Medical Virology Lec.1

General properties of virus, structure, Classification, of DNA&RNA viruses.

Medical virology : The science that deal with the study of the medically important viruses, which infect humans.

Viruses: **'virus' (from the Latin 'poison')**

Viruses may be defined as a cellular organisms whose genomes consist of **nucleic acid**, and which obligatory **replicate inside host cells** using **host metabolic machinery** and **ribosome's** to form a **pool of components** which assemble into particles called **virions**, which serve to **protect the genome** and to **transfer it to other cells**. Viruses do not **respire**, **move,** and nor do they **grow**. They are distinct from other so-called virus-like agents such as **viroids , plasmids and prions**.

Viruses are distenect from living organism : Viruses are submicroscopic, obligate intracellular parasites. Clearly, it is not a problem to differentiate viruses from higher macroscopic organisms. Even within a broad definition of microbiology encompassing **prokaryotic** organisms and microscopic **eukaryotes** such as algae, protozoa, and fungi. A few groups of prokaryotic organisms, however, have specialized intracellular parasitic life cycles and confound the above definition.

These are the Rickettsiae and Chlamydiae—obligate intracellular parasitic bacteria which have evolved to be so cell-associated that they can exist outside the cells of their hosts for only a short period of time before losing viability. Therefore, it is necessary to add further clauses to the definition of what constitutes a virus:

- **Virus particles are produced from the assembly of preformed components, whereas other agents grow from an increase in the integrated sum of their components and reproduce by division.**
- **Virus particles (virions) themselves do not grow or undergo division.**
- **Viruses lack the genetic information that encodes apparatus necessary for the generation of metabolic energy or for protein synthesis (ribosomes).**

No known virus has the biochemical or genetic potential to generate the energy necessary to drive all biological processes (e.g., macromolecular synthesis).They are therefore absolutely dependent on the host cell for this function. **It is often asked whether viruses are alive or not. One view is that inside the host cell viruses are alive, whereas outside it they are merely complex assemblages of metabolically inert chemicals**. That is not to say that

chemical changes do not occur in extracellular virus particles, as will be explained elsewhere, but these are in no sense the ‘growth 'of a living organism.

NANOMETER 10^{-9}meter. 1nm = 10Å. 1000nm = 1µm.

The differences between virus and other microorganism

	Growth on artificial media	Division by binary fission	Whether they have both DNA and RNA	Whether they have ribosomes	Have muramic acid	Sensitivity to antibiotics
Bacteria	+	+	+	+	+	+
Mycoplasma	+	+	+	+	-	+
Rickettsia	-	+	+	+	+	+
Chlamydia	-	+	+	+	-	+
Viruses	-	-	-	- *	-	-

Viruses properties.

1. They are obligate intracellular parasites.
2. Probably there are no cells in nature that escape infection by one or more kinds of viruses. (Viruses that infect bacteria are called **bacteriophages**.) .Outside the cell, they consist of particles called **virions**.
3. They are not cells. They are very simple structures consisting essentially of a nucleic acid genome, protected by a shell of protein. The genome consists of only one type of nucleic acid: either RNA or DNA. Most DNA viruses are double stranded and most RNA viruses have a single stranded genome.
4. They are metabolically inert and can only replicate once they are inside a host cell.
5. They have no organelles.
6. They are very small, sizes range from 20 to 200 nm. This is beyond the resolving power of the light microscope.

Factors which affect host range: Viruses infect all major groups of organisms: vertebrates, invertebrates, plants, fungi, bacteria.

- Whether the virus can get into the host cell
- If the virus can enter the cell, is the appropriate cellular machinery available for the virus to replicate?
- If the virus can replicate, can infectious virus get out of the cell and spread the infection?

VIRUS STRUCTURE

1. **Nucleic acid -**contains 3-400 genes

Deoxyribonucleic Acid (DNA) -unique features

- Single and/or double stranded
- Circular or linear
- Bound protein molecules

Ribonucleic Acid (RNA) - Unique features

- Single or double stranded
- Segmented or unsegmented
- Bound protein molecules

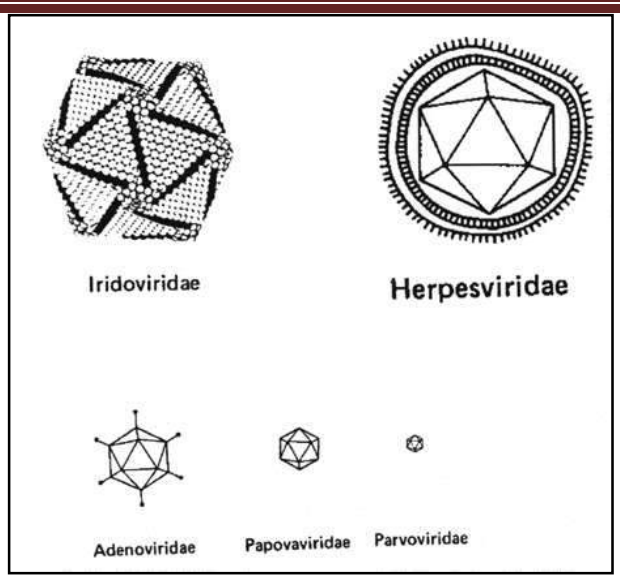
2. **Capsid** -The capsid accounts for most of the virion mass. It is the protein coat of the virus. It is a complex and highly organized entity which gives form to the virus. Subunits called protomeres aggregate to form capsomeres which in turn aggregate to form the capsid.

3**. Envelope** -this is an amorphous structure composed of lipid, protein and carbohydrate which lies to the outside of the capsid. It contains a mosaic of antigens from the host and the virus. A naked virus is one without an envelope.

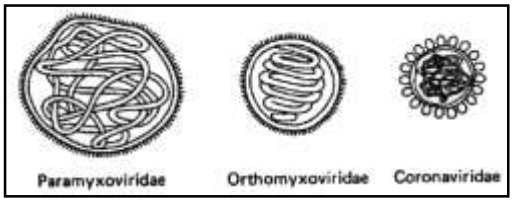
4**. Spikes.** These are glycoprotein projections which have enzymatic and/or adsorption and/or hemagglutinating activity. They arise from the envelope and are highly antigenic.

- **Morphology (Symmetry)**

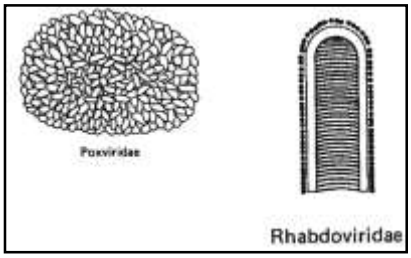
1. **Icosahedral** -The protomeres aggregate in groups of five or six to form the capsomere. In electron micrographs, capsomeres are recognized as regularly spaced rings with a central hole. The shape and dimensions of the icosahedron depends on characteristics of its protomeres. All icosahedral capsids have 12 corners each occupied by a penton capsomere and 20 triangular faces, each containing the same number of hexon capsomeres. Icosahedral symmetry is identical to cubic symmetry.



2. **Helical** -The protomeres are not grouped in capsomeres, but are bound to each other so as to form a ribbon-like structure. This structure folds into a helix because the protomeres are thicker at one end than at the other. The diameter of the helical capsid is determined by characteristics of its protomeres, while its length is determined by the length of the nucleic acid it encloses.

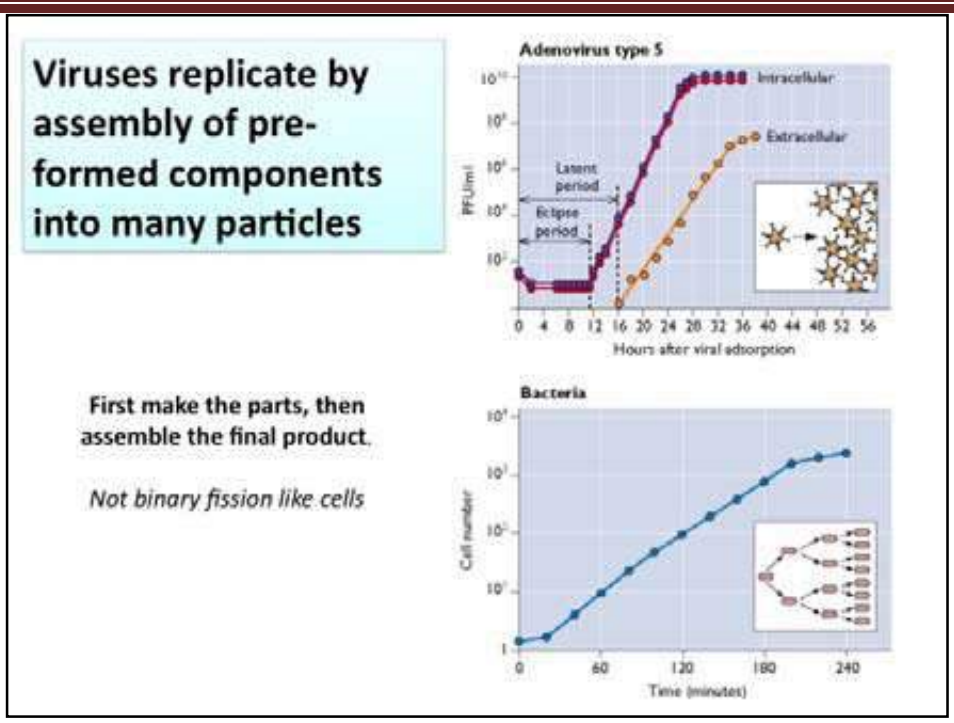


3. **Complex** -e.g., that exhibited by poxvirus and rhabdovirus. This group comprises all those viruses which do not fit into either of the above two groups.



Viral replication

Viral growth curve: The curve shows the amount of virus produced at different times of infection. it was plot the amount of versus the time there will be no virus detected after 3-4 hours from entry to cell (eclipse period) ,mean while there is accumulation of N A. inside the cells ,then virus are produced & exit from the cell (Rize period). The time required for these periods varies according to the type of virus form **1 min. (bacteriophage) to 12 hours (human's virus).**



Replication Cycle

1. Adsorption -Viruses can enter cells via phagocytosis, viropexis or adsorption. Adsorption is the most common process and the most highly specific process. It requires the interaction of a unique protein on the surface of the virus with a highly specific receptor site on the surface of the cell.

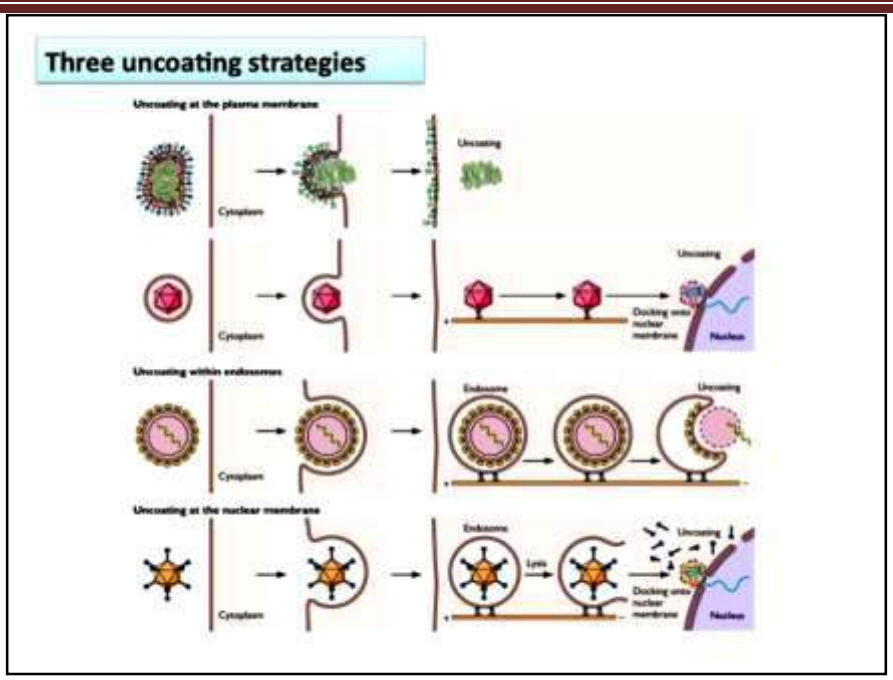
2. Penetration -This occurs by one or more processes.

- Enveloped viruses fuse their envelope with the membrane of the host cell. This involves local digestion of the viral and cellular membranes, fusion of the membranes and concomitant release of the nucleocapsid into the cytoplasm.

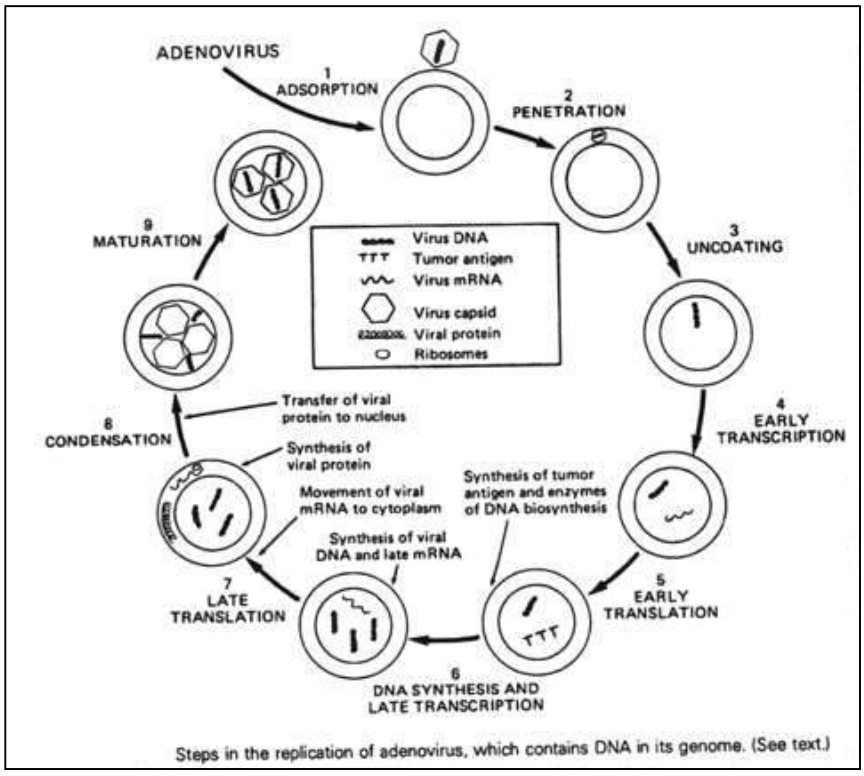
- Naked viruses bind to receptor sites on the cellular membrane, digest the membrane and enter into the cytoplasm intact.

- Both naked and enveloped viruses can be ingested by phagocytic cells. However, in this process they enter the cytoplasm enclosed in a cytoplasmic membrane derived from the phagocytic cell.

3. Uncoating -During this stage cellular proteolytic enzymes digest the capsid away from the nucleic acid. This always occurs in the cytoplasm of the host cell. The period of the replication cycle between the end of the Uncoating stage and maturation of new viral particles is termed the eclipse. Thus during the eclipse stage, no complete viral particles can be viewed within the cell.



4. **Replication of nucleic acid.** Replication of viral nucleic acid is a complex and variable process. The specific process depends on the nucleic acid type.

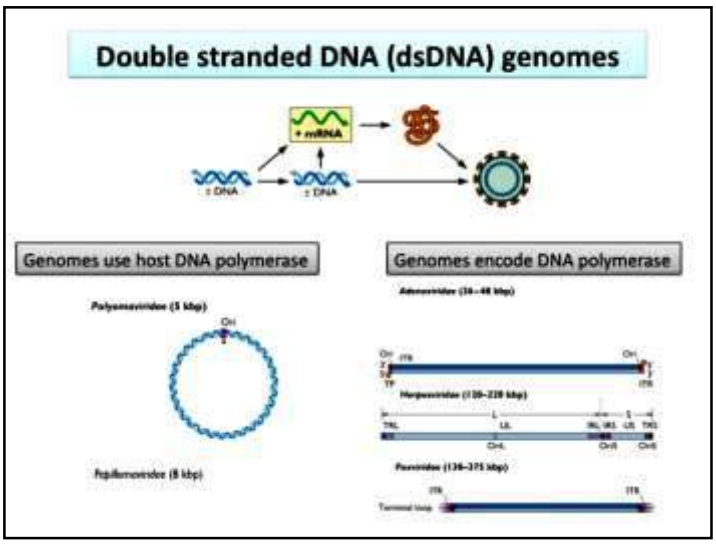


Steps in the replication of adenovirus, which contains DNA in its genome. (See text.)

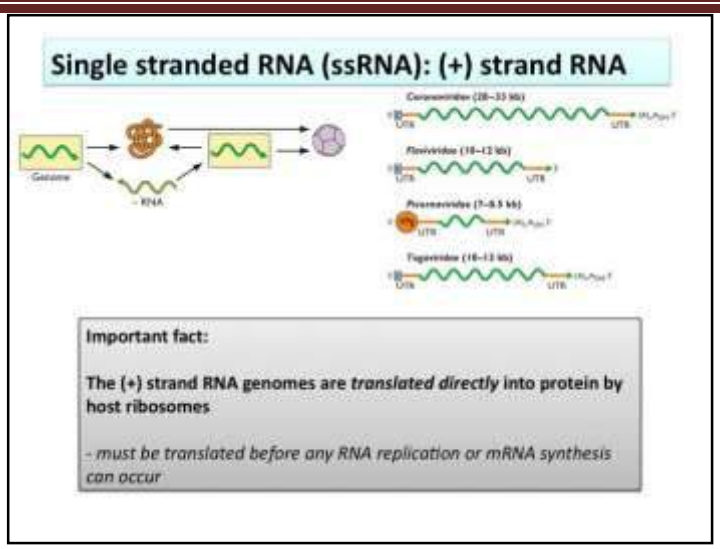
DNA virus replication -with the exception of the poxviruses, all DNA viruses replicate in the nucleus. In some cases one of the DNA strands is transcribed (in others both strands of a small part of the DNA may be transcribed) (step 4) into specific mRNA, which in turn is translated (step 5) to synthesize virus-specific proteins such as tumor antigen and enzymes necessary for biosynthesis of virus DNA. This period encompasses the early virus functions. Host cell DNA synthesis is temporarily elevated and is then suppressed as the cell shifts over to the manufacture of viral DNA (step 6). As the viral DNA continues to be transcribed, late virus functions become apparent. Messenger RNA transcribed

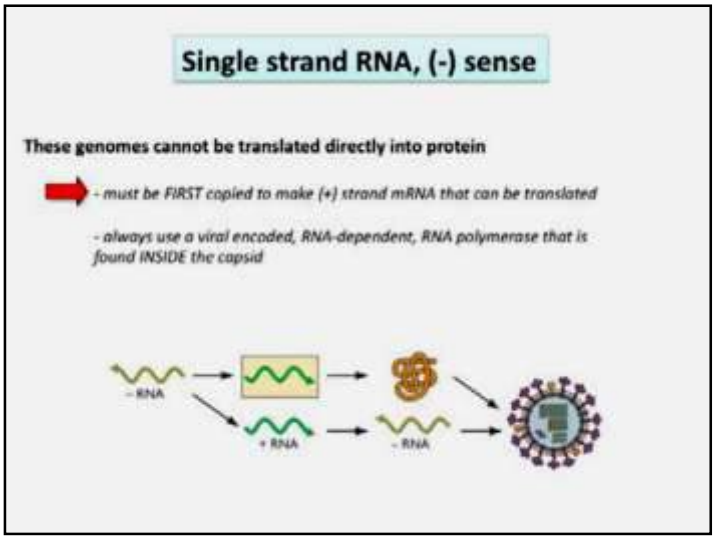
during the later phase of infection (step 6) migrates to the cytoplasm and is translated (step 7). Proteins for virus capsids are synthesized and are transported to the nucleus to be incorporated into the complete virion (step 8). Assembly of the protein subunits around the viral DNA results in the formation of complete virions (step 9), which are released after cell lysis.

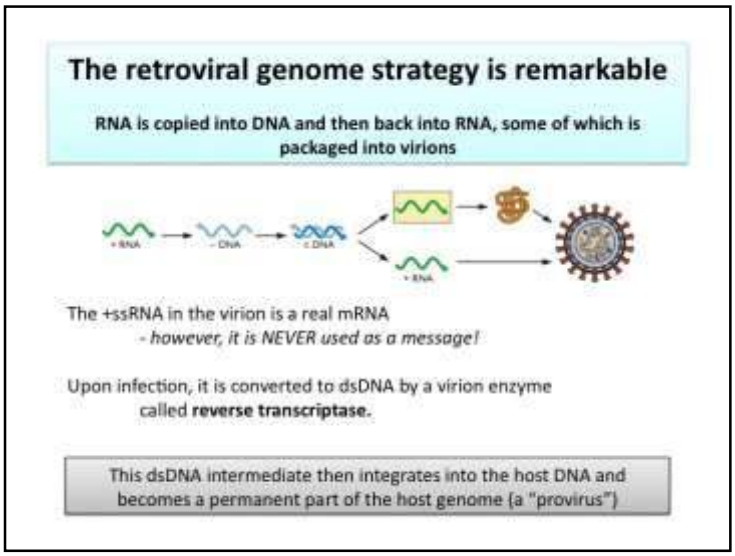
The single-stranded DNA viruses first form a double stranded DNA, utilizing a host DNA-dependent DNA polymerase. They then undergo a typical replication cycle.



RNA virus replication -with the exception of the orthomyxoviruses and retroviruses, all RNA viruses replicate in the cytoplasm of the host cell. The exact process varies with the species of virus. The single-stranded RNA that is released after Uncoating will act as either: (a) the mRNA to synthesize viral-coded proteins; or (b) a template to synthesize mRNA; or (c) a template to synthesize double stranded RNA, which is then used as a template to synthesize mRNA; or (d) a template to synthesize double-stranded DNA, which is then utilized as a template to synthesize mRNA. This latter process occurs only with the retroviruses (on cornaviruses).







The replication of poliovirus, which contains a single-stranded RNA as its genome, provides a useful example. All of the steps are dependent of host DNA and occur in the cell cytoplasm. Polioviruses absorb to cells at specific cell receptor sites (step 1), losing in the process one virus polypeptide. The sites are

specific for virus coat-cell interactions. After attachment, the virus particles are taken into the cell by viropexis (similar to pinocytosis) (step 2), and the viral RNA is uncoated (step 3). The single-stranded RNA then serves as its own messenger RNA. This messenger RNA is translated (step 4), resulting in the formation of an RNA-dependent RNA polymerase that catalyzes the production of a replication intermediate (RI), a partially double-stranded molecule consisting of a complete RNA strand and numerous partially completed strands (step 5). At the same time, inhibitors of cellular RNA and protein synthesis are produced. Synthesis of (+) and (-) strands of RNA occurs by similar mechanisms. The RI consists of one complete (-) strand and many small pieces of newly synthesized (+) strand RNA (step 6). The replicative form (RF) consists of two complete RNA strands, one (+) and one (-).

The single (+) strand RNA is made in large amounts and may perform any one of three functions: (a) serve as messenger RNA for synthesis of structural proteins; b) serve as template for continued RNA replication; or (c) become encapsulated, resulting in mature progeny virions. The synthesis of viral capsid proteins (step 7) is initiated at about the same time as RNA synthesis.

The entire poliovirus genome acts as its own mRNA, forming a polysome of approximately 350S, and is translated to form a single large polypeptide that is subsequently cleaved to produce the various viral capsid polypeptides. Thus, the poliovirus genome serves as a polycistronic messenger molecule. Poliovirus contains four polypeptides.

5. Maturation and Release

- **Naked viruses** -Maturation consists of two main processes: the assembly of the capsid, and its association with the nucleic acid. Maturation occurs at the site of nucleic acid replication. After they are assembled into mature viruses, naked virions may become concentrated in large numbers at the site of maturation, forming inclusion bodies. Naked virions are released in different ways, which depend on the virus and the cell type. Generally, RNA-containing naked viruses are released rapidly after maturation and there is little intracellular accumulation; therefore, these viruses do not form predominant inclusion bodies. On the other hand, DNA-containing naked icosahedral viruses that mature in the nucleus do not reach the cell surface as rapidly, and are released when the cells undergo autolysis or in some cases are extruded without lyses. In either case they tend to accumulate within the infected cells over a long period of time. Thus, they generally produce highly visible inclusion bodies.

- **Enveloped viruses** -In the maturation of enveloped viruses, a capsid must first be assembled around the nucleic acid to form the nucleocapsid, which is then surrounded by the envelope. During the assembly of the nucleocapsid, virus-coded envelope proteins are also synthesized. These migrate to the plasma membrane (if assembly occurs in the cytoplasm) or to the nuclear membrane (if assembly occurs in the nucleus) and become incorporated

into that membrane. Envelopes are formed around the nucleocapsids by budding of cellular membranes. **Note**: Enveloped viruses will have an antigenic mosaicism characteristic of the virus and the host cell. Viruses are slowly and continuously released by the budding process with the results that:

(a) The cell is not lysed.
(b) Little intracellular accumulation of virus occurs.
(c) Inclusion bodies are not as evident as with naked viruses.

- **Complex viruses** -These viruses, of which the poxvirus is a good example, begin the maturation process by forming multilayered membranes around the DNA. These layers differentiate into two membranes: The inner one contains the characteristic nucleoid, while the external one acquires the characteristic pattern of the surface of the virion.
- These form very characteristic cytoplasmic inclusion bodies. The viruses are generally released from the cell via cell lysis.

Summary

1. Viruses contain either DNA or RNA as their genetic material, but not both. This nucleic acid usually has unique chemical and/or physical features which makes it distinguishable from human nucleic acid.

2. Viral nucleic acid is enclosed in a capsid made up of protein subunits called protomeres.

3. Some species of viruses have a membrane, the envelope, surrounding the capsid; other species do not have an envelope, i.e., they are naked. Enveloped viruses have glyco-protein spikes arising from their envelope. These spikes have enzymatic, absorptive, hemagglutinating and/or antigenic activity.

4. The morphology of a virus is determined by the arrangement of the protomeres. When protomeres aggregate into units of five or six (capsomeres) and then condense to form a geometric figure having 20 equal triangular faces and 12 apices, the virus is said to have icosahedral (cubic) morphology. When protomeres aggregate to form a capped tube, they are said to have helical morphology. Any other arrangement of the protomeres results in a complex morphology.

5. All viruses undergo a replication cycle in their human host cell consisting of adsorption, penetration, Uncoating, nucleic acid replication, maturation and release stages.

6. During the viral replication cycle, an accumulation of mature viruses, incomplete viruses and viral parts occurs within the cell. This accumulation is the inclusion body. The size, shape, location and chemical

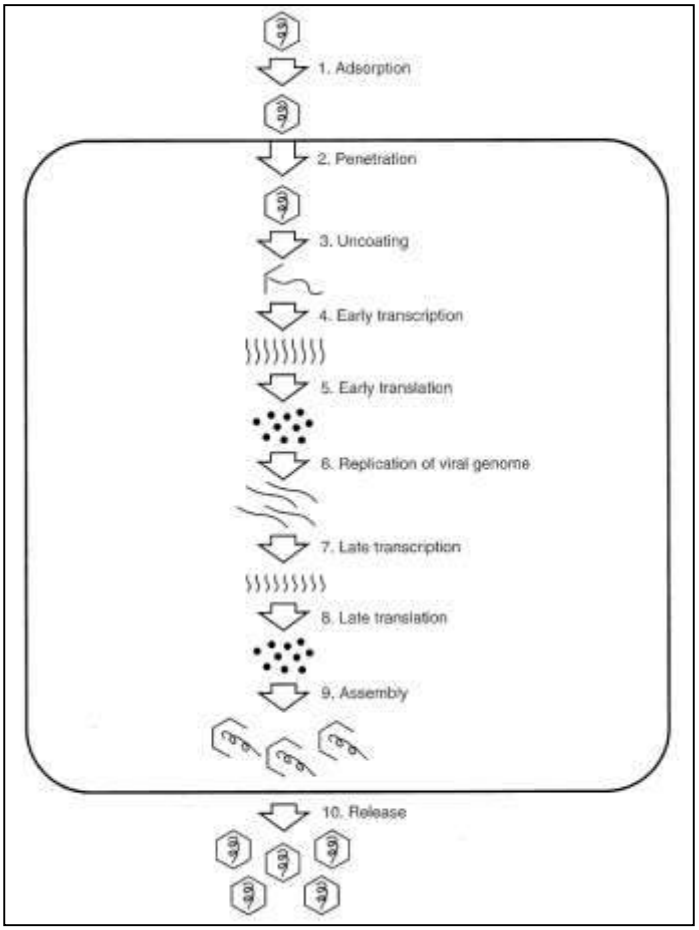
properties of the inclusion body are used by the pathologist to diagnose viral infectious disease.

7. A virally-infected cell generally presents three signals that it is infected. The first is the production of double-stranded RNA, which induces interferon; the second is the expression of viral protein on the surface of the plasma membrane, thus causing activation of cytotoxic T-cells, natural killer cells and sometimes induction of antibody synthesis. The third is the formation of an inclusion body either within the cytoplasm or the nucleus or very rarely within both the cytoplasm and nucleus.

8. In general, all DNA-containing viruses replicate in the host cell nucleus. The exceptions to the rule are the poxviruses.

9. In general, all RNA-containing viruses replicate in the host cell cytoplasm. The exceptions to the rule are the retroviruses and the orthomyxoviruses.

Viroids : Viroids are responsible for causing serious diseases in many plants. They consist of naked RNA which does not code for any protein, nor is protein associated with it. Essentially, each viroid particle is a circular ssRNA molecule



GLOSSARY

- **Capsid** the protein coat that surrounds the nucleic acid of a virus.

- **Capsomers** substructures of virus particles. composed of aggregates of polypeptide chains that interact to form the basic structural units of the capsid.
- **Case fatality rate** (=cfr) the proportion of clinically apparent cases which result in death.
- **Cytopathic effect** (=cpe) cpe consists of morphologic alterations of host cells, may result in cell death.
- **Envelope** a host-cell-derived membrane, containing virus specific antigens, that is acquired during virus maturation.
- **Fomite** an object (e.g. furniture, book) that is not harmful in itself but which can harbor pathogenic organisms and thus may be involved in transmission of an infection.
- **Genome** a set of genes.
- **Giant cells + syncytium.**
- **Hemadsorption** the attachment of red blood cells to the surface of host cells.
- **Hemagglutination** aggregation of red blood cells.
- **Icosahedron** a geometric figure composed of 12 vertices, 20 triangular faces and 30 edges.
- **Inclusion bodies** usually sites of virus synthesis or assembly; may be of diagnostic value (e.g. negri bodies in rabies infection).
- **Nucleocapsid** the virus structure composed of the nucleic acid surrounded by the capsid.
- **Monolayer** sheet of cells forming a continuous layer one cell thick on a solid (e.g.glass or plastic) surface. cells may be e.g. fibroblast, epithelial, epitheliod in nature. they may exist in either primary or continuous (transformed) state.
- **Peplomers** spikes (peplos = envelope).
- **Plaque** a defined area of cell destruction resulting from virus infection in vitro.
- **Plaque forming unit (=pfu)** a measure of **infectious** virus particles. One plaque forming unit is equivalent to one infectious virus particle.
- **Pock** a discrete pustular lesion found in the chorioallantoic membrane or skin following infection with certain viruses.
- **Spikes** surface projection of varying lengths spaced at regular intervals on the viral envelope, also called peplomers. consist of viral glycoproteins.
- **Structural proteins** those proteins which are present in the virion. **this includes proteins present in low amounts.** 'structural proteins' do not necessarily play a skeletal role in maintaining a virus's shape.
- **Syncytium** a multinucleated protoplasmic mass formed by the fusion of originally separate cells.
- **Viral hemagglutinin** a virally coded protein on the outer surface of some viruses which reacts with a surface determinant on red cells. since such a virion will have many copies of the surface hemagglutinin, it can bind to more than one red blood cell, thus causing hemagglutination.

- **Viral infectious dose** the amount of virus required to cause a demonstrable infection in 50% of the inoculated animals (id_{50}) or tissue culture cells ($tcid_{50}$).
- **Viremia** presence of virus particles in the blood.
- **Virion** the mature virus particle, with all of its structural components intact.
- **Virus** a small, obligate intracellular parasite that depends on a living host cell for energy, precursors, enzymes, and ribosomes to multiply. it consists of a single type of nucleic acid, either dna or rna, and a protein coat surrounding the nucleic acid. in addition, some viruses have an envelope.

Medical Virology **Lec.2**

Pathogenicity of viral infection

Viral pathogenesis :The study of the Capability & manner of viruses to infect and cause disease.

Virulence **:** The degree to which a virus causes disease. Strains of virus differ greatly in their ability to cause disease

Mechanisms of Infection and Spread of Viruses through the Body

I. Routes of Entry

A. Respiratory Tract

Protective mechanisms

- Large particles (10 μm and above) are filtered out on mucocilliary layer of nasal turbinates. Then swept down into esophagus
- Intermediate particles (5-10 μm) are usually trapped on mucocilliary layer of trachea and bronchioles. From there they are swept into esophagus.
- Smaller particles often make it to the alveoli of lungs where they can set up infection or be destroyed by alveolar macrophages.
- lungs and other mucosal tissue are sites of secretory immunoglobulin (IgA) which will facilitate viral killing

B. Alimentary tract:

Viruses are either ingested directly, carried into stomach by cilliary action of oropharynx, or introduced through the anus. Major players here are enterovirus, reovirus, adenovirus and HIV in systemic infections, and coronavirus and rotavirus in localized infections. In the case or reovirus, it actually uses the proteases of the intestinal lumen to initiate uncoating to the subviral particle. A common theme here is that most enveloped viruses do not initiate infection via the alimentary tract because detergents such as bile salts disrupt their membrane. Enteric coronaviruses, such as transmissible gastroenteritis virus are exceptions. Esophagus is usually not infected because of thick layer of epithelial tissue .Constant movement of contents through tract provides many opportunities for viral attachment. Some enteric viruses move through the gut lining via mucosa cell transcytosis. Other viruses (such as human rotavirus and corona virus (TGE), infect the mucosa cells and cause mucosal inflammation and diarrhea.

Protective Mechanisms

- Thick layer of esophagus
- Secretary antibodies (IgA)
- Chemicals - bile, stomach acid, proteases

C. Skin, genital tract, and conjunctiva

Skin: Tough outer layer is nearly impenetrable - entry is through cuts, scrapes, bites (insect or animal), iatrogenic (human intervention - needles). Some viruses produce localized infection in skin (papilloma), but most move through the skin and into deeper layers and eventually into bloodstream (viremia).

Genital tract: genital tract is route of entry for important pathogens such as HSV, papilloma, HIV, HTLV, Hepatitis B and C. Sexual activity can cause minute drops in vagina and urethra, through which the virus may enter. Some virus stay local (papillomavirus), others spread systemically (HIV, Hep B and C, HTLV)

Eyes: Conjunctiva has protective mechanisms (lysozyme in tears, washing, eyelid wiping, etc) and is not usually a route of infection. A few viruses, however, can infect here. Usually the infection is through a small tear in the conjunctiva and even then the infection is usually initiated by direct inoculation (physically touching something that has the virus on it).

II. Mechanisms of Spread in the Body

A. Viruses have the choice of setting up infection at the point they entered the body or entering the blood stream and setting up an infection at another point.

The directionality of budding is very important to how a virus spreads. If it spreads at the apical surface it is usually released into a lumen where it can spread quickly on the lumenal epithelial surface, or even be shed to the exterior of the host. If it buds basolaterally, it will encounter slow movement and a myriad of host defenses. In many cases the budding dictates the type of infection that is set up.

B. Local Spread on Epithelial Surfaces

This does not happen much on skin because it is difficult for the virus to be transported without the aid of water. Poxviruses, such as smallpox, enter lymphatic system and are spread throughout the body. In internal epithelium the surface is coated with water and this make spread much easier. Therefore these infections tend to have a shorter incubation time. In the case of paramyxoviruses, influenza virus, and rotavirus the epithelial tissue is infected but there is no invasion beyond this layer -- possibly because of lack of cellular receptors in the deeper tissue layers, or possibly due to higher temperatures in the deeper cellular tissue. However, they can still be quite severe.

C. Subepithelial Invasion and Lymphatic Spread

Lymph system is a system of ducts, vessels, and glands that lies just below the basement membrane of the skin. The major purpose in in immunosurveillance -- detecting and getting rid of foreigh invaders. Many immune cells can be found

in lymph nodes and lymph fluid. Macrophages are probably the primary defenders. They eat the viruses and use the protein parts to activate the immune response.

Viruses evade the lymph system in two primary ways. They can directly infect the immune cells (which eventually find their way into the bloodstream), or they can pass quickly through the lymph system (evading the macrophages) and into the blood stream.

D. Primary and Secondary Viremia

First entry of virus into the bloodstream is primary viremia (can be active or passive). This viremia may be subclinical and is the route by which viruses get to their sites of infection. After the infection occurs, then a secondary viremia can take place because of shedding of virus from the infected organ. This secodary viremia can then be the cause of infection at yet another site of the body.

Viruses may circulate freely in the blood (hepadnaviruses, togaviruses, flaviviruses, and enteroviruses), or they may associate with leukocytes (WBC), platelets, or erythrocytes and be harbored by them (HIV, Rift Valley Fever, Colorado tick fever). The latter viral infections are more difficult to clear and tend to be more persistent infections.

Macrophages have a lot to do with the type of infection that may be caused after a primary viremia. The factors that are important are the area of the body in which the infection occurs (different types of macrophages in different parts of the body), the susceptibility of the macrophages to infection, the state of their activation, and the age and genetics of the host. In most cases, macrophages are efficient destroyers of virus, but in some cases, as in dengue fever, they may serve as a host and carry the virus to different parts of the body.

Because your immune system is constantly fighting viruses in the bloodstream there must be some sort of mechanism to constantly put out virus to maintain a viremia. This is particularly important in spread to certain parts of the body such as the central nervous system, where a constant viremia is necessary for the virus to be able to cross the blood/brain barrier. The viremia is usually maintained by either direct infection of blood cells (leukocytes usually) or infection of another organ which constantly is shedding virus into the bloodstream.

E. Secondary sites of infection.

- **Skin** - this usually results in some sort of rash made up of macules, papules, vesicles or pustules.
- **CNS** - spread is usually from blood vessels in meninges and infection of neurons in cerebrospinal fluid, or directly from blood vessels of the brain and spinal cord. Spread is usually either by infection of endothelial cells or

transport directly through the endothelial layer. Rarely by infected leukocytes moving into brain.

- Another important route is travel of virus up neurons (rabies, varicella, herpes simplex)
- Meningitis is infection of lining of brain and CNS (meninges)
- Encephalitis is infection of brain
- Other organs - liver (hepatitis), heart (carditis), lungs (pneumonia), salivary glands (mumps), testes (orchitis)

III. Virus Shedding

Necessary for maintenance of infection in poulation.

A. Repiratory and oropharyngeal secreations: mucus or saliva from coughing sneezing and talking - measles. Chickenpox, rubella. Direct transmission of saliva or mucus - herpesviruses, CMV, EBV

B. Feces : Enteric viruses - can often persist for longer periods of time (nonenveloped)

C. Skin : Direct contact needed for transmission - molluscum contagiosum, warts, genital herpes, and poxviruses.

D. Urine : Viruria is principal mode of shedding in arenavirus infections of rodents. Mumps virus and CMV in humans

E. Milk : CMV in mother's milk

F. Genital secretions : HIV, HSV I, HSV II, papillomaviruses, hepatitis B and C, HTLV

G. Blood and body fluids : Heptitis B, C, D, HIV, HTLV. Luckily some of the more fatal hemorrhagic fevers can only be transmitted this way.

Pathogenic Properties of Viruses

1. Viruses avoid the host’s immune response by growing inside cells.
2. Viruses gain access to host cells because they have attachment sites for receptors on the host cell.
3. Visible signs of viral infections are called cytopathic effects (CPE).
4. Some viruses cause cytocidal effects (cell death), and others cause noncytocidal effects (damage but not death).
5. Cytopathic effects include the stopping of mitosis, lysis, and the formation of inclusion bodies, cell fusion, antigenic changes, chromosomal changes, and transformation.

Persistent viral infections : In contrast to acute viral infections, persistent infections last for long periods, and occur when the primary infection is not cleared by the adaptive immune response. Varicella-zoster virus, measles virus,

HIV-1, and human cytomegalovirus are examples of viruses that cause typical persistent infections.

A chronic infection is a type of persistent infection that is eventually cleared, while latent or slow infections last the life of the host. There is no single mechanism responsible for establishing a persistent infection; a key feature is reduction in host defenses and the ability of the virus to kill cells.

- In some persistent viral infections there are alternate cycles of virion production and quiescence. An example is **Epstein-Barr virus**, the agent of infectious mononucleosis. After the initial bout of fever, sore throat, and swollen lymph glands, the virus establishes a dormant infection in which the viral genome persists in cells of the immune system. Periodically the infection is reactivated and infectious virions are shed in the absence of clinical symptoms. These reactivations lead to transmission of the infection to new hosts.
- Many infections persist because viral replication interferes with the function of cytotoxic T-lymphocytes (CTLs), immune cells that are extremely important for clearing viral infections. Infected cells are recognized when CTLs detect viral antigens on the cell surface. This recognition process requires presentation of the viral peptides by **major histocompatibilty complex (MHC) class I proteins**. Many viral proteins interfere with different steps of the MHC class I pathway, including the synthesis, processing, and trafficking of the protein. Even transport to the cell surface of viral peptides – produced from viral proteins by the large protein complex known as the proteasome – may be blocked.
- An amazing example of such immune modulation occurs in cells infected with cytomegalovirus (CMV). This beta herpes virus causes a common childhood infection of little consequence in healthy individuals. The infection is never cleared, and the virus persistently infects salivary and mammary glands and the kidney. When latently infected individuals are immunosuppressed by drugs or HIV infection, viral replication ensues with life-threatening consequences. CMV persists in the host because the viral genome encodes multiple proteins that interfere with MHC class I presentation of viral antigens. Two viral proteins cause degradation of MHC class I proteins before they reach the cell surface.
- There are many more examples of how virus infections modulate the immune response, leading to persistent infection. Not surprisingly, many of the processing or regulatory steps that are targets of viral modulation were not even known until it was discovered that they were blocked by virus infection.

Cytopathic effects [CPE]

1.Cytocidal effects : Cytocidal effects are cytopathic effects that lead to host cell death.

- **Noncytocidal effects** are cytopathic effects that do not lead to cell death. Basically, viral infection can lead to cell abnormalities (biochemical and morphological) and/or cell death.
- **Syncytia [giant cells] : Big cells:** Syncytia multi-nucleated , giant cells formed through the fusion of host cells .

2.Inclusion bodies :Intracelluar granules: Inclusion bodies are intracellular granules whose presence is a result of viral infection . Inclusion bodies do not necessarily represent acute virus-induced damage of host cells, but instead can be a more benign effect. The characterization of inclusion bodies is useful for the identification of some viral infections .

3.Cell-mediated immunity

a. Destruction of virus infected cells: Virus infected cells may be recognized by the immune system, which leads to the destruction of the virus infected cells. This immune system action is a very important mechanism by which viral infection can lead to cytopathic, particularly cytocidal effects. Significant host damage can result from the effects of cell-mediated immunity.

b.Various mechanisms: The various mechanisms (two general mechanisms) by which the destruction of virus-infected cells is mediated are termed **cell-mediated immunity.** Mechanisms of cell mediated immunity involve the distinguishing of virus infected cells from uninfected cells through the recognition of viral proteins found associated with the cell .These proteins are recognized by separate mechanisms leading to the distinguishing of these cell-mediated immunity into two distinct mechanisms:

- Those in which viral proteins are recognized by soluble antibody.
- Those in which viral proteins are recognized by something other than soluble antibody

4. **Antibody-dependent cellular cytoxicity [ADCC, natural killer cells] .Antibody tagged cell destruction:** Mechanisms of cell-mediated immunity in which viral proteins are recognized by soluble antibody are termed antibody-dependent cellular cytoxicity (or ADCC) or mediated by natural killer cells.

Mechanism:

ADCC is a consequence of three distinct steps: viral proteins find their way to the surface of infected cells as a consequence of membrane proteins being left behind in the infected-cell membrane following either adsorption or budding. These proteins are recognized as foreign by the body and antibody is produced which recognizes (i.e., binds) these proteins. Natural killer cells

recognize the presence of antibody bound on the surface of cells; they then follow:

- A cell to which an antibody is bound is either not-self, or is self but infected by a pathogen
- Destroy that cell in order to interupt pathogen replication.

5. Cyototoxic T cell-mediated immunity [major histocompatibility complex, MHC, cytoxic T lymphocyte, CTL]

- **Antibody independent:** In contrast with ADCC, cytotoxic T cell-mediated immunity is not a response to soluble antibody binding. In fact, cytotoxic T cell-mediated immunity is a mechanism by which cells harboring foreign proteins, but not displaying those proteins intact on their surface, may be recognized and targetted for destruction.

Mechanism: Cytoxic T cell-mediated immunity occurs via the following steps:

- All of the proteins produced in a cell are ultimately targetted for destruction and broken down; partially broken down intracellular proteins are sequestered by vesicle manipulating machinery and brought into contact with major histocompatibility complex (MHC) proteins made by the host . In conjunction with the MHC proteins these protein fragments are displayed on the surface of cells. White blood cells called cyotoxic T cells (or cytotoxic T lymphocytes or CTLs) are capable of reversibly interacting with surface-presented MHC proteins.

Recognition is said to occur if strong interaction with the MHC protein-foreign protein fragment occurs: the immune system efficiently culls CTLs capable of recognizing host proteins displayed by MHC proteins. As a consequence, the host CTL populations tend to be much more capable of recognizing foreign proteins displayed by MHC proteins than they are of recognizing proteins normally produced by host cells, upon sufficiently strong binding, the CTL is induced to effect the destruction of the host cell . In this way, host cells displaying (sufficiently) aberrant proteins may be eliminated, thus interfering with viral replication and propagation

Latent infection

Selection against viral protein synthesis: The net effect of cell-mediated immunity is to strongly select against the synthesis of virus proteins and the release of virus progeny. For many viral infections this selection is sufficient to markedly reduce viral replication and allow the clearance of the infection. That is, one mechanism through which an individual may recover from a virus

infection is via the destruction of virus-infected cells through the action of cell-mediated immunity.

For some viruses, however, this selection simply results in a predominance of latent forms, ones which are neither replicating nor translating proteins. Thus, some viral infections can become latent, only to re-emerge upon immunodepression.

Interferon

Interferon (IFN) is any of a class of proteins naturally produced by the cells of the vertebrate immune system (leucocytes, T cells), fibroblasts) in response to challenges by foreign agents (antigens) such as viruses, bacteria, and parasites and their products, as well as in response to tumor cells. Interferons belong to the large class of glycoproteins known as cytokines.

While interferons are naturally produced by the cells of the immune system, they also can be synthetically produced. Mass production of interferons, utilizing recombinant DNA technology, has allowed various interferons to be used for combating such diseases as hepatitis B, hepatitis C, Kaposi's sarcoma, multiple sclerosis, and human papillomavirus.

The interferon system is not only complex and remarkably coordinated with other parts of the immune system, but allows a very rapid response to viral invaders.

Medical Virology Lec.3

Control of Viral Infections and Diseases

Immunoprophylaxis : Immunoprophylaxis against viral illnesses includes the use of vaccines or antibody-containing preparations to provide immune protection against a specific disease.

Active Prophylaxis (Vaccines): Active immunization involves administering a virus preparation that stimulates the body's immune system to produce its own specific immunity. Viral vaccines now available for use include the following types:

(1) Attenuated live viruses.

(2) Killed viruses.

(3) Recombinant produced antigens. A vaccinee is a person who has been vaccinated**.**

Immune Response to Vaccines: Vaccination evokes an antibody response and stimulates T lymphocytes. Vaccine effectiveness is assessed in terms of percentage of recipients protected and the duration and degree of protection. Most effective viral vaccines protect more than 90 percent of recipients and produce fairly durable immunity.

Passive Prophylaxis : Passive immunity is conferred by administering antibodies formed in another host. Human immunoglobulins remain a mainstay of passive prophylaxis (and occasionally therapy) for viral illnesses; they are usually used to protect individuals who have been exposed to a disease and cannot be protected by vaccination.

Sanitation and Vector Control

Many viral diseases are controlled by reducing exposure to the virus by:

(1) Eliminating nonhuman reservoirs.

(2) Eliminating the vector .

(3) Improving sanitation.

Antiviral Chemotherapy : There are three types of antiviral agents:

(1) Virucidal agents, which directly inactivate viruses.

(2) Antiviral agents, which inhibit viral replication.

(3) Immunomodulators, which boost the host immune response.

Interferons : Virus-infected cells and cells induced with other agents, e.g., double-stranded polynucleotides, can secrete proteins called interferons, which protect normal cells from viral infection. Therapeutic administration of interferon alpha has proven effective for several human viral illnesses.

Cytokines : Cytokines are molecules produced by cells which modify the biological responses of the same or other cells.

Introduction : Viral diseases range from trivial infections to plagues that alter the course of history. Because of the enormous variations in viruses and in their epidemiology and pathogenesis, there is no single, magic-bullet approach to control. Each virus presents its own set of problems. This chapter covers methods useful to various degrees in controlling selected viral diseases. The most spectacular progress so far has involved vaccines. Vector control and sanitation have contributed greatly. Also, a number of therapeutic antiviral agents are now available, including some for very serious infections such as human immunodeficiency virus type 1 (HIV-1) infection. In addition, interferon alpha is now available for the therapy of several viral diseases.

Immunoprophylaxis

Immunoprophylaxis against viral illnesses includes the use of vaccines or antibody-containing preparations to provide a susceptible individual with immunologic protection against a specific disease. Immunization against viral illnesses can be either active or passive. With active immunity, protection is achieved by stimulating the body's immune system to produce its own antibodies by immunization with a virus preparation. Passive immunity is conferred by administering antibodies formed in another host. For example, an antibody-containing gamma globulin preparation may protect a susceptible individual exposed to a viral illness.

Active Prophylaxis (Vaccines)

(1) Attenuated live viral vaccines

Most live vaccines contain viruses that have been attenuated by laboratory manipulation. These attenuated viruses can infect and replicate in the recipient and produce a protective immune response without causing disease. Live attenuated viral vaccines can often confer lifelong immunity after one immunization series. However, because live viruses can multiply in the body, there is always the possibility that they may revert to a more pathogenic form. Adequate laboratory and animal testing and extensive clinical studies must be performed to assess this possibility. In addition, new recombinant technologies facilitate direct alteration of viral genetic structure, thus permitting scientists to produce attenuated viruses in which the genetic regions likely to lead to pathogenic reversion are modified or deleted.

(2) Killed (inactivated) viral vaccines

Killed viral vaccines contain either whole virus particles, inactivated by chemical or physical means, or some component(s) of the virus. Completely inactivated viral vaccines cannot cause infection. However, they do not generally produce lifelong immunity following one immunization series; additional doses are usually required. In addition, because killed virus does not multiply in the host, the inoculum itself must provide a sufficiently large concentration of viral antigens to induce the desired immune response.

(3) Recombinant-produced antigens

Application of a recombinant DNA strategy to develop new vaccines is performed by identifying the specific component(s) that can elicit the production of protective antibodies, and then cloning and expressing the gene encoding that protein and assembly of a complex in some cases. This approach has made possible a safe and effective recombinant vaccine against hepatitis B virus, which has replaced the vaccine derived from the plasma of hepatitis B virus-infected individuals.

Immune Response to Vaccines

Vaccination evokes an antibody response which is, in turn, a measure of the effectiveness of the vaccine in stimulating B lymphocytes. Antiviral antibodies are classified as IgA, IgM, or IgG and can be measured by various techniques. Some antibody categories (IgA and IgM) are normally more abundant in respiratory and intestinal secretions; others (mainly IgG) are more abundant in the circulatory system.

Vaccines also stimulate T lymphocytes, leading to cell-mediated responses that influence protection. Antibody assays are now routine laboratory procedures, but measuring cellular immunity in vitro usually requires the utilization of complex laboratory techniques. In general, despite the complexities of the immune system, resistance to the vaccine-preventable viral diseases often correlates well with the presence of circulating antiviral antibodies, which are easily measured.

Effectiveness is a key concern with any vaccine. Here the standard for comparison is usually the immunity conferred by the natural disease; an example of an exception is rabies. Both epidemiologic and laboratory methods are used to generate comparative data. Vaccine-induced immunity can be defined by the percentage of recipients protected, the projected duration of protection, and the degree of protection. Most viral vaccines considered effective protect more than 90 percent of recipients, and the immunity produced appears to be fairly durable, lasting several years or more. However, vaccines usually do not induce an immunologic response entirely comparable to that seen in the natural disease. Immunity to viral diseases should not be thought of as absolute. Persons immune due to the natural infection, as well as, vaccines,

sometimes experience subclinical reinfection if exposed. Evaluating the protection conferred by a vaccine often involves measuring the frequency and extent to which subclinical reinfection can override vaccine-induced resistance.

Often, upon revaccination or reinfection, a boost in IgG antibodies is observed with little or no detectable IgM response, suggesting prior exposure with antibody priming. Such anamnestic responses may be seen in individuals who lack detectable antibody prior to reexposure. Therefore, the absence of measurable antibody may not mean that an individual is unprotected.

Immune responses to viral vaccines may be influenced by a number of factors related to the vaccine as well as to the host. As already discussed, the magnitude and duration of immunity differ significantly between live and killed vaccines. The immune response to vaccines can be enhanced by adding adjuvant substances such as aluminum salts (e.g., hepatitis B vaccine). The route of administration of a vaccine can also influence the immunogenicity of some vaccines. Also, maternal antibodies acquired transplacentally can interfere with responses to measles, mumps, and rubella (MMR) vaccine, as demonstrated by lower response rates when the vaccine is administered earlier than 15 months of age. In this case, it is thought that the antibodies interfere with the post-vaccination replication of these live vaccine viruses in the host.

Antiviral Chemotherapy : Antiviral chemotherapeutic agents can be divided into three categories:

- **Virucidal agents**, Virucidal agents directly inactivate intact viruses. Although some of these agents have clinical usefulness (e.g., topical treatment of warts with podophyllin, which destroys both virus and host tissues), most virucides have no demonstrated therapeutic value.
- **Antiviral agents**: Antiviral agents inhibit viral replication at the cellular level, interrupting one or more steps in the life cycle of the virus. These agents have a limited spectrum of activity and, because most of them also interrupt host cell function, they are toxic to various degrees. The emergence of drug resistant viruses may occur during clinical use that further limits the effectiveness of various antivirals.
- **Immunomodulators**: Immunomodulators such as interferons that alter the host immune responses to infection could, in principle, be protective, and several are under investigation.

A number of antiviral agents with demonstrated effectiveness are now available . These antiviral agents improve the clinical course of disease, but typically have important limitations especially as therapeutics for chronic or latent infections. For example, the four nucleoside analog drugs now available for the therapy of HIV-1 do not prevent the ultimate worsening of disease. The concept of a targeted approach is now practical since information concerning the structure and replication of viruses and the spatial configuration and function of their proteins is available. Such data may be useful in identifying specific target sites for antiviral agents.

Medical Virology **Lec.4**

VIRAL GENETICS

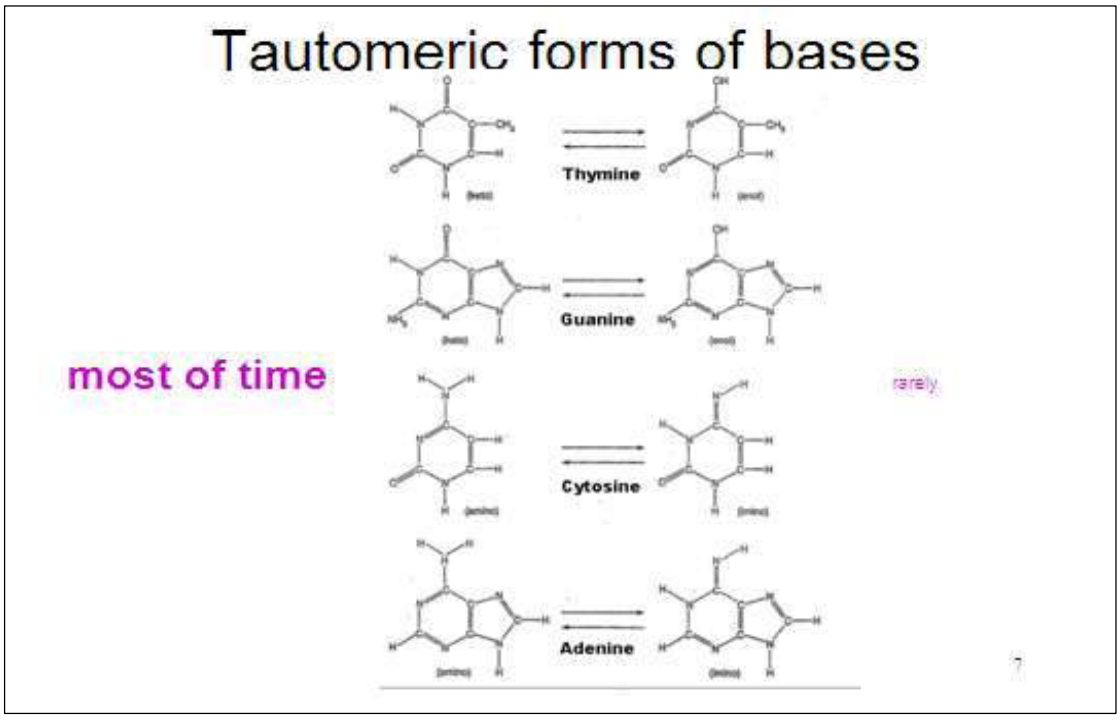
MUTANTS

a) Origin

Spontaneous mutations

- **tautomeric form of bases**
- **polymerase errors**

These arise naturally during viral replication: e.g. due to errors by the genome-replicating polymerase or a a result of the incorporation of tautomeric forms of the bases



DNA viruses tend to more genetically stable than RNA viruses. There are error correction mechanisms in the host cell for DNA repair, but probably not for RNA.

Some RNA viruses are remarkably invariant in nature. Probably these viruses have the same high mutation rate as other RNA viruses, but are so precisely adapted for transmission and replication that fairly minor changes result in failure to compete successfully with parental (wild-type, wt) virus.

Mutations that are induced by physical or chemical means

Chemical: Agents acting directly on bases, e.g. nitrous acid Agents acting indirectly, e.g. base analogs which mispair more frequently than normal bases thus generating mutations

Physical: Agents such as UV light or X-rays

Types of mutation

a) Point mutation
b) Insertion mutation
c) Deletion mutation

Examples of the kinds of phenotypic changes seen in virus mutants

(**phenotype =** the observed properties of an organism)

- **Conditional lethal mutants:** These mutants multiply under some conditions but not others (whereas the wild-type virus grows under both sets of conditions).
- **Temperature sensitive** (TS) mutants - These will grow at low temperature e.g. 31 degrees C but not at e.g. 39 degrees C, wild type grows at 31 and 39 degrees C. It appears that the reason for this is often that the altered protein cannot maintain a functional conformation at the elevated temperature.
- **host range** - These mutants will only grow in a subset of the cell types in which the wild type virus will grow - such mutants provide a means to investigate the role of the host cell in viral infection
- **Plaque size:** Plaques may be larger or smaller than in the wild type virus, sometimes such mutants show altered pathogenicity.
- **Drug resistance:** This is important in the development of antiviral agents - the possibility of drug resistant mutants arising must always be considered.
- **Enzyme-deficient mutants:** Some viral enzymes are not always essential and so we can isolate viable enzyme-deficient mutants; e.g. herpes simplex virus thymidine kinase is usually not required in tissue culture but it is important in infection of neuronal cells
- **"Hot" mutants:** These grow better at elevated temperatures than the wild type virus. They may be more virulent since host fever may have little effect on the mutants but may slow down the replication of wild type virions.
- **Attenuated mutants:** Many viral mutants cause much milder symptoms (or no symptoms) compared to the parental virus - these are said to be attenuated. These have a potential role in vaccine development and they also are useful tools in determining why the parental virus is harmful

EXCHANGE OF GENETIC MATERIAL

Recombination: Exchange of genetic information between two genomes.

"Classic" recombination: This involves breaking of covalent bonds within the nucleic acid, exchange of genetic information, and reforming of covalent bonds. This kind of break/join recombination is common in DNA viruses or those RNA viruses which have a DNA phase (retroviruses). The host cell has recombination systems for DNA.

Recombination of this type is very rare in RNA viruses (there are probably no host enzymes for RNA recombination). Picornaviruses show a form of very low efficiency recombination. The mechanism is not identical to the standard DNA mechanism, and is probably a "copy choice" kind of mechanism (figure 1) in which the polymerase switches templates while copying the RNA.

Recombination is also common in the coronaviruses - again the mechanism is different from the situation with DNA and probably is a consequence of the unusual way in which RNA is synthesized in this virus.

So far, there is no evidence for recombination in the negative stranded RNA viruses giving rise to viable viruses (In these viruses, the genomic RNA is packaged in nucleocapsids and is not readily available for base pairing).

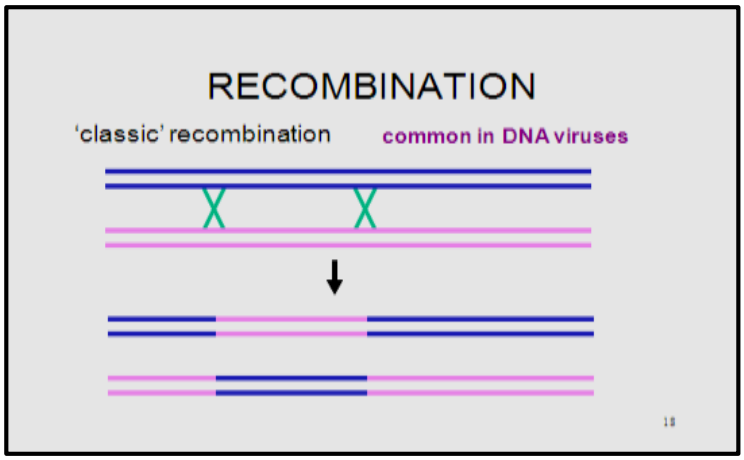


Figure 1: Copy choice recombination

Various uses for recombination techniques

a) Mapping genomes (the further apart two genes are, the more likely it is that there will be a recombination event between them).

b) Marker rescue - DNA fragments from wild type virus can recombine with mutant virus to generate wild type virus - this provides a means to assign a gene function to a particular region of the genome. This also provides a means to insert foreign material into a gene (figure 2).

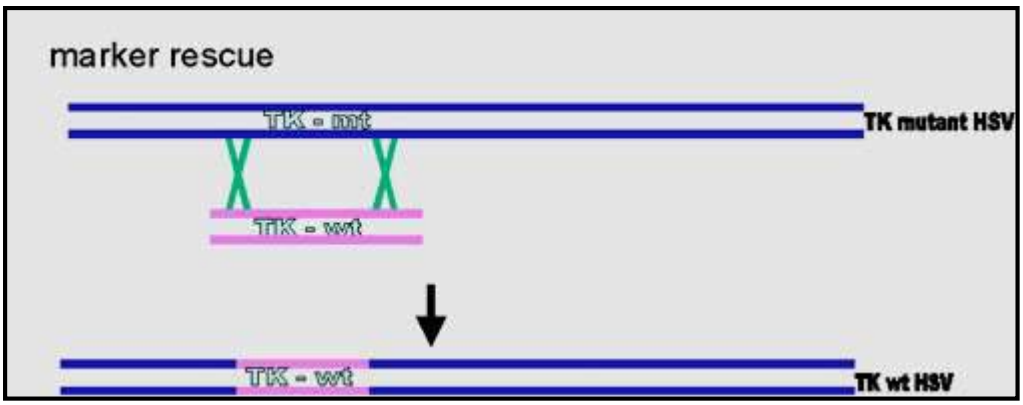


Figure2: Marker rescue

Recombination enables a virus to pick up genetic information from viruses of the same type and occasionally from unrelated viruses or even the host genome (as occurs in some retroviruses).

Reassortments

- Form of recombination (non classical)
- Very efficient
- Segmented viruses only
- Can occur naturally
- Used in some new vaccines : eg for influenza and rotaviruses

If a virus has a segmented genome and if two variants of that virus infect a single cell, progeny virions can result with some segments from one parent, some from the other.

This is an efficient process - but is limited to viruses with segmented genomes - so far the only human viruses characterized with segmented genomes are RNA viruses e.g. orthomyxoviruses, reoviruses, arenaviruses, bunya viruses.

Reassortment may play an important role in nature in generating novel reassortants and has also been useful in laboratory experiments (figure 3,4). It has also been exploited in assigning functions to different segments of the genome. For example, in a reassorted virus if one segment comes from virus A and the rest from virus B, we can see which properties resemble virus A and which virus B.

Reassortment is a non-classical kind of recombination

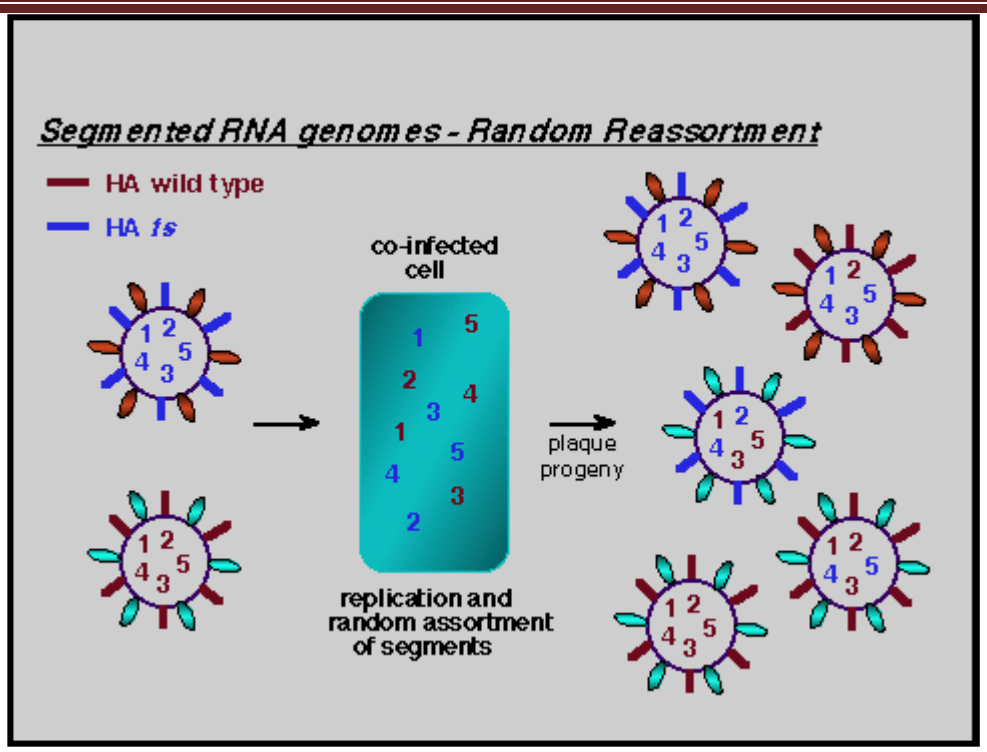


Figure 3: Reassortment of viral genome in segmented virus

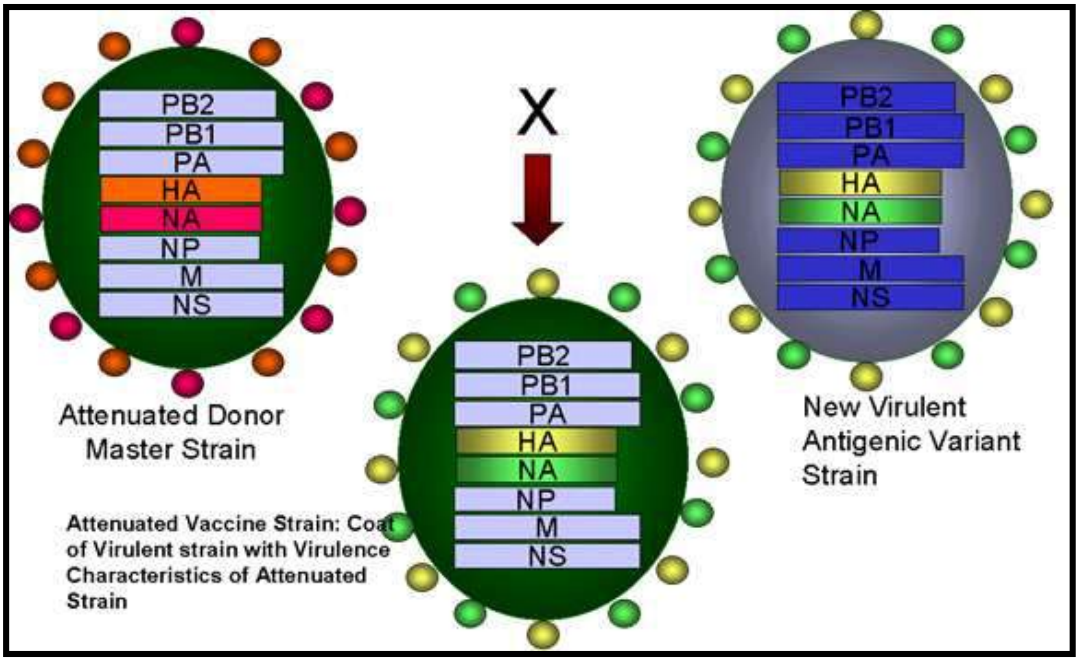


Figure 4: Reassortment of genes between the attenuated strain of influenza virus and a new virulent strain in the formation of an attenuated influenza vaccine

Applied genetics : There is vaccine called Flumist (LAIV, approved June 2003) for influenza virus which involves some of the principles discussed above. The vaccine is trivalent – it contains 3 strains of influenza virus:

The viruses are cold adapted strains which can grow well at 25 degrees C and so grow in the upper respiratory tract where it is cooler. The viruses are

temperature-sensitive and grow poorly in the warmer lower respiratory tract. The viruses are attenuated strains and much less pathogenic than wild-type virus. This is due to multiple changes in the various genome segments.

Antibodies to the influenza virus surface proteins (HA - hemagglutinin and NA - neuraminidase) are important in protection against infection. The HA and NA change from year to year. The vaccine technology uses reassortment to generate reassortant viruses which have six gene segments from the attenuated, cold-adapted virus and the HA and NA coding segments from the virus which is likely to be a problem in the up-coming influenza season.

This vaccine is a live vaccine and is given intranasally as a spray and can induce mucosal and systemic immunity. A live, attenuated reassortant vaccine has recently (2006) been approved for rotaviruses (RotaTeq from Merke). Another attenuated vaccine, Rotarix (Glaxo), is in development.

Complementation

Interaction at a functional level NOT at the nucleic acid level. For example, if we take two mutants with a ts (temperature-sensitive) lesion in different genes, neither can grow at a high (non-permissive) temperature. If we infect the same cell with both mutants, each mutant can provide the missing function of the other and therefore they can replicate (nevertheless, the progeny virions will still contain ts mutant genomes and be temperature-sensitive).

We can use complementation to group ts mutants, since ts mutants in the same gene will usually not be able to complement each other. This is a basic tool in genetics to determine if mutations are in the same or a different gene and to determine the minimum number genes affecting a function.

Multiplicity reactivation

If double stranded DNA viruses are inactivated using ultraviolet irradiation, we often see reactivation if we infect cells with the inactivated virus at a very high multiplicity of infection (i.e. a lot of virus particles per cell) - this is because inactivated viruses cooperate in some way. Probably complementation allows viruses to grow initially, as genes inactivated in one virion may still be active in one of the others. As the number of genomes present increases due to replication, recombination can occur, resulting in new genotypes, and sometimes regenerating the wild type virus.

Defective viruses

- lack gene(s) necessary for a complete infectious cycle
- 'helper' virus provides missing functions

Defective viruses lack the full complement of genes necessary for a complete infectious cycle (many are deletion mutants) - and so they need another virus to provide the missing functions - this second virus is called a helper virus.

Defective viruses must provide the necessary signals for a polymerase to replicate their genome and for their genome to be packaged but need provide no more. Some defective viruses do more for themselves.

Some examples of defective viruses:

Some retroviruses have picked up host cell sequences but have lost some viral functions. These need a closely related virus which retains these functions as a helper.

Some defective viruses can use unrelated viruses as a helper: For example, hepatitis delta virus (an RNA virus) does not code for its own envelope proteins but uses the envelope of hepatitis B virus (a DNA virus).

Defective interfering particles

The replication of the helper virus may be less effective than if the defective virus (particle) was not there. This is because the defective particle is competing with the helper for the functions that the helper provides. This phenomenon is known as interference, and defective particles which cause this phenomenon are known as "defective interfering" (DI) particles. Not all defective viruses interfere, but many do.

Note that it is possible that defective interfering particles could modulate natural infections.

Phenotypic mixing

If two different viruses infect a cell, progeny viruses may contain coat components derived from both parents and so they will have coat properties of both parents. This is called phenotypic mixing (figure 5). **It involves no alteration in genetic material,** the progeny of such virions will be determined by which parental genome is packaged and not by the nature of the envelope.

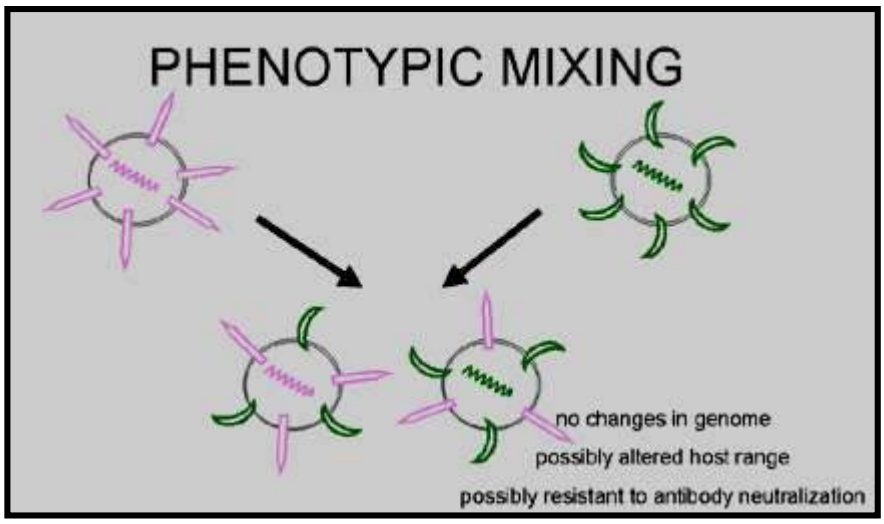


Figure 5: phenotype mixing between two different viruses infecting the same cell

Phenotypic mixing may occur between related viruses, e.g. different members of the Picornavirus family, or between genetically unrelated viruses, e.g. Rhabdo- and Paramyxo- viruses. In the latter case the two viruses involved are usually enveloped since it seems there are fewer restraints on packaging nucleocapsids in other viruses' envelopes than on packaging nucleic acids in other viruses' icosahedral capsids

We can also get the situation where a coat is entirely that of another virus, e.g. a retrovirus nucleocapsid in a rhabdovirus envelope. This kind of phenotypic mixing is sometimes referred to as pseudotype (pseudovirion) formation (figure 6). The pseudotype described above will show the adsorption-penetration-surface antigenicity characteristics of the rhabdovirus and will then, upon infection, behave as a retrovirus and produce progeny retroviruses. This results in pseudotypes having an altered host range/tissue tropism on a temporary basis

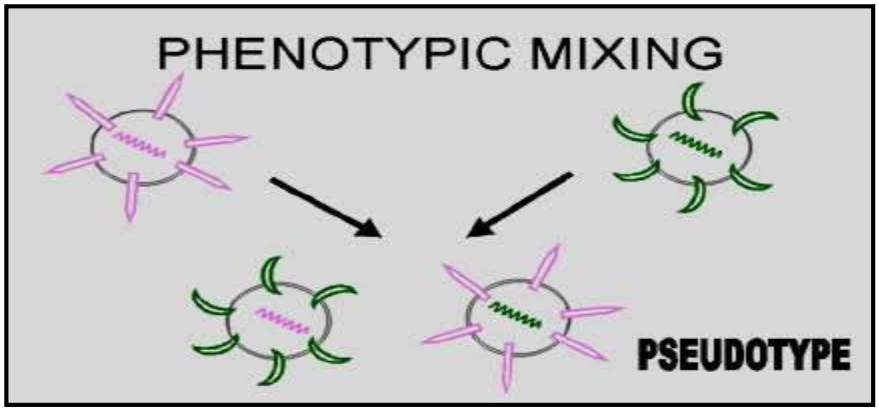


Figure 6:phenotype mixing to form pseudotype

We can also get the situation where a coat is entirely that of another virus, e.g. a retrovirus nucleocapsid in a rhabdovirus envelope. This kind of phenotypic mixing is sometimes referred to as pseudotype (pseudovirion) formation (figure 6). The pseudotype described above will show the adsorption-penetration-surface antigenicity characteristics of the rhabdovirus and will then, upon infection, behave as a retrovirus and produce progeny retroviruses. This results in pseudotypes having an altered host range/tissue tropism on a temporary basis.

Medical Virology **Lec.5**

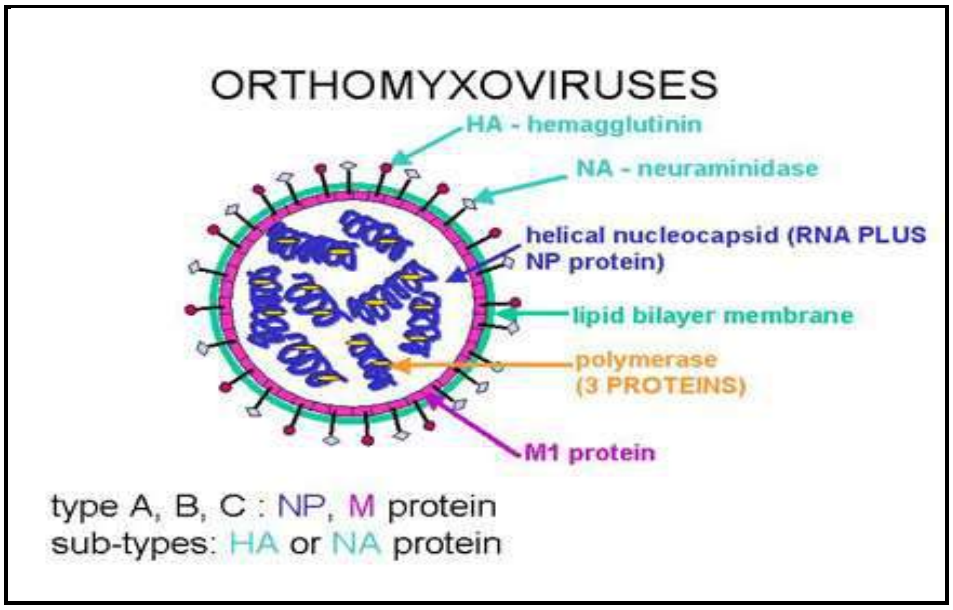
RNA Viruses INFLUENZA VIRUSES

ORTHOMYXOVIRIDAE

Ortho =True or real

Myxo = Affinity to mucins

INFLUENZA VIRUSES



Introduction : True influenza is an acute infectious disease caused by a member of the orthomyxovirus family: influenza virus A, B or, to a much lesser extent, influenza virus C. However, the term 'flu' is often used for any febrile respiratory illness with systemic symptoms which may be caused be a myriad of bacterial or viral agents as well as influenza. Influenza outbreaks usually occur in the winter in temperate climates.

ORTHOMXYOVIRUSES

Influenza viruses

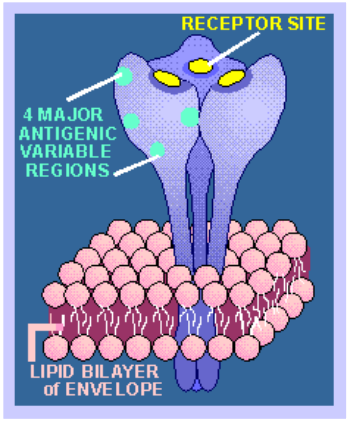
Orthomyxoviruses are divided into three types: influenza A, B and C. Only influenza virus type A and B are of medical importance. Type A influenza viruses differ from type B viruses in that they have an animal reservoir and are divided into subtypes . The single stranded, negative sense RNA genomes of influenza A and B viruses occur as eight separate segments; influenza C viruses contain seven segments of RNA, lacking a neuraminidase gene .

Morphology of an influenza virion: The virion is generally rounded but may be long and filamentous.
A **single-stranded RNA** genome Negative polarity is closely associated with a helical nucleoprotein **(NP)**, and is present in **eight separate segments of ribonucleoprotein (RNP),** each of which has to be present for successful replication. The segmented genome is enclosed within an outer lipoprotein **envelope**. The lipoprotein envelope makes the virion rather labile - susceptible to heat, drying, detergents and solvents.

An antigenic protein called the **matrix protein (MP)** lines the inside of the envelope and is chemically bound to the RNP. The envelope carries two types of protruding spikes included:

Haemagglutinin (HA): Atrimeric protein which consist of **16** major antigenic types. The haemagglutinin functions during attachment of the virus particle to the cell membrane, and can combine with specific receptors on a variety of cells including red blood cells. Variability in HA primarily responsible for the continual evolution of new strains and subsequent influenza epidemic. Haemaglutinine (HA or H) is a glycoprotein containing either 2 or 3 glycosylation sites. It spans the lipid membrane so that the major part, which contains at least 5 antigenic domains, is presented at the outer surface. The HA serve as a receptor by binding to sialic acid (N-acetyl-neuramic acid) and induce penetration of the interior of the virus particle by membrane fusion .

The HA is synthesized as precursor protein and cleaved by cellular serine proteases into the functional proteins HA1 and HA2. The amino acid sequence at the cleavage site determines HA processing by cellular proteases, and thus the organ tropism .



Nuraminidase (NA):

A cylender stalk is topped with tetrameric box-shaped, protein of which there are **9** major antigenic types, and which has enzymic properties as the

name implies. The function of NA at the end of viral life cycle .It is a sialidase enzyme that removes sialic acid from glycol-conjugates , facilitates release of virus particles from infected cell surfaces during budding process and helps prevent self aggregation of virions by removing sialic acid resides from viral glycoprotein's. It is possible that NA help the virus negotiate through mucin layer in respiratory tract to reach the target epithelial cells.

Influenza virus neuraminidase is a major surface glycoprotein of the influenza A and B viruses (about 50 copies per virions). On the upper surface of the enzyme with respect to its orientation on the viral membrane, contain many of the antigenically and enzymatically important amino acids .

The Life Cycle of Influenza Virus

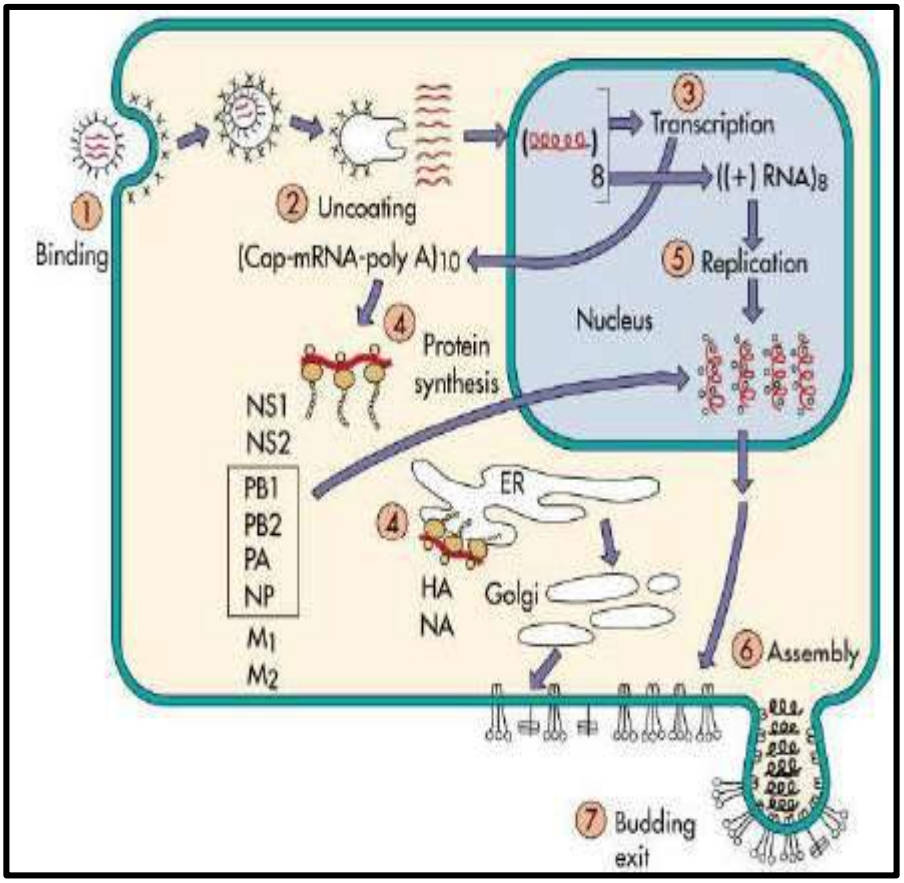
In human, influenza viruses are transmitted by the respiratory rout. Host cellular receptors consist of oligosaccharides residing on the surface of respiratory epithelial cells . Endocytosis is a multi-step process consisting of surface receptor-mediated binding, internalization, and intracellular trafficking. Clathren-mediated and Clathren-independent endocytosis have been demonstrated .

Fusion of influenza virus to the endosome is triggered by low PH conditions and mediated by the fusion peptide of HA2 after cleavage of HA, creating a pore in the endosome through fusion of viral and endosomal membranes . Uncoating process involve the influenza virus tetrameric M2 protein, which is involved in the release of RNP into the host cell cytoplasm through ion channel activity .

Viral RNA serves as a template for the production of messenger RNA (mRNA) and subsequent transcription, as well as for the generation of complementary RNA (cRNA) , which is positive sense and function as a template for the generation of more vRNA (viral replication) . Viral replication requires the synthesis of vRNA , which is primer-independent and occurs through a cRNA intermediate .

Two models for packing of viral RNA segments exist, and include the “random incorporation”, the “selective incorporation” models . Because the HA binds cell surface sialic acid receptor, virions must be released.

The NA functions as a sialidase and cleave sialic acids from the host cell and viral glycoprotein’s to minimize viral aggregation at the cell surface . Balance between the HA and NA is thus required for optimal receptor binding and destruction .



Types of influenza virus

Influenza A viruses

Influenza A viruses include the avian, swine, equine and canine influenza viruses, as well as the human influenza A viruses. Influenza A viruses are classified into subtypes based on two surface antigens, the hemagglutinin (H) and neuraminidase (N) protein. There are 16 hemagglutinin antigens (H1-H16) and 9 neuraminidase (N1-N9) . H1N1, H1N2 and H3N2 viruses are currently in general circulation in humans. H1N2 viruses appeared most recently.

Influenza B viruses

Influenza B viruses are mainly found in humans. These viruses can cause epidemics in human population, but have not, to date, been responsible for pandemics. They have also been found in animal . Influenza B viruses are categorized into lineages rather than subtypes. They are also classified into strains . Influenza B viruses undergo antigenic drift, though it occurs more slowly than in influenza A viruses.

Influenza C viruses

Influenza C viruses are mainly associated with disease in humans. Until recently, they had never been associated with large scale epidemics. Influenza C viruses have also been found in Animals . The viruses are not classified into subtypes, but are classified into strains.

COMPARISON OF INFLUENZA A, B AND C			
	TYPE A	**TYPE B**	**TYPE C**
Severity of illness	++++	++	+
Animal reservoir	Yes	No	no
Human pandemics	Yes	No	no
Human epidemics	Yes	Yes	no (sporadic)
Antigenic changes	shift, drift	Drift	drift
Segmented genome	Yes	Yes	yes
Amantadine, rimantadine	sensitive	no effect	no effect
Zanamivir (Relenza)	sensitive	sensitive	
Surface glycoproteins	2	2	(1)

Antigenic shift and drift in influenza viruses

Influenza A viruses change frequently and strains evolve as they accumulate point mutations during virus replication; this process is sometimes called ‘antigenic drift. A more abrupt change can occur during genetic reassortment . Reassortment is possible whenever two different influenza viruses infect a cell simultaneously; when the new viruses (the ‘progeny’) are assembled, they may contain some genes from one parent virus and some genes from the other .

Reassortment between different strains results in the periodic emergence of novel strains. Reassortment between subtypes can result in the emergence of a new subtype also occur between avian, swine, equine, canine and human influenza A viruses. This type of reassortment can result in a ‘hybrid’ virus with, for example, both avian and human influenza virus proteins. An abrupt change in the subtypes found in a host species is called an ‘antigenic shift.’ Antigenic shifts can result from mechanisms Genetic reassortment between subtypes.

Viral Transmission

Influenza viruses are transmitted in aerosols created by coughing and sneezing, and by contact with nasal discharges, either directly or on fomites. Close contact and closed environments favor transmission . Person-to-person transmission occurs with the H1N1 virus that is currently circulating in humans.

Clinical findings

- **Incubation Period :** The incubation period for human influenza is usually short; most infections appear after one to four days . The incubation period for the novel H1N1 virus circulating in humans appears to be 2 to 7 days .
- **Clinical Signs &Pathogenicity**

Uncomplicated infections with human influenza A or B viruses are usually characterized by upper respiratory symptoms, which may include fever, chills, anorexia, headache, myalgia, weakness, sneezing, rhinitis, sore throat and a nonproductive cough . Nausea, vomiting and otitis media are common in children, and febrile seizures have been reported in severe cases. Most people recover in one to seven days, but in some cases, the symptoms may last up to two weeks or longer .

More severe symptoms, including pneumonia, can be seen in individuals with chronic respiratory or heart disease. Secondary bacterial or viral infections may also occur. In addition, influenza A has been associated with encephalopathy, transverse myelitis, Reye syndrome, myocarditis, pericarditis and myositis .

Swine flu H1N1

The novel swine origin 2009 influenza A (H1N1) virus was identified in April 2009, and it is currently causing the first influenza pandemic of the 21st century. The virus is a completely new reassortant virus . The majority of the human population does not have preexisting immunity against it and the case fatality rate of the current pandemic virus infection is still unclear, but it is estimated to be somewhat higher than that of seasonal influenza virus infections . The pandemic 2009 A (H1N1) virus causes an uncomplicated respiratory tract illness with symptoms similar to those caused by seasonal influenza viruses. However, gastrointestinal symptoms atypical to seasonal influenza have been detected in a significant proportion of cases .

The pandemic 2009 (H1N1) influenza A virus originates from a swine influenza A virus strain. It underwent multiple reassortment events in pigs and then transferred into the human population, The new virus has gene segments from the North American triple-reassortant and Eurasian swine H1N1 viruses . Relatively little is known about the pathogenesis and transmission of the pandemic 2009 A (H1N1) virus in humans . The 2009 A (H1N1) HA showed a glycan binding pattern similar to that of the HA from the pandemic 1918 A (H1N1) virus though its affinity to α2, 6 glycan was much lower than that of the 1918 virus HA **.** The transmission of the pandemic 2009 A (H1N1) virus via respiratory droplets was as efficient as that of a seasonal A (H1N1) virus .

The Dendratic Cells and macrophages reside beneath the epithelium of the respiratory organs, and these cells are thus potential targets for influenza viruses. From the epithelial cells influenza viruses spread in DCs and macrophages, which coordinate the development of an effective innate immune response against the virus **.** During influenza virus infection, DCs and macrophages secrete antiviral cytokines such as interferons (IFNs) and tumor necrosis factor alpha (TNF-α) and DCs and macrophages activate virus-destroying NK cells and T cells with the cytokines they secrete and via direct cell-to-cell contacts **.**

The pandemic (H1N1) virus infects and replicates very well in human monocyte-derived DCs and macrophages. The virus induced a relatively weak innate immune response in these cells, as evidenced by a poor expression of antiviral and proinflammatory cytokine genes. However, like seasonal influenza A viruses, the pandemic 2009 (H1N1) virus was extremely sensitive to the antiviral actions of type I IFNs (IFN-α/β) and Interestingly, the virus was even more sensitive to antiviral IFN-λ than a seasonal A (H1N1) virus .

Laboratory Diagnosis of Human Influenza

Specimen collection

- **Respiratory specimens:** Respiratory specimens obtained within four days of onset of symptoms and different types of respiratory specimens can be used such as nasal washes and nasopharyngeal aspirates tend to be more sensitive than pharyngeal swabs. In patients that are intubated, tracheal aspirates and bronchial lavages can be collected .
- **Blood specimens :** Acute and convalescent serum samples 14 − 21 days apart should be collected to demonstrate a significant (at least fourfold) rise in strain-specific antibody titer .

Isolation methods

- **Embryonated egg culture**

Specimens are inoculated into the amniotic cavity of 10-12 day embryonated chicken eggs. High yields of virus can be harvested after 3 days of incubation .Since this technique requires the supply of fertilized chicken eggs and special incubators it is no longer used for the routine diagnosis of influenza infection .

- **Cell culture**

Conventional culture: Various cell-lines are utilized to isolate influenza viruses, most commonly primary monkey kidney cells and Madin-Darby canine kidney (MDCK) cells, Conventional cell culture takes up to two weeks but has a very high sensitivity, after infection, the cell monolayer is studied under the microscope at regular intervals, as infection of cells gives a visible cytopathic effect (CPE): cells become more refractile and rounded and eventually loose cells can be seen in the growth medium, the presence of influenza virus as a cause of the CPE must be verified , techniques commonly used for this purpose are haemagglutination (HA).

Laboratory Tests

Direct methods

- **Immunofluorescence**
- **Enzyme immuno assays or Immunochromatography assays**
- **Reverse transcription polymerase chain reaction (RT-PCR)**

Serology

Serology refers to the detection of influenza virus-specific antibodies in serum (or other body fluids).Serology can either detect total antibodies or be class-specific (IgG, IgA, or IgM). Different serological techniques are available for influenza diagnosis: haemagglutination inhibition (HI), compliment fixation (CF), enzyme immunoassays (EIA) and indirect immunofluorescence. Serological diagnosis has little value in diagnosing acute influenza. In order to diagnose acute infection, an at least four-fold rise in titer needs to be demonstrate, which necessitates both an acute and a convalescent specimen . Serology has greater clinical value in pediatric patients without previous exposure to influenza .

Treatment

Four antiviral drugs are available for influenza treatment in the U.S. Amantadine and rimantadine (adamantanes) are active against human influenza A viruses, if treatment is begun within the first 48 hours.

Vaccines

Live vaccines

Cold-adapted live attenuated influenza virus (CAIV) vaccines, for intranasal administration, have been available in the USA since July 2003, and in the former Soviet Union, live attenuated influenza vaccines have been in use for several years. The vaccine consists of a master attenuated virus into which the HA and NA genes have been inserted .

Killed vaccines

Killed virus vaccines can be divided into whole virus vaccines, and split or subunit vaccines. Whole virus vaccines were the first to be developed. Whole virus vaccines are safe and well tolerated, with an efficacy of 60-90 % in children and adults.

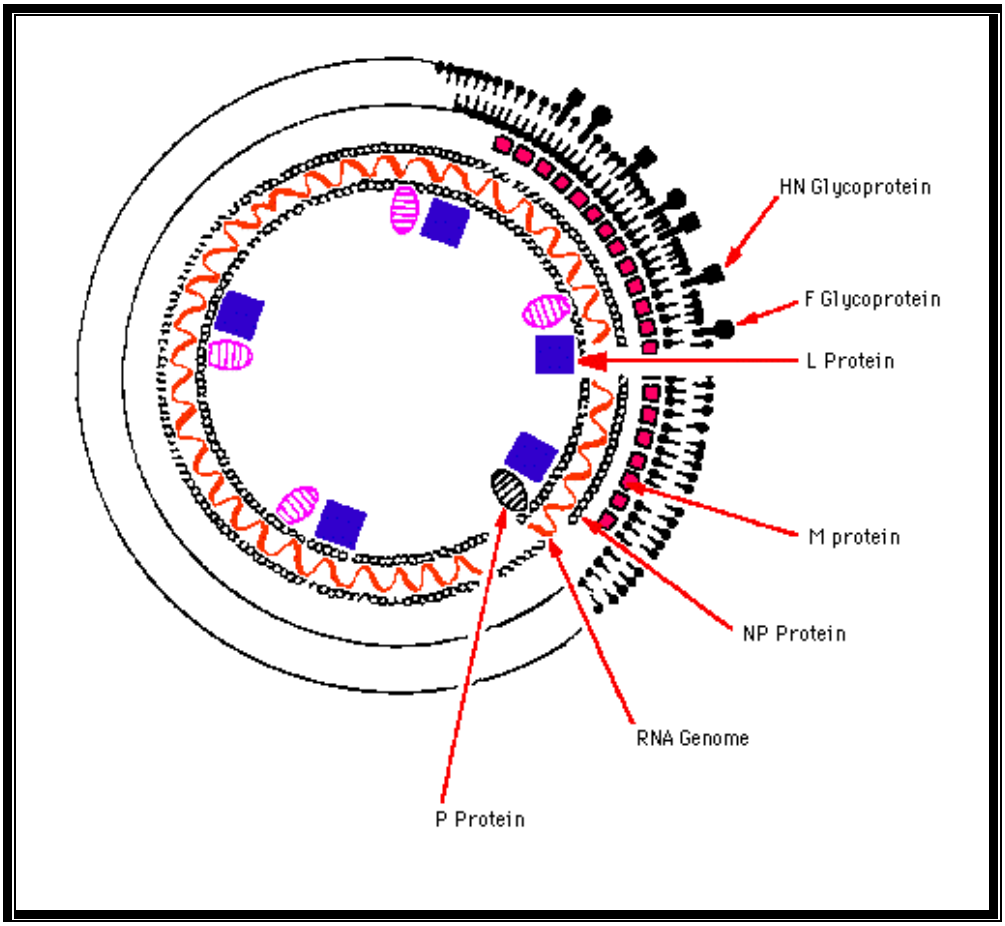
Medical Virology **Lec.6**

RNA Viruses **PARAMYXOVIRIDAE**

PARAMYXOVIRUS FAMILY

GENUS	MEMBERS	GLYCOPROTEINS
Paramyxovirus	mumps human parainfluenza viruses (HPIV 1-4)	HN, F
Morbillivirus	Measles	H, F
Pneumovirus	respiratory syncytial virus	G, F

Morphology:



Generally fairly similar to influenza: roughly spherical sometimes filamentous, easily distorted. A bit larger than influenza virus - 100-300nm. Composed of inner helical nucleocapsid containing **Genome ,linear minus sense(-ve ssRNA) .**

HN - haemagglutinin + neuraminidase activities;

F - consists of 2 disulphide-linked subunits (**F1** + **F2**) - responsible for cell fusion + haemolytic function.

The **M** (matrix) protein lines the inner surface of the envelope.

NP - nucleoprotein.

L and **P** - polymerase activity

Replication: Very similar for all viruses in this group. Unlike influenza, all the action occurs in the cytoplasm. However, the overall strategy very similar to influenza, A large excess of nucleocapsids are produced in infected cells, which form characteristic cytoplasmic inclusion bodies. Syncytium formation is quite common by (F glycoprotein).

Spread: The human paramyxoviruses are essentially diseases of human only, and are spread by droplets from the nose and mouth to close contacts. Many of them are highly infectious and go around the community in epidemics - often seasonal, eg. Winter coughs and colds. Fomites might also assist spread.

Pathogenesis:

1- Parainfluenzaviruses 1-4:

Pneumovirus (sub-genus of paramyxoviridae)
Enveloped, ssRNA viruses. Lacks the HN glycoprotein typical of the para-myxovirus group, but contains the fusion protein.
Gets its name from the fact that it causes large syncitia in cell culture

Epidemiology: The virus has a world wide distribution. It is the prime cause of bronchiolitis in young infants. There is no protection against RSV from maternal antibody and infants exposed in the first 6 months of life can develop life threatening disease. The virus is highly infectious and most children are exposed in their first year of life. Infection occurs in seasonal epidemics, mainly during the winter months. Re-infections are common thoughout life, but are of lesser severity, often sub-clinical.

Spread: Respiratory droplets, fomites NB A patient may shed virus for up to three weeks post infection.

Clinical syndromes:

- Bronchiolitis, Broncho-pneumonia: infants < 6 months of age.
- Laryngo Tracheo bronchitis: infants, young children .

- Acute bronchitis: adults, especially the elderly.
- Common cold syndrome: re-exposure in children and adults

Vaccine attempts: In the 1960s an attempt was made to produce a vaccine for RSV, using killed whole virus. Unfortunately, following exposure to live virus, vaccinated children developed more severe disease than the unvaccinated

children. The killed virus vaccine appears to have sensitized these children and induced a hypersensitivity response. This drew attention to the possibility that much of the lung damage caused by RSV could be due to immune mechanisms. Because of the importance of RSV in childhood infections, intensive efforts to make a vaccine have continued, however, no vaccine is as yet in general use.

2 - Mumps

British "to mump" - to grimace or grin, from the appearance of the patient as a result of parotid gland swelling. (Note: Other agents can also cause parotitis).

Recognized as early as the 5th century B.C. by Hippocrates who described a mild epidemic illness involving swelling near the ears. Humans are believed to be the only natural reservoir for the virus .Transmission via saliva and respiratory secretions; less infectious than measles/chickenpox - more adult cases.

Symptoms: Typically causes painful swelling of parotid glands 16-18 days after infection. This is preceded by primary replication of the virus in epithelial cells of the U.R.T. and local lymph nodes, followed by viraemia. In children, mumps is usually self-limited, but in adults (post-puberty) a proportion of cases have more serous sequalae: orchitis (20-30% of males - rarely resulting in sterility); meningitis, encephalitis, pancreatitis, myocarditis, nephritis - <1% adult cases.

Prevention: one serotype therefore vaccines are viable - both formalin-inactivated and live attenuated vaccine (MMR) exist.

PATHOGENESIS AND DISEASE

Mumps is very contagious and is probably usually acquired from respiratory secretions and saliva via aerosols or fomites. The virus is secreted in urine and so urine is a possible source of infection. Virus infects upper/lower respiratory tract leading to local replication. The virus spreads to lymphoid tissue which, in turn, leads to viremia. The virus thus spreads to a variety of sites, including salivary, other glands and other body sites.

The average time to full manifestation of disease is 2-3 wks but there may be fever, anorexia, malaise, myalgia during prodromal phase.

Diagnosis

Approximately 30% of infections are subclinical. Parotitis is suggestive (30-40% infections). The disease is confirmed by isolating the virus or by **serology (HI, CF, enzyme immunoasssay).**

Complement fixing antibody to the S (soluble) antigen (nucleocapsid protein) is seen for a few months after infection and is used to diagnose a recent infection. However, one needs to be careful as there is some cross reaction with other human parainfluenza virus nucleocapsid proteins. CF antibody to the viral envelope (V antigen) persists.

Epidemiology: Man is the only known natural host. Many (~30%) infections are subclinical. Single serotype.

Mumps is contagious from ~7 days before infection and becomes clinically apparent at ~9 days afterwards.

Prevention : Attenuated vaccine. The vaccine virus does not spread to contacts and gives long-term immunity. It is given as MMR vaccine (three live, attenuated viruses:**m**umps, **m**easles and **r**ubella).

Vaccine is contraindicated in immunosuppressed patients and in pregnant women.

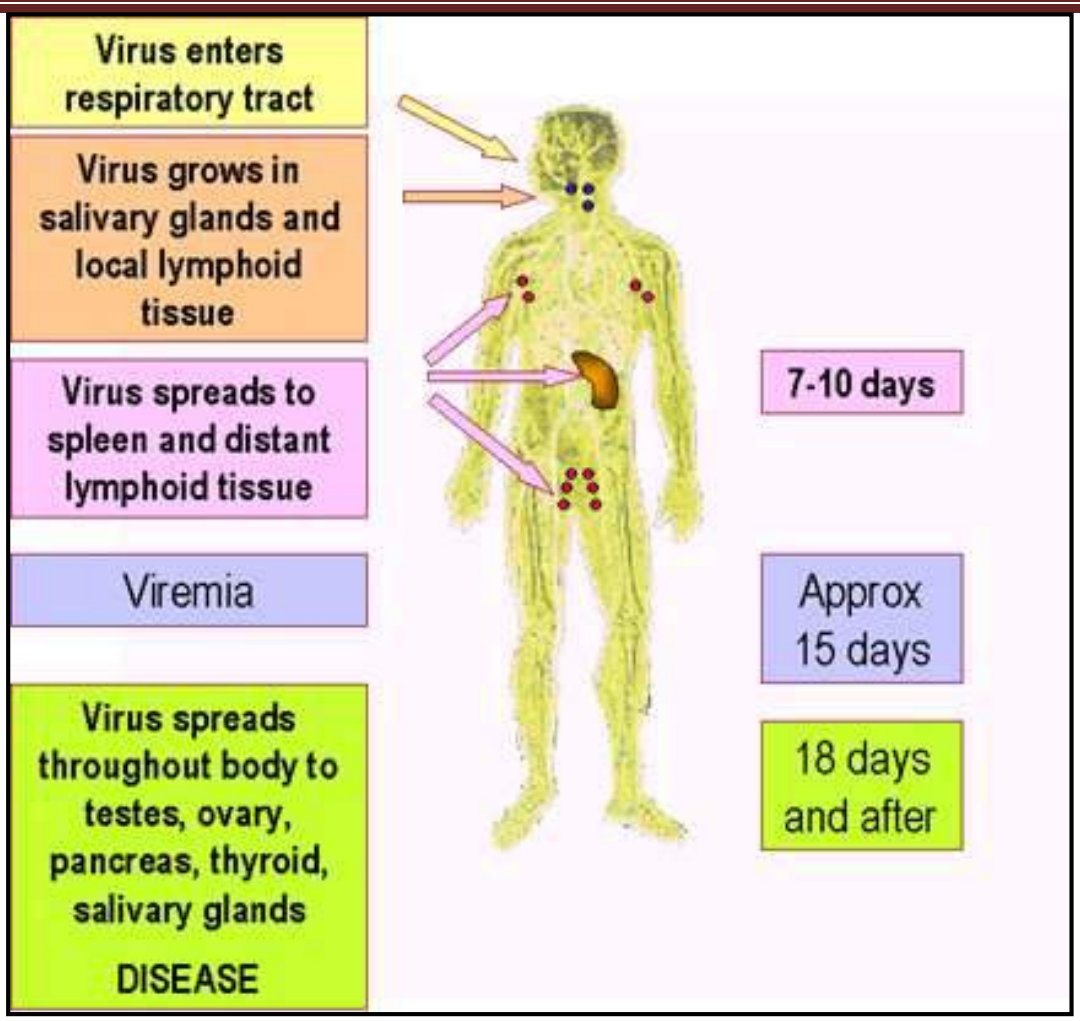
Treatment: There is no specific treatment for mumps.

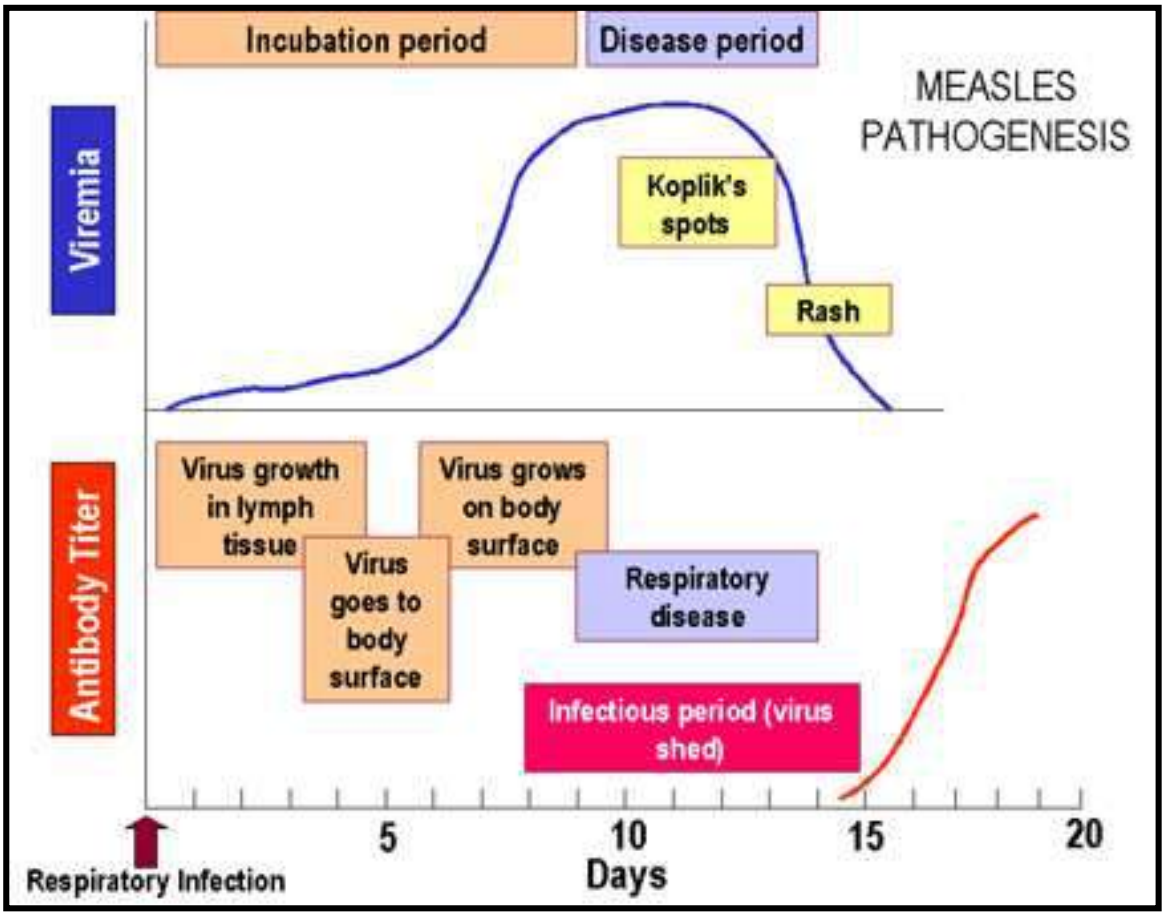
3 MEASLES (RUBEOLA)

Measles infection was distinguished from smallpox as early as the 9th century by an Arab physician by the name of Abu Becr (or Rhazes of Baghdad).

One of the most infectious diseases known. $>10^6$ deaths in children in the third world. Transmission and initial stages of disease similar to mumps, but this virus can also infect via the eye and multiply in the conjunctivae. Viraemia following primary local multiplication results in widespread distribution to many organs.

Measles virus replicates in the cytoplasm, but inclusions containing nucleocapsid protein can accumulate in the nucleus. It is not known if this has any effect on the host cell, but histologically typically giant cells with cytoplasmic and nuclear inclusion bodies are seen.


Virus enters respiratory tract
Virus grows in salivary glands and local lymphoid tissue
Virus spreads to spleen and distant lymphoid tissue
Viremia
Virus spreads throughout body to testes, ovary, pancreas, thyroid, salivary glands
DISEASE
7-10 days
Approx 15 days
18 days and after


Incubation period
Disease period
MEASLES PATHOGENESIS
Viremia
Koplik's spots
Rash
Antibody Titer
Virus growth in lymph tissue
Virus goes to body surface
Virus grows on body surface
Respiratory disease
Infectious period (virus shed)
Respiratory Infection
5
10
15
20
Days

Pathogenesis and disease:

Infection is via an aerosol route and the virus is very contagious. The virus replicates initially in the upper/lower respiratory tract. This is followed by replication in lymphoid tissues leading to viremia and growth in a variety of epithelial sites. The disease develops 1-2 weeks after infection.

The pathogenesis of measles. The virus invades the body via blood vessels and reaches surface epithelium first in the respiratory tract where there are only 1-2 layers of epithelial cells then in mucosae (Koplik's spots) and finally in the skin (rash). Adapted from Mims et al. Medical Microbiology, 1993, Mosby

Uncomplicated disease : Fever , Respiratory tract symptoms: running nose (coryza), cough,Conjunctivitis Koplik's spots on mucosal membranes - small (1-3mm), irregular, bright red spots, with bluish-white speck at center - may get enormous number, red areas may become confluent . Maculopapular rash (extends from face to extremities), seems to be associated with T-cells targeting infected endothelial cells in small blood vessels. Infection is prostrating.
Recovery is usually rapid, cell mediated response important (patients with agamma-globulinemia recover normally).Tends to be more severe in adults than children.

Complications of measles : If patient has an impaired cell-mediated immune response, there is continued growth in lungs leading to giant cell pneumonia (such patients may not have a rash). This is rare, but often fatal.

Since virus grows in epithelia of the nasopharynx, middle ear, lung, all of these sites may then be susceptible to secondary bacterial infection. Otitis media and bacterial pneumonia are quite common.

Outcome is affected by the nourishment of the patient and access to medical care. Measles is still a major killer in underdeveloped countries and several studies in areas with severe vitamin A deficiency problems have found that vitamin A treatment of children with measles has resulted in reduction in morbidity and mortality. Pneumonia accounts for 60% of deaths from measles.

1 in 1000 cases may get encephalitis a few days after the rash disappears. Most patients (90%) survive encephalitis but there may be complications - deafness, seizures, mental disorders.

SSPE : Very rarely (7 in 1,000,000 cases) the patient may get **s**ubacute **s**clerosing**p**an**e**ncephalitis (SSPE). This develops 1-10 years after initial infection. It is a progressive, fatal disease. Risk factors include acquiring primary measles at an early age. The incidence of SSPE has decreased since vaccination. SSPE is associated with defective forms of the virus in the brain and so it is difficult to isolate infectious virus from such patients. Certain viral proteins are often not expressed, the M protein being frequently absent.

Diagnosis: Clinical picture is the first part of diagnosis (that is: exposure plus upper respiratory tract symptoms, Koplik's spots and rash (which is usually quite characteristic for physicians familiar with measles)). This diagnosis is confirmed by serodiagnosis or isolation. Serodiagnosis is simpler but two samples are needed, one 10-21days post rash, and so takes longer. It is recommended that all suspect cases in the United States be confirmed by laboratory testing

Prevention : There is an attenuated virus vaccine. It is currently recommended to give a first dose of the vaccine at 12-15 months. If given earlier, the recipient does not mount a strong immune response to the vaccine. A second dose is administered at 4-6 yrs of age, before the recipient enters kindergarten or first grade. This reduces the proportion of persons who remain susceptible due to primary vaccine failure. The vaccine gives long term immunity and does not spread from the vaccinee.

Immune serum globulin can be used for at risk patients during an outbreak; that is those less than 1 year old or with impaired cellular immunity.

Measles vaccine can cause problems (e.g. fatal giant cell pneumonia) in those with severely compromised cell-mediated immunity. No inactivated vaccine is available, due to past problems in which subsequent infection with naturally acquired measles was sometimes associated with an atypical, severe form of measles.

Treatment: No antiviral therapy available for primary disease. Treat complications appropriately.

4_Respiratory Syncytia virus (RSV):

A major cause of L.R.T. disease in infants and young children. Infects human, with disease production. In culture, causes characteristic syncytial masses - hence the name. Highly infectious, transmission by respiratory secretions. Primary multiplication occurs in epithelial cells of U.R.T. producing a mild illness. In ~50% children less than 8 months old, virus subsequently spreads into the L.R.T. causing bronchitis, pneumonia and croup. **Has been suggested as a possible factor in cot death in about 1% of infants who develop serious illness.** Finally, it should be noted that the elderly and immunosuppressed transplant patients are at risk for developing pneumonia due to RSV infection.

Laboratory Diagnosis of a paramyxovirus:

Viral Detection Isolation of a paramyxovirus from a patient is strong evidence for a cause of a respiratory infection. All the Paramyxoviruses are to be found in the respiratory tract secretions - and this is the best material to send to the

laboratory. The paramyxoviruses are unstable and do not survive well outside cells.

1. Direct Examination: Viral antigens or viral infected cells in the secretions may be directly and rapidly detected by immunofluorescence or ELIZA tests.

2. Culture: Primary Monkey kidney cells will support replication of all the Paramyxoviruses and many of the other respiratory viruses. An intriguing property of most Paramyxo's is their ability to induce **cell fusion**. Neighboring cells join up to form large **multinucleate syncytia** or giant cells.

Multinucleated giant cells can sometimes be seen in lung sections from children dying with Paramyxovirus infection in the lung. Not all Paramyxos do this readily, and haemadsorption is usually used to detect Paramyxovirus infection in culture cells.

3.Serological Diagnosis: Antibody determinations in acute and convalescent blood specimens by complement fixation, ELIZA or haemagglutination inhibition may sometimes be helpful in arriving at a specific diagnosis.

Vaccination

The Mumps Measles &Rubella (MMR) vaccine prevents measles and two other viral diseases — mumps and rubella. These three vaccines are safe given together. MMR is an attenuated (weakened) live virus vaccine. This means that after injection, the viruses grows, and causes a harmless infection in the vaccinated person with very few, if any symptoms. The person's immune system fights the infection caused by these weakened viruses and immunity develops which lasts throughout that person’s life.

Most infants born will receive passive protection against measles, mumps, and rubella in the form of antibodies from their mothers. These antibodies can destroy the vaccine virus if they are present when the vaccine is administered and cause it to be ineffective. By 12 months of age, almost all infants have lost this passive protection. The second dose of MMR can be given anytime, usually administered before the child begins kindergarten or first grade (4-5 years of age) or before entry to middle school (11-12 years of age). The age at which the second dose is required is generally mandated by state school entry requirements.

Medical Virology Lec.7

RUBELLA (GERMAN MEASLES) VIRUS

Rubella virus is the only member of the Rubrivirus genus of the Togavirus family.

Rubella which means "little red" and is also known as German measles.

Was originally though to be a variant of measles. It is a mild disease in children and adults, but can cause devastating problems if it infects the fetus, especially if infection is in the first few weeks of pregnancy

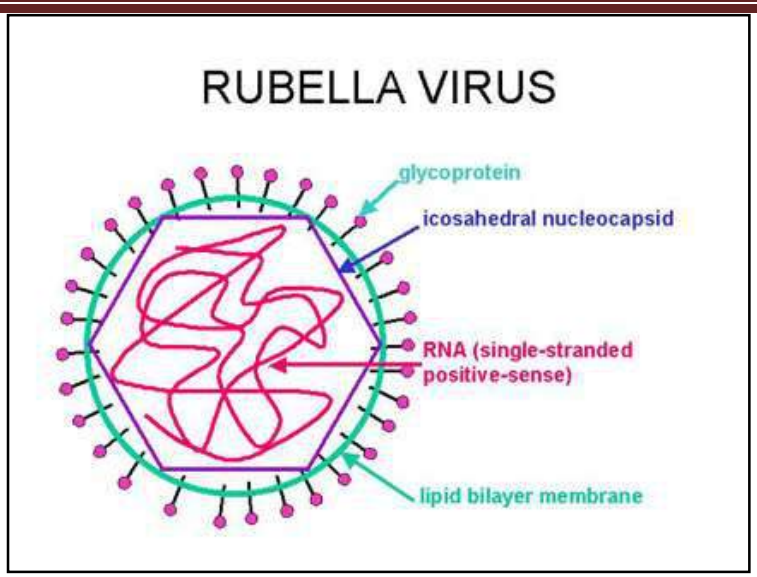
, **classified as a togavirus, genus Rubivirus**. It is an enveloped RNA virus, with a single antigenic type that does not cross-react with other members of the togavirus group. Rubella virus is relatively unstable and is inactivated by lipid solvents, trypsin, formalin, ultraviolet light, extremes of pH and heat, and amantadine.

THE VIRUS

Rubella virus was first isolated in 1962 . The only member of the Rubivirus genus of the Togavirus family. Unlike most Togaviruses it is NOT arthropod-borne, but is acquired via the respiratory route. It is an enveloped **(toga=cloak), non-segmented, positive sense, RNA virus and replicates in the cytoplasm.** Its nucleocapsid has **icosahedral** symmetry .There is only one major antigenic type.

Structure : The spherical virus particles (virions) of Togaviridae have a diameter of 50 to 70 nm and are covered by a lipid membrane (viral envelope), derived from the host cell membrane. There are prominent "spikes" (projections) of 6 nm composed of the viral envelope proteins E1 and E2 embedded in the membrane. Inside the lipid envelope is a capsid of 40 nm in diameter.

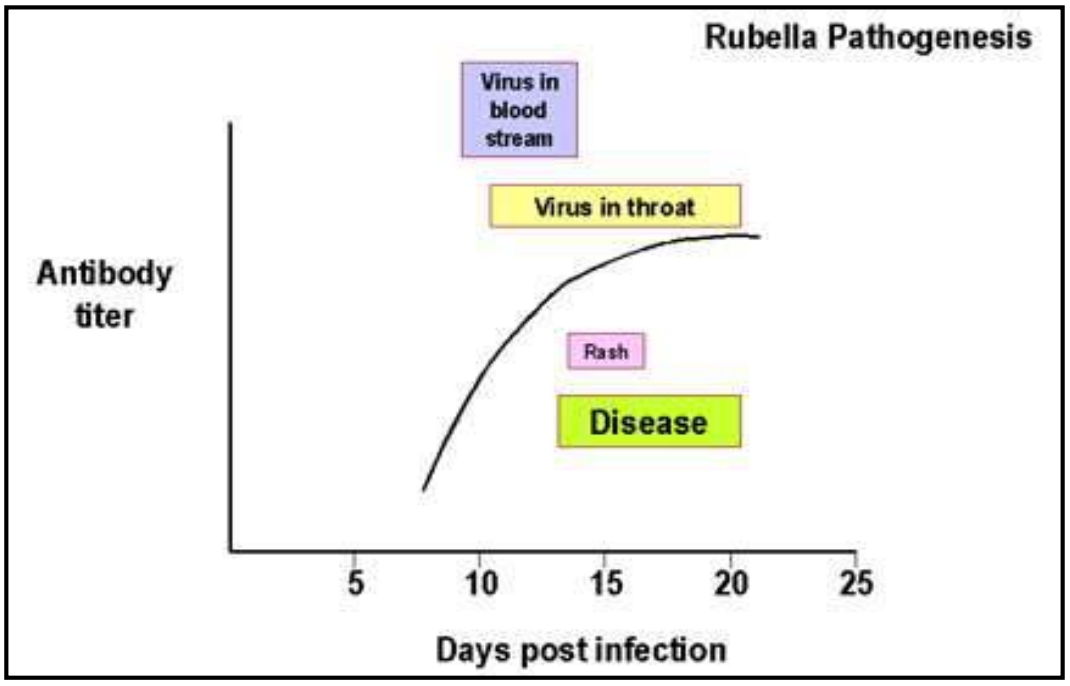
The capsid protein and the two glycosylated envelope proteins E1 and E2 make up for the three structural proteins.



Replication : Togaviruses attach to the cell surface via specific receptors and are taken up by an endosome being formed. At the neutral pH outside of the cell the E2 envelope protein covers the E1 protein. The dropping pH inside the endosome frees the outer domain of E1 and causes the fusion of the viral envelope with the endosomal membrane. Thus, the capsid reaches the cytosol, decays and releases the genome

The (+)ssRNA (positive, singlestranded RNA) at first only acts as a template for the translation of the non-structural proteins, which are synthesized as a large polyprotein and are then cut into single proteins. The sequences for the structural proteins are first replicated by the viral RNA polymerase (Replicase) via a complementary (-)ssRNA as a template and translated as a separate short mRNA. This short subgenomic RNA is additionally packed in a virion.

PATHOGENESIS AND DISEASE



Rubella virus is the pathogenic agent of the disease Rubella, and is the cause of congenital rubella syndrome when infection occurs during the first weeks of pregnancy. Humans are the only known host of this virus.

This again, is a benign childhood disease. The only danger is infection in the fetus of a pregnant woman in the first trimester.

The incubation period of rubella is 14 days with a range of 12 to 23 days. Symptoms are often mild, and 20%-50% of cases may be subclinical or unapparent. In children, rash is usually the first manifestation and a precursor of symptoms is rare. In older children and adults, there is often a 1-5 day precursor of low-grade fever, malaise, abnormal enlargement of the lymph nodes and upper respiratory symptoms preceding the rash. The rash of rubella usually occurs initially on the face and then progresses from head to foot. It lasts about 3 days and is occasionally marked by itching. The rash is fainter than measles rash and does not coalesce. Arthralgia and arthritis occur so frequently in adults that they are considered by many to be an integral part of the illness rather than a complication. Other symptoms of rubella include conjunctivitis, testalgia, or orchitis. Forschheimer spots may be noted on the soft palate, but are not diagnostic for rubella. Rubella is a human disease. There is no known animal reservoir. A true carrier state has not been described. However Infants with Congenital rubella syndrome shed large quantities of virus from body secretions for up to one year and can therefore transmit rubella to persons caring for them who are susceptible to the disease.

The molecular basis for the causation of congenital rubella syndrome are not yet completely clear, but in vitro studies with cell lines showed that Rubella virus has an apoptotic effect on certain cell types. There is evidence for a p53-dependent mechanism.

Children and adults

Man is the only host. Rubella virus is spread via an aerosol route and occurs throughout the world.

The initial site of infection is the upper respiratory tract. The virus replicates locally (in the epithelium, lymph nodes) leading to viremia and spread to other tissues. As a result the disease symptoms develop. Rash (if it occurs) starts after an incubation period of approximately 2 weeks (12 to 23 days) from the initial infection. There is probably an immunological basis for the rash (since it occurs as antibody titers rise).

The patient is infectious from about 1 week before onset of rash to about 1 week after. There is usually no prodrome in young children but in older children and adults disease results in low grade fever, rash, sore throat and lymphoadenopathy. Maculopapular rash begins on the face and lasts from 12 hours to 5days. Some individuals (especially adults and especially women) get arthralgia and sometimes arthritis which usually clears up in a few weeks.

Recovery: T-cell immunity plays an important role in recovery. IgM may persist for up to a year. There are also IgG, IgA responses.

Complications: Complications are extremely rarely (1 in 6000 cases). Rubella encephalopathy (headache, vomiting, stiff neck, lethargy, convulsions) may occur about 6 days after rash. It usually lasts only a few days and most patients recover (no sequelae). If death occurs, it is within few days of onset of symptoms. Other rare complications include orchitis, neuritis and panencephalitis.

Fetus: The risk to a fetus is highest in the first few weeks of pregnancy and then declines in terms of both frequency and severity, although there is still some risk in second trimester. The virus infects the placenta and then spreads to the fetus. In an outbreak of rubella in the United States in the mid 1960's, there were over 12 million cases of rubella and 20,000 cases of congenital rubella syndrome. If non-immune mothers are infected in the first trimester, up to 80% of neonates may have sequelae.

There may other problems including bone lesions, pneumonitis etc. In most cases, there is neural involvement - lethargy, irritability, motor tone problems, mental retardation, motor disabilities, abnormal posture, and neurosensory hearing loss.

Virus from congenital infections persists after birth. Those with congenital infections can infect others after birth for a year or more. Virus occurs in naso-pharyngeal secretions, urine and feces. Later on, patients with congenital rubella syndrome may develop additional complications including diabetes mellitus (up to 20%), thyroid dysfunction, growth hormone deficiency, ocular complications.

Progressive rubella panencephalitis: This is an extremely rare slow virus disease. It usually develops in the teens with death within 8 years. Most often it is associated with congenital rubella and may be associated with childhood rubella.

Epidemiology : Man is the only host and rubella occurs world wide. Periodic epidemics occur in an unvaccinated population. Natural infection protects for life (there is a single serotype).

Diagnosis of rubella

Many (possibly 50%) infections are apparently subclinical and many infections go unrecognized, even if symptoms develop (rash is not always present).

Infections with many other agents give similar symptoms to rubella (e.g. infection with human parvovirus, certain arboviruses, many of the enterovirus group of picornaviruses, some adenoviruses, EBV, scarlet fever, toxic drug reactions).

Serological tests, RT-PCR or isolation of virus (immunofluorescence) are needed to confirm infection of individual.

Prevention

A live vaccine (attenuated strain) is available. The vaccine virus is grown in human diploid fibroblasts. Since there is only one serotype, a univalent attenuated vaccine can provide lifelong immunity. The vaccine strain does not spread to family members.

It is important that women are vaccinated prior to their first pregnancy. United States recommendations are for childhood vaccination to prevent epidemics, combined with vaccination of susceptible, non-pregnant adolescent and adult females.

TREATMENT: There is no specific treatment. Supportive care should be used.