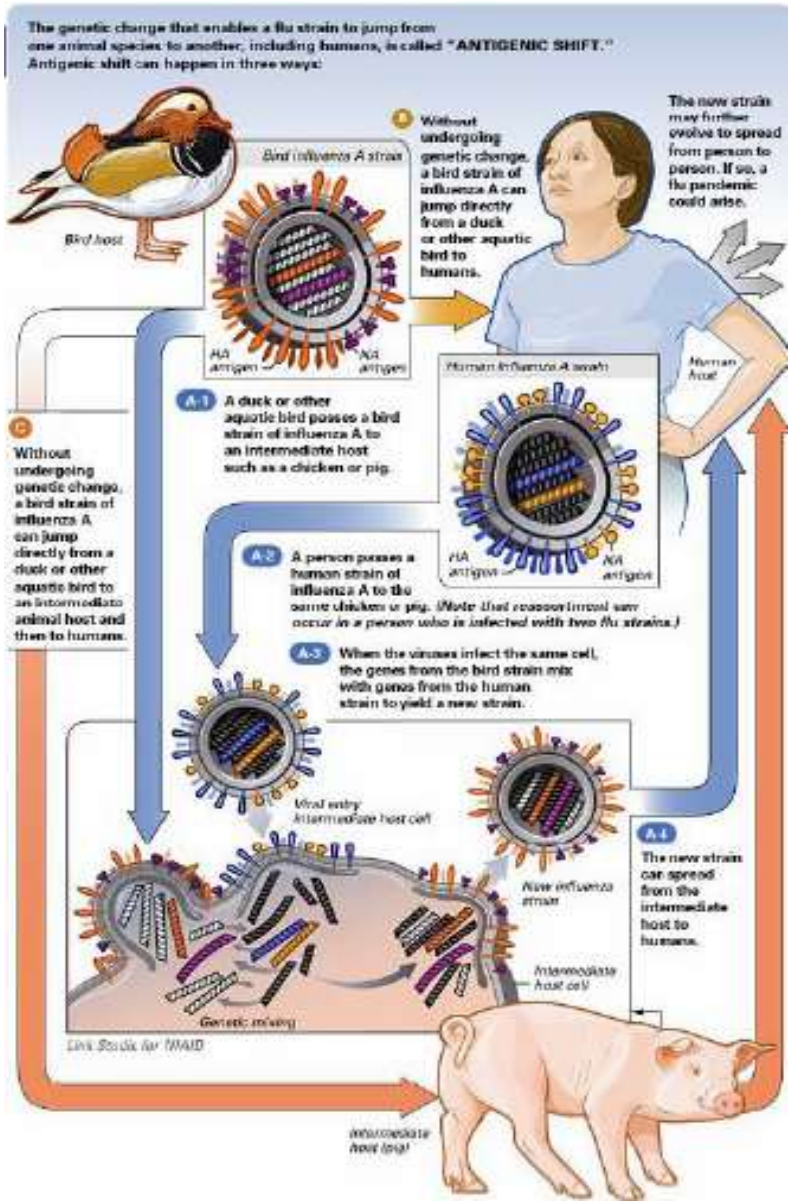
- Avian subtypes :
  - 2 H subtypes (H5 and H7)
  - 7 N subtypes

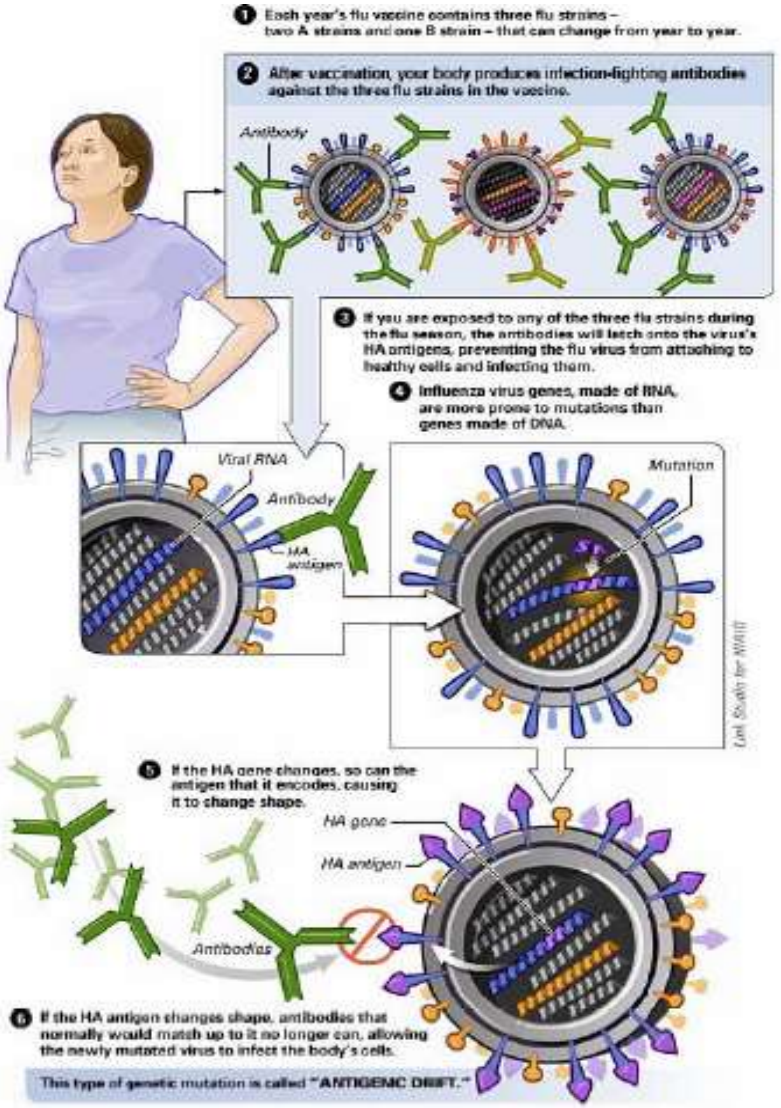
- Only H1, H2 and H3 H subtypes and N1 and N2 N subtypes are associated with stable human infection

Influenza A

- Antigenic drift
  - Subtle changes in H, N or non structural genes
  - Cause by point mutations
  - Occurs every few years
  - Allow viral maintenance in a population
  - Responsible for seasonal outbreaks

- Antigenic shift
  - Major antigenic changes
    - Due to reassortment or whole gene 'swapping'
    - New subtype may develop mutations too
  - Correlates with epidemics and pandemics
    - Little or no immunity





Antigenic Shift

Antigenic Drift

Influenza Viruses in Birds, Pigs and Humans

Characteristics of Influenza Viruses

- Three major groups based on ribonuclear antigens
  - A: Most studied, Human, swine, avian, equine, marine mammals
  - B: Humans only
  - C: Humans, swine
- Subtype A
  - Greatest virulence and epidemic spread
- Specificity
  - Receptors
    - α-2-6 in humans
    - α-2-3 in birds
    - α-2-6, α-2-3 in pigs

Human influenza A virus

- Only H1, H2 and H3 H subtypes and N1 and N2 N subtypes are associated with stable human infection

- Circulating strains
  - H1N1, H3N2, H1N2
  - Antigenically and genetically distinct from swine counterparts
  - H3N2-intorduced in 1968 (Hong Kong Pandemic)
    - Preceded H2N2
    - Reassortment between avian and human influenza A

- Transmission
  - Person to person via respiratory route by aerosols

Avian Influenza Viruses among Birds

- Contact with infected birds
  - contaminated nasal, respiratory, or fecal material
  - usually fecal-oral transmission
- Indirect spread
  - virus-contaminated water and fomites
    - Virus suspensions in water have been shown to retain infectivity for more than 100 days at 17° C
  - In contrast to influenza virus infections in mammals (humans, swine, and horses) - primarily transmission by aerosols
- Transmission from wild birds to domestic poultry
  - greatest where domestic birds roam freely, share a water supply with wild birds, or use a water or food supply that might become contaminated by droppings from infected wild bird carriers
- 2002-2005 SE Asia, 150 million birds infected (H5N1)

Highly Pathogenic Avian Influenza A Viruses

- 'fowl plague'
- low pathogenic avian influenza virus, LPAIV (mild) > highly susceptible poultry species > a series of mutation (several cycles of infection) > highly pathogenic avian influenza viruses, HPAIV (overwhelming systemic and rapidly fatal disease)
- HPAI in poultry is characterized by a sudden onset, severe illness of a short duration, and a mortality approaching virtually 100 % in vulnerable species.
- H5 and H7

Why avian viruses at present rarely infect and spread between humans

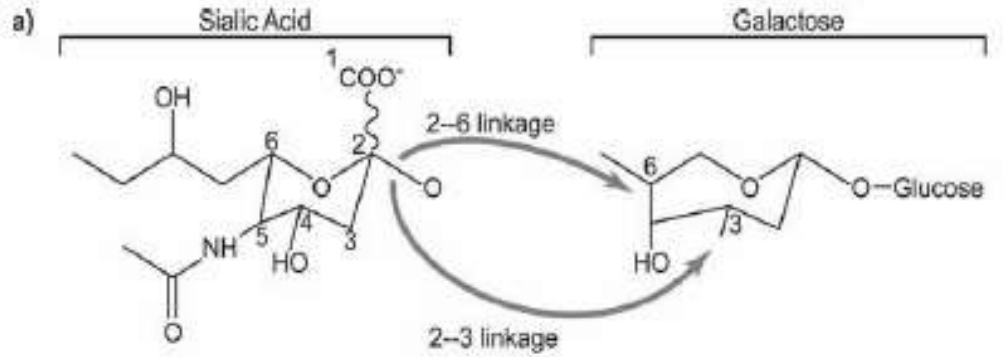
- Avian and human flu viruses seem to target different regions of a patient's respiratory tract.
  - SA α 2,6Gal dominant on epithelial cells in human nasal mucosa, with SA α 2,3Gal occasionally detected
    - Paranasal sinuses, pharynx, trachea, bronchi
  - SA α 2,3Gal found in lower respiratory tract
    - non-ciliated cuboidal bronchiolar cells at the junction between the respiratory bronchiole and alveolus
    - Alveolar wall
- Human-derived viruses
  - preferentially recognize SA α 2,6Gal
  - bind extensively to epithelial cells in the bronchi
  - Bind to a lesser degree to alveolar cells
- Avian viruses
  - preferentially recognize SA α 2,3Gal
  - bind extensively to alveolar cells but less widely to bronchial epithelial cells
  - More difficult to transmit via the upper respiratory tract
- A/Hong Kong/213/03 H5N1 (human isolate)
  - recognizes both SA α 2,3Gal and SA α 2,6Gal
  - binds extensively to both bronchial and alveolar cells

Pigs

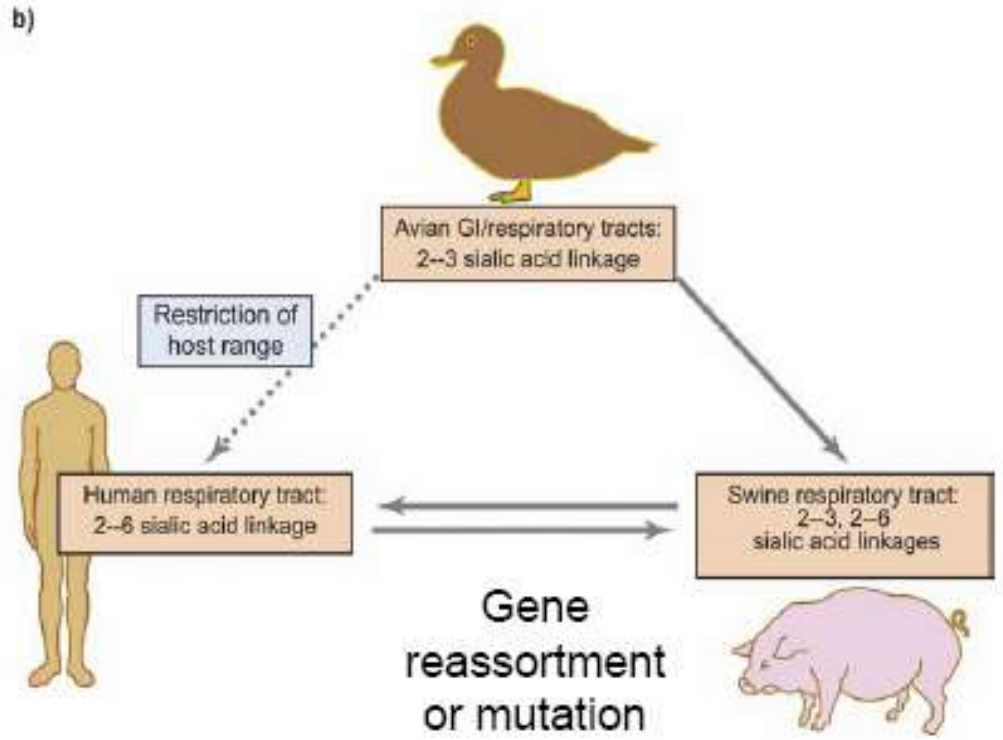
- Respiratory epithelial cells in the pig contain both 2,3- and 2,6 linkages, susceptible to both human and avian influenza viruses .
- a potential source of new pandemic strains
  - mixed infection of avian and human strains, potentially resulting in new reassortant viruses.
  - purely avian strains can adapt to human receptor recognition.

H1N1, H3N2, H1N2

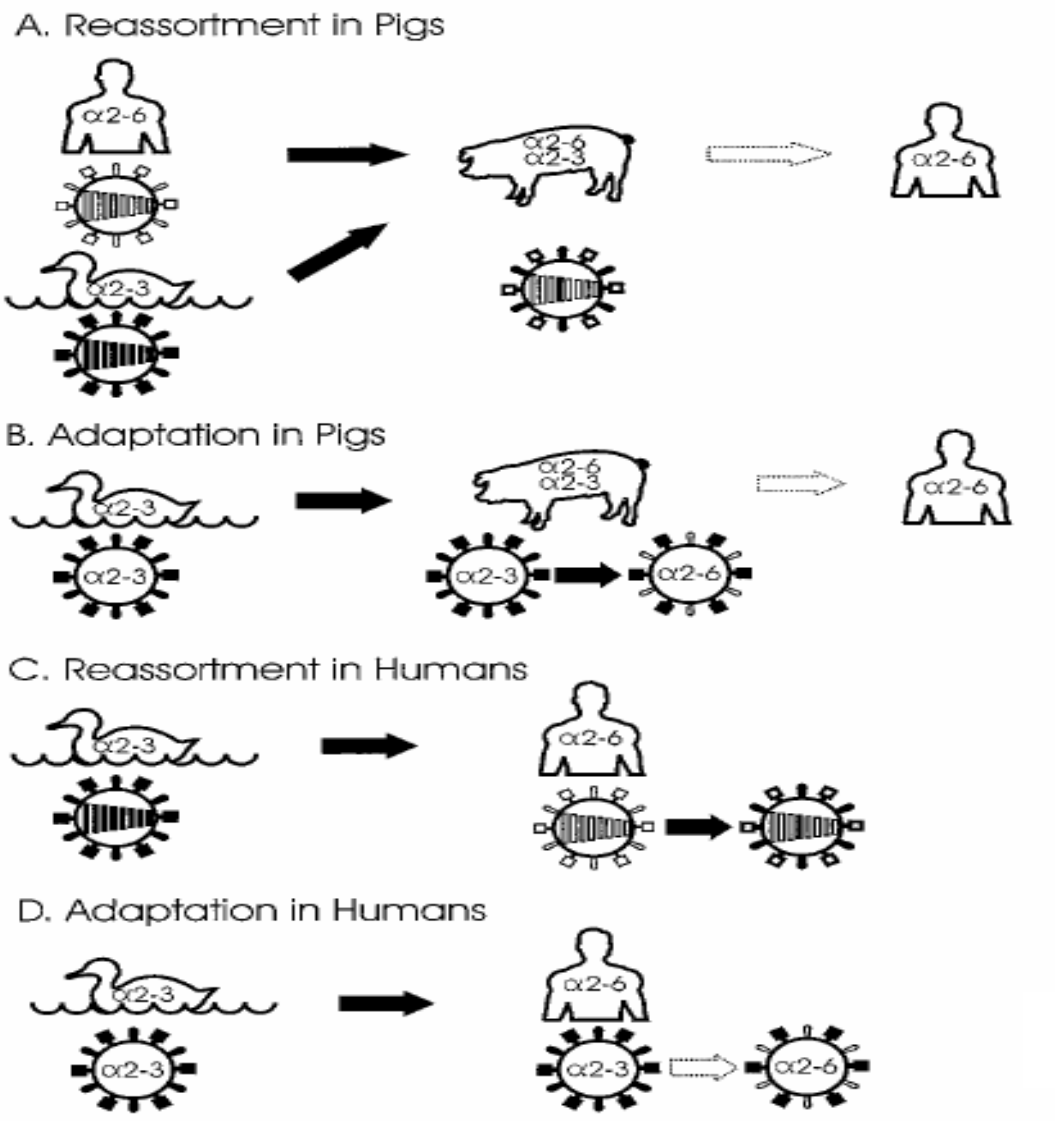
Sialic acid residues can be covalently attached to galactose residues of integral glycoproteins and glycolipids via either 2–3 or 2–6 α linkages.

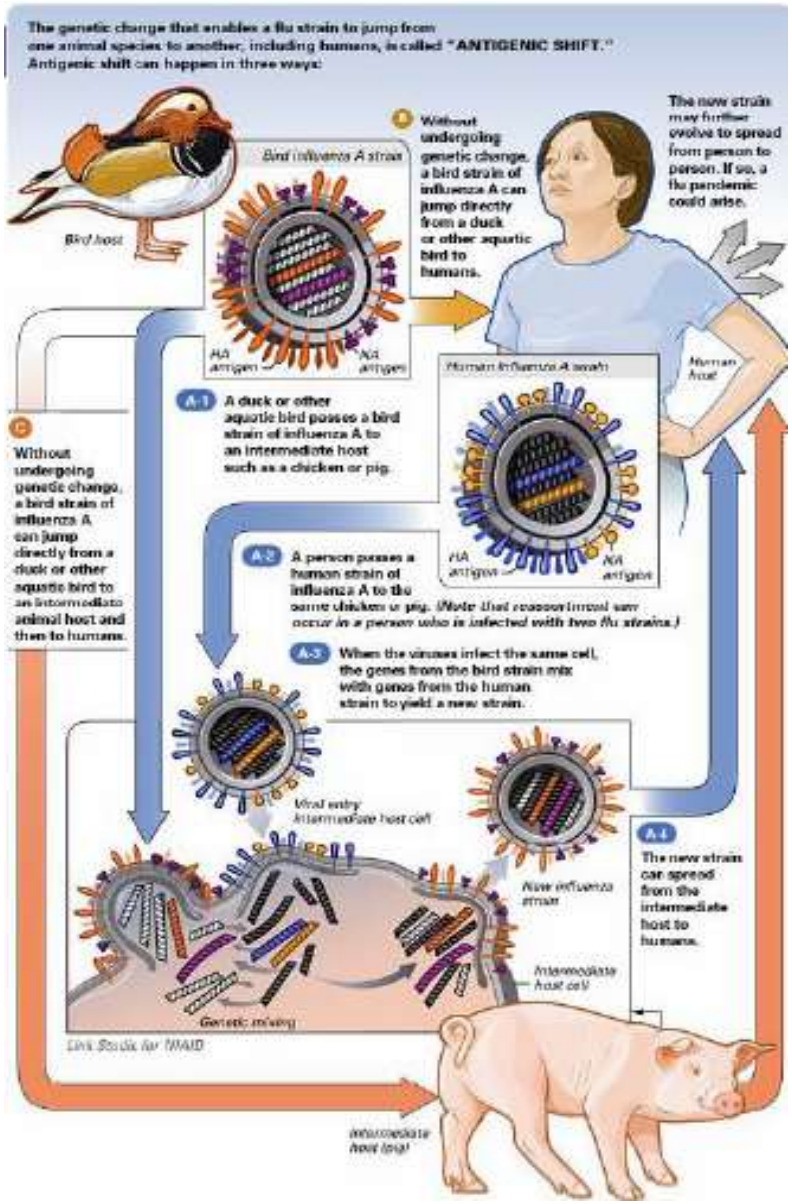


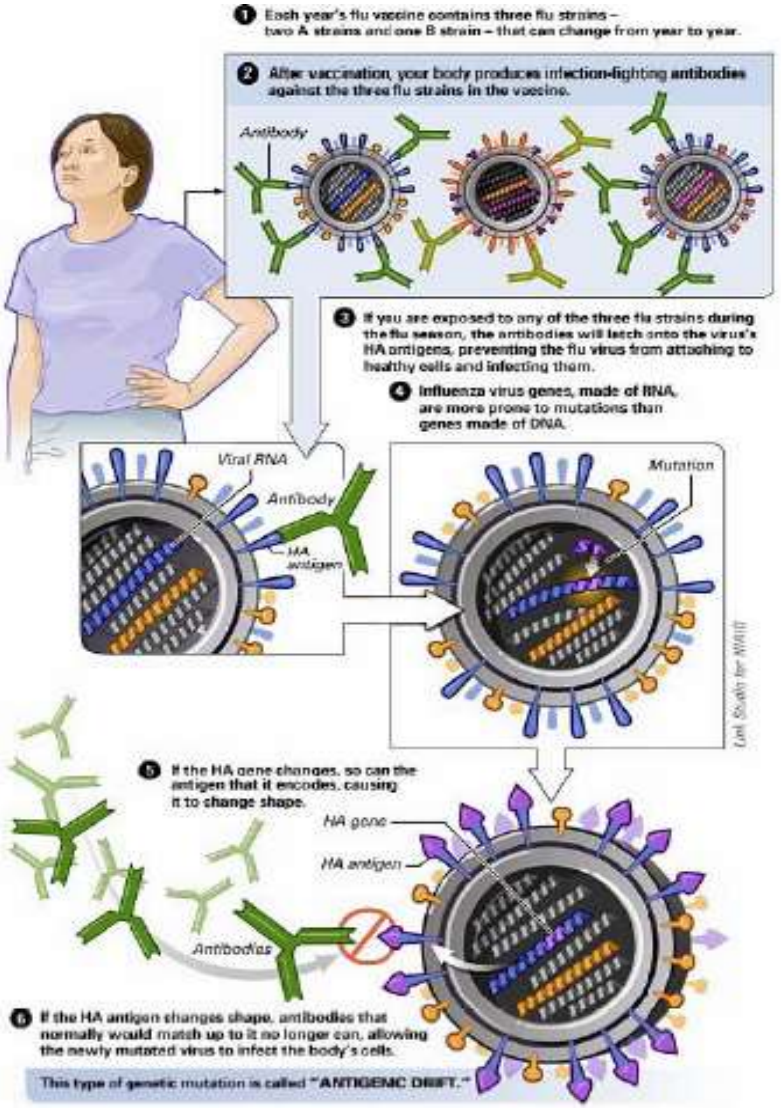
The avian, human, and swine upper respiratory tract epitheliae preferentially express 2–3 linkages, 2–6 linkages, and both 2–3 and 2–6 linkages respectively



Molecular basis for generation in pigs of Influenza A with pandemic potential







Antigenic Shift

Antigenic Drift

Influenza Pandemics

2005

- New influenza A virus strain spreads rapidly throughout the world

- Three to four times each century
  - We are due?
  - Any time of the year
  - Excess morbidity and mortality

CHARACTERISTICS OF SEASONAL INFLUENZA EPIDEMICS AND THE THREE INFLUENZA PANDEMICS OF THE TWENTIETH CENTURY

	Influenza virus type	Estimated rates of symptomatic infection (%)	Estimated total excess deaths worldwide	Case fatality rates (%)	Mortality pattern
Seasonal influenza epidemics	Various A & B viruses	5–20*	Up to ½ million annually*	~0.001*	Excess mortality rates are 50–200 times greater in persons ≥65 years old compared with those <65 years. However, in the first 2–7 years following a pandemic, mortality rates in persons ≥65 years old may only be 2–30 times greater than those <65 years. The majority of excess deaths (more than 85%) occur among the elderly and those with high-risk medical conditions.
Spanish influenza pandemic 1918–1919	A/H1N1	20–40	>40 million	>2.5	Excess mortality rates were 3 times greater in persons <65 years old compared with persons ≥65 years old. 99% of excess deaths occurred in people <65 years. In Australia, 60% of excess deaths occurred in healthy persons 20–45 years.
Asian influenza pandemic 1957–1958	A/H2N2	10–60	2 million	0.01–0.05	Excess mortality rates were 18 times greater in persons ≥65 years compared with those <65 years. 36% of excess deaths were in persons <65 years old.
Hong Kong influenza pandemic 1968–1969	A/H3N2	25–30	1 million	0.01–0.05	Excess mortality rates were 13 times greater in persons ≥65 years compared with those <65 years. 41% of excess deaths were in persons <65 years old.

* Mortality due to seasonal influenza epidemics varies greatly depending on the predominant type/subtype of virus circulating and, for influenza A, the time since the subtype evolved in the human population.

Data sources: Mandell et al[1]; Taubenberger and Morens[2]; Department of Health and Ageing[11]; World Health Organization[4, 12, 13]; and Simonsen et al.[14]

Clarifications

- Seasonal flu
  - Influenza viruses circulating in humans
    - Maintained by antigenic drift

Avian flu ≠ Pandemic flu

- Avian flu
  - Bird flu
    - Occurs in aquatic birds (waterfowl)
    - Drifts and shifts cause transmission and pathogenesis in other species

- Pandemic flu
  - New influenza A virus strain spreads rapidly throughout the world
  - Caused by shifts
    - Transmission and pathogenesis
    - Limited immunity

Pandemic Threat of 2005

- Avian H5N1 epidemics in poultry
  - Ability to cross species barrier
  - Level of concern

- Criteria needed for pandemic virus
  - Emergence of a new virus subtype in which population has no or little immunity
  - Ability to replicate and cause serious illness in humans
  - Efficient transmission from human to human
    - H5N1 only met the first two criteria

- Predictions
  - It cannot be predicted whether H5N1 will meet third criterion
  - Historical data: no pandemic preceded by pathogenic avian epidemic
  - Genetic alterations in H5N1 need to occur
    - Risk and timing cannot be predicted

H1N1

- Swine influenza
- Became a pandemic in **2009**
- Despite H1N1 becoming pandemic it has a much lower virulence than N5N1 and caused fewer deaths
- Discussion of emergency of pandemic avian influenza?

End