Ministry of Higher Education and Scientific Research
University of Baghdad
College of Science
Department of Biology

Advanced Virology
2020-2021

الدراسات العليا/ طلاب الماجستير
الاحياء المجهرية
الفصل الدراسي الثاني

أ.د. رغد حربي مهدي العزاوي

أ.د. نوال محمد عتبة

Week1–Virology

VirologyCourseforPostgraduates

University ofBaghdad

Dr.Raghad H.Al-azzawi

April,2021

Learning outcomes

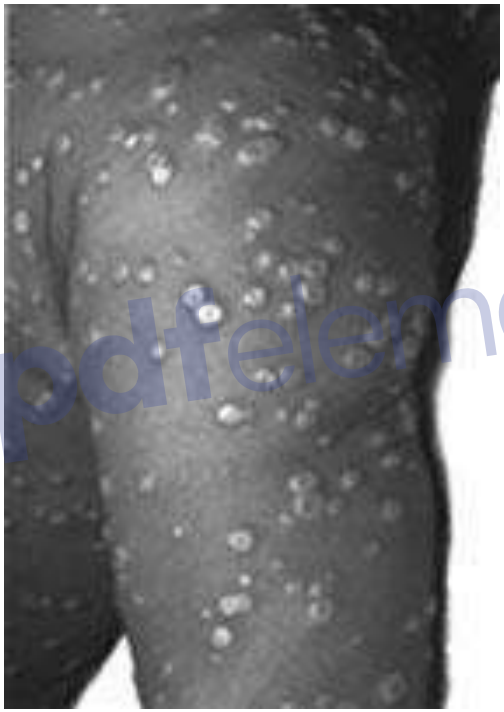
- [ ] How to define a virus? What are subviral agents?
- [ ] Know major events and discoveries Viral Features & characteristics What is structure of viruses?
- [ ] How to classify viruses?
- [ ] Know major events and discoveries Viral technologies and cultivation Viral vaccines
- [ ] Viral disinfectants and sterilization
- [ ]
- [ ]
- [ ]
- [ ]
- [ ]
- [ ]
- [ ]
- [ ]

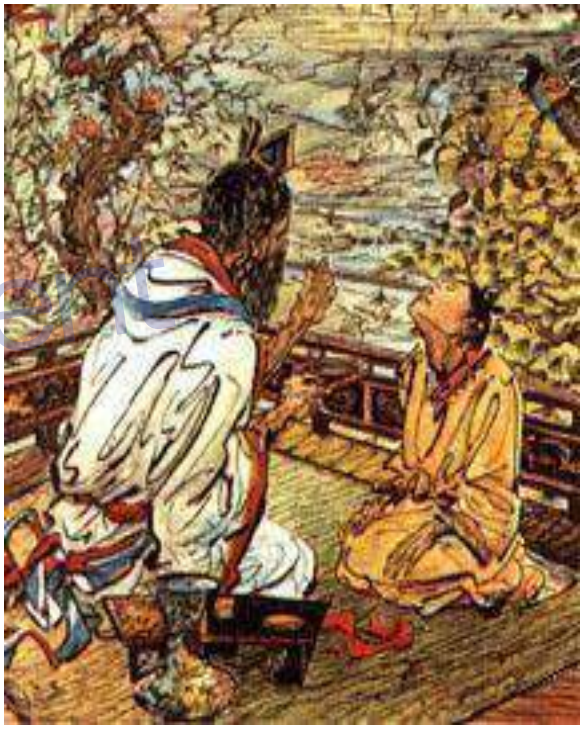
TheOriginandHistoryofViruses

The first written record of a virus infection consists of a heiroglyph from Memphis, the capital of ancient Egypt,

- drawn in approximately 3700BC,
- depictsatemplepriestcalled**Rumashowingtypical clinicalsignsofparalyticpoliomyelitis.**
- Small pox, the Pharoh Ramses V
- Smallpox was endemic in China by 1000BC (Variolation)
- **The "germ theory" of disease by Robert Koch & Louis Pasteur in the 1880s**

Poliomyelitis&SmallPox





Koch&Pasteur14.3.2013

RobertKoch(1843-1910)

LouisPasteur(1822-1895)

Koch‟sPostulates4famouscriteria

1. **Theagentmustbepresentineverycaseof the disease.**
2. **Theagentmustbeisolatedfromthehost & grown invitro.**
3. **Thediseasemustbereproducedwhenapure culture of the agent is inoculated into a healthy susceptible host.**
4. **Thesameagentmustberecoveredonce againfromtheexperimentallyinfectedhost.**

Pasteur identify a „virus“

- He worked on Rabies
- Identified as being caused a _virus‘
- **Virus: from the Latin for _poison„**
- However, No one yet can discriminate between bacterial & other agents of disease
- In 1892, **Dmitri Iwanowski (a Russian Botanist):**
  - Extracts from disease Tobacco plants transmits disease to other plants
  - Beginning of Virology
- in 1898, **Martinus Beijerinick confirmed the tobacco mosaic virus & referred to as contagium vivum fluidum 'soluble living germ'**

1898, **Freidrich Loeffler & Paul Frosch showed that a similar agent was responsible for foot-and-mouth disease (FMD) in cattle.**

- **Landsteiner & Popper** (1909), who showed that poliomyelitis was caused by a 'filterable agent'
  - The first human disease to be recognized as having a viral cause.
- **Frederick Twort (in 1915) & Felix d'Herelle (in 1917) were the first to recognize viruses which infect bacteria (bacteriophage)**

Major Discoveries

- The first vaccine
- Cell culture
- Plaque assays
- Serological & immunological
- Monoclonal antibodies
- Molecular technology

Definitions **in virology:**

Virus in Latin **word** **means poison.**

Definition: Obligate intracellular parasite composed of: Nucleic acid - either DNA or RNA and Protein coat.

Consist of a genome, *either* RNA or DNA that is surrounded by a protective protein shell (capsid).

This shell often is surrounded by an envelope that consists of protein and lipid.

They multiply only in living cells. Totally dependent upon host cell organelles and energy.

TABLE 13.1 Viruses and Bacteria Compared

	Bacteria		Viruses
	Typical Bacteria	Rickettsias/ Chlamydias	
Intracellular parasite	No	Yes	Yes
Plasma membrane	Yes	Yes	No
Binary fission	Yes	Yes	No
Pass through bacteriological filters	No	No/Yes	Yes
Possess both DNA and RNA	Yes	Yes	No
ATP-generating metabolism	Yes	Yes/No	No
Ribosomes	Yes	Yes	No
Sensitive to antibiotics	Yes	Yes	No
Sensitive to interferon	No	No	Yes

The NA is the genome that contains the information necessary for virus multiplication; the protein is arranged around the genome in the form of a shell that is the *capsid*. The shell plus the NA is the *nucleocapsid*. Some virus particles consist of a naked nucleocapsid, many others posses an *envelope* that is acquired from the host as the virus buds out. The complete particle is the *Virion*.

Capsid: composed of repeating subunits, identical or belonging to only a few different proteins. The simplest are composed of a single protein molecule; more complex units are composed of many subunits of either identical of different protein molecules that are called *capsomers*. The function of the capsid is to protect genomes and to help get genome into host cells.

Envelope: **only 7 families of animals viruses exist as naked nucleocapsids, all the others are enclosed by lipid envelopes that are acquired by the budding of viruses through the host cell membrane. These may be virus modified to contain viral proteins (spikes), but also have host cell components. Envelope helps in attachment to host cells. There is a virus specific matrix protein between the nucleocapsid and the envelope.**

Origins

Viruses are found wherever there is life and have probably existed since living cells first evolved. The origin of viruses is unclear because they do not form fossils, so molecular techniques have been used to compare the DNA or RNA of viruses and are a useful means of investigating how they arose. There are three main hypotheses that try to explain the origins of viruses.

Regressive hypothesis

Viruses may have once been small cells that parasitised larger cells. Over time, genes not required by their parasitism were lost. The bacteria rickettsia and chlamydia are living cells that, like viruses, can reproduce only inside host cells. They lend support to this hypothesis, as their dependence on parasitism is likely to have caused the loss of genes that enabled them to survive outside a cell. This is also called the *degeneracy hypothesis*, or *reduction hypothesis*.

Some viruses may have evolved from bits of DNA or RNA that "escaped" from the genes of a larger organism. The escaped DNA could have come from plasmids (pieces of naked DNA that can move*between* cells) or transposons (molecules of DNA that replicate and move around to different positions *within* the genes of the cell). Once called "jumping genes", transposons are examples ofmobile genetic elements and could be the origin of some viruses. They were discovered in maize by Barbara McClintock in 1950. This is sometimes called the *vagrancy hypothesis*, or the*escape hypothesis*.

Coevolution hypothesis

This is also called the *virus-first hypothesis* and proposes that viruses may have evolved from complex molecules of protein and nucleic acid at the same time as cells first appeared on earth and would have been dependent on cellular life for billions of years. Viroids are molecules of RNA that are not classified as viruses because they lack a protein coat. However, they have characteristics that are common to several viruses and are often called subviral agents. Viroids are important pathogens of plants. They do not code for proteins but interact with the host cell and use the host machinery for their replication. The hepatitis delta virus of humans has an RNA genome similar to viroids but has a protein coat derived from hepatitis B virus and cannot produce one of its own. It is, therefore, a defective virus and cannot replicate without the help of hepatitis B virus. In similar manner, the virophage 'sputnik' is dependent on mimivirus, which infects the protozoan *Acanthamoeba castellanii*. These viruses that are dependent on the presence of other virus species in the host cell are called *satellites* and may represent evolutionary intermediates of viroids and viruses.

Viriods: are responsible for causing serious diseases in many plants, they consist of naked DNA which does not code for any protein, nor is protein associated with it. Each viriod particles a ssRNA molecule contain 250-400 nucleotides. They are highly resistance to enzymatic degradation because they have no free ends and because they have a very tightly secondary structure, all viriod strains have similar characteristics. There genome has no AUG initiation codon for protein syntheses; there is no evidence that the RNA is translated. Theu replicate in the nucleus infected cells by host enzymes through double stranded intermediates. The replication is blocked by alpha amantine which inhibits RNA polymerase Π (the RNA polymerase responsible for generating the transcripts for mRNA).

Satellites

- Contain nucleic acid
- Depend on co-infection with a helper virus
- May be encapsidated (satellite virus)
- Mostly in plants, can be human e.g. hepatitis delta virus
- If nucleic acid only = virusoid

Defective virus:

*a VIRUS that can infect a HOST CELL but that lacks one or more essential functions for its REPLICATION. Such a virus may, however, replicate and produce progeny in the presence of another non-defective virus called a HELPER VIRUS that can provide the missing function(s).(2005)

*one unable to replicate on its own and needing to be complemented by a 'helper' virus for replication.(2007)

Defective virus:

* **a virus particle that contains insufficient nucleic acid to provide for production of all essential viral components; consequently, infectious virus is not produced except under certain conditions (for example, when the host cell is infected with a "helper" virus also).(2012)**

***A virus particle that contains insufficient nucleic acid to provide for production of all essential viral components.(2012)**

Prions are infectious protein molecules that do not contain DNA or RNA. They can cause infections such as scrapie in sheep, bovine spongiform encephalopathy ("mad cow" disease) in cattle, and chronic wasting disease in deer; in humans prionic diseases include Kuru, Creutzfeldt–Jakob disease, and Gerstmann–Sträussler–Scheinker syndrome. They are able to replicate because some proteins can exist in two different shapes and the prion changes the normal shape of a host protein into the prion shape. This starts a chain reaction where each prion protein converts many host proteins into more prions, and these new prions then go on to convert even more protein into prions; all known prion diseases are fatal. Although prions are fundamentally different from viruses and viroids, their discovery gives credence to the theory that viruses could have evolved from self-replicating molecules.

Host range:

Host range of viruses is the spectrum of host cell that the virus can infect. Most viruses are able to infect specific types of cells of only one host species.

- **Viruses that infect bacteria are bacteriophages or phages.**
- **Virus may infect invertebrates, vertebrates, plants and fungi.**

Size:

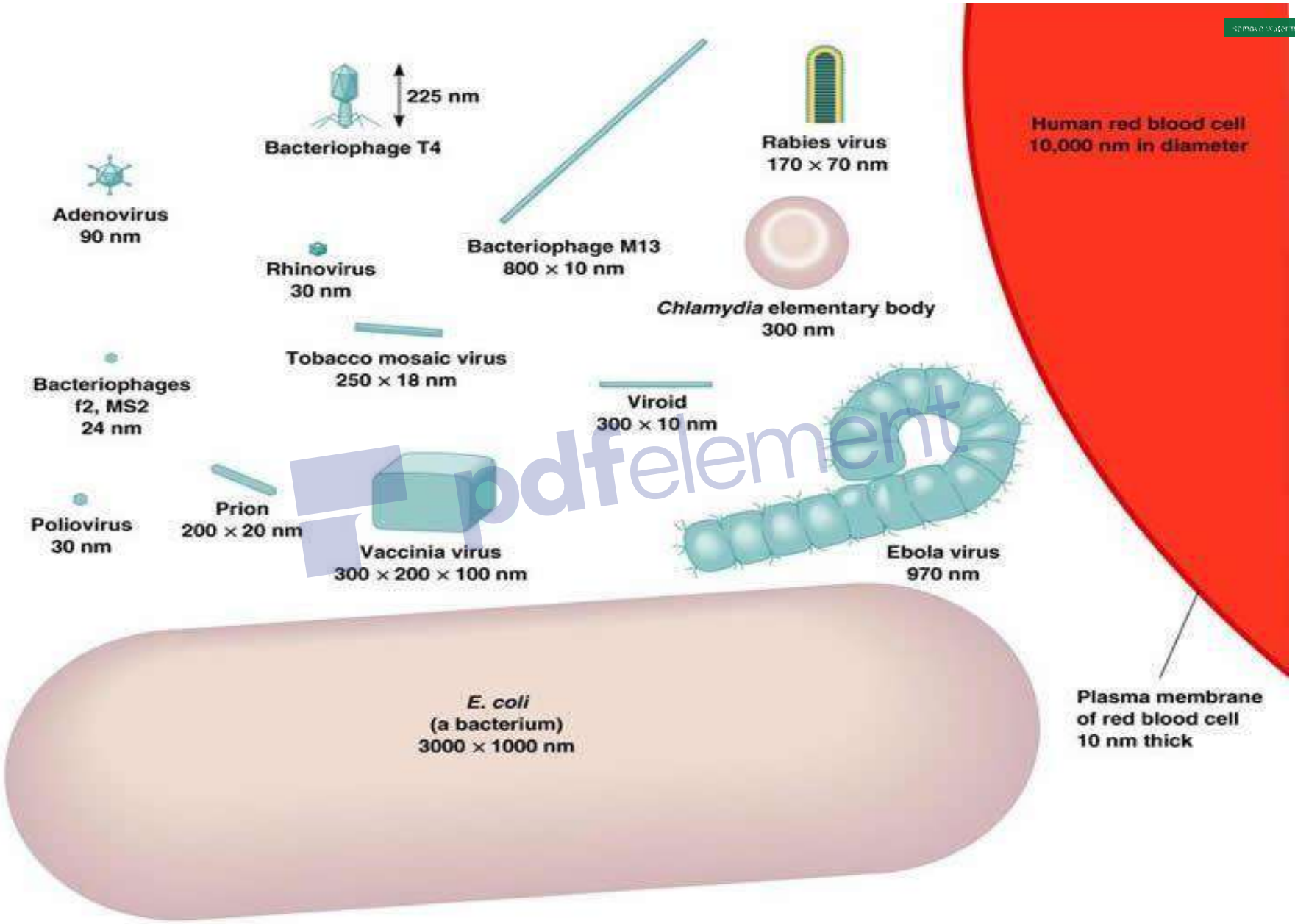
Viruses vary considerably in their sizes, although most are quite smaller than bacteria, some of large viruses are about the same size of bacteria, they range from 20-14000 nm in length.

The sizes of the viruses can be measured by some methods as:

1- Direct observation by electron microscope: the electron microscope uses electrons rather than light waves and electromagnetic lenses rather than glass lenses. The electron beam obtained has much shorter wavelength than that of light so the objective much smaller than the wave length of visible or ultraviolet light can be visualized. Viruses can be visualized in preparations from tissue extract sand in ultra thin sections of infected cells.

2- Filtration through membranes of graded porosity: Membranes are available with pores of different sizes. The sizes of the limiting APD (average pore diameter) multiplied by 0.064 yields the diameter of viral particle. This method gives only the approximate estimate of size.

3- Sedimentation in ultracentrifuge: In an ultracentrifuge forces of more than 100.000 times gravity may be used to drive the particles to the bottom of the tube. The relationship between the size of a particle and its rate of sedimentation permits determination of particle size. The physical structure of the virus will affect the size estimate obtained.

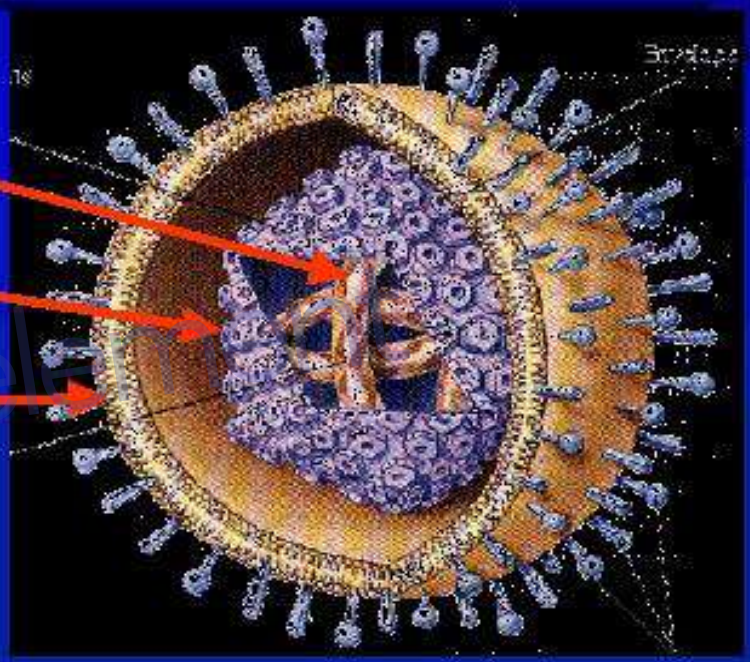

225 nm
Bacteriophage T4
Adenovirus
90 nm
Rhinovirus
30 nm
Bacteriophage M13
800 × 10 nm
Rabies virus
170 × 70 nm
Human red blood cell
10,000 nm in diameter
Chlamydia elementary body
300 nm
Tobacco mosaic virus
250 × 18 nm
Bacteriophages
f2, MS2
24 nm
Viroid
300 × 10 nm
Poliovirus
30 nm
Prion
200 × 20 nm
Vaccinia virus
300 × 200 × 100 nm
Ebola virus
970 nm
E. coli
(a bacterium)
3000 × 1000 nm
Plasma membrane
of red blood cell
10 nm thick

Viral structure

Virion: complete infectious virus particle, consists of nucleic acid core surrounded by a protective coat of protein called a capsid. These are (capsid) formed from identical protein subunits called capsomers. The complete set of virion is known nucleocapsid. In turn the nucleocapsid may be naked or enveloped by a loose covering. Viruses are classified by differences in the structures of these coats.

COMPONENTS OF VIRUSES

- core
  - nucleic acid
- capsid
  - protein coat
- envelope
  - lipid bilayer membrane
  - not all viruses have an envelope



Component of viruses.

Nucleic acid

-Viral genomes are either DNA or RNA (not both).

-Nucleic acid may be single- or double-stranded.

-Nucleic acid may be circular or linear or separate molecules.

-Nucleic acid: protein ranges from about 1% - 50%.

Envelope – the outer covering of some viruses, the envelope is derived from the host cell plasma membrane when the virus buds out. Some enveloped viruses have spikes, which are viral glycoproteins that project from the envelope.

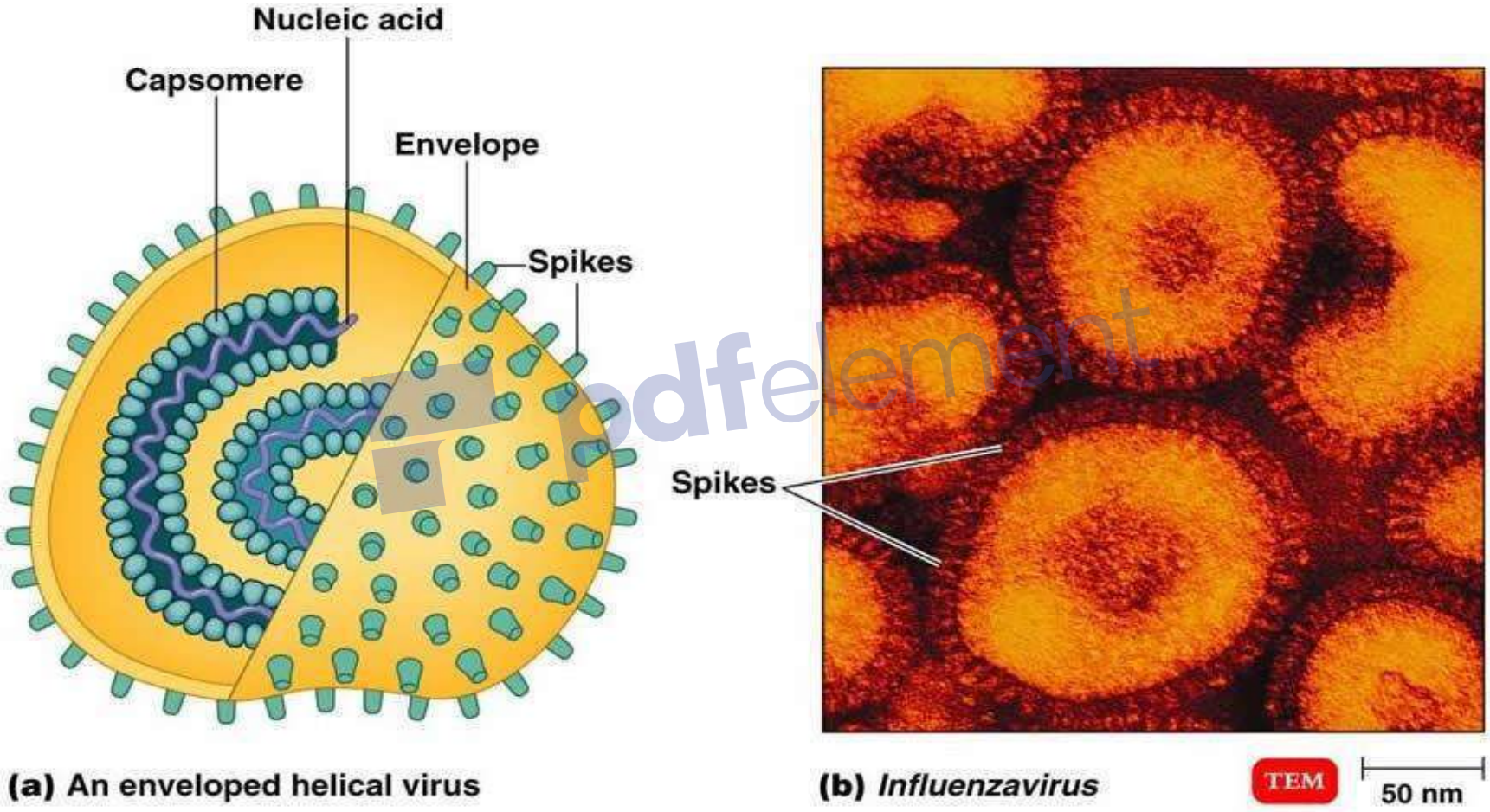
Influenzavirus has two kinds of spikes, H (hemagglutinin) and N (neuraminidase). The H spike allows the virus to attach to host cells (and red blood cells), the N spike is an enzyme that allows the mature viral particles to escape from the host cell.

Non-enveloped or naked viruses are protected by their capsid alone.

Chemically the envelope is made up of proteins and glycoproteins. Due to the presence of lipid the envelope seems flexible and loose. Envelope is composed of both the host and viral components i.e. protein (virus specific) and carbohydrates (host specific). There are certain projections on the envelope known as spikes which are arranged into distinct units.

Some species of virus envelop themselves in a modified form of one of the cell membranes, either the outer membrane surrounding an infected host cell or internal membranes such as nuclear membrane or endoplasmic reticulum, thus gaining an outer lipid bilayer known as a viral envelope. This membrane is studded with proteins coded for by the viral genome and host genome; the lipid membrane itself and any carbohydrates present originate entirely from the host. The influenza virus and HIV use this strategy. Most enveloped viruses are dependent on the envelope for their infectivity.

The envelope is a common feature in animal viruses not plants viruses.



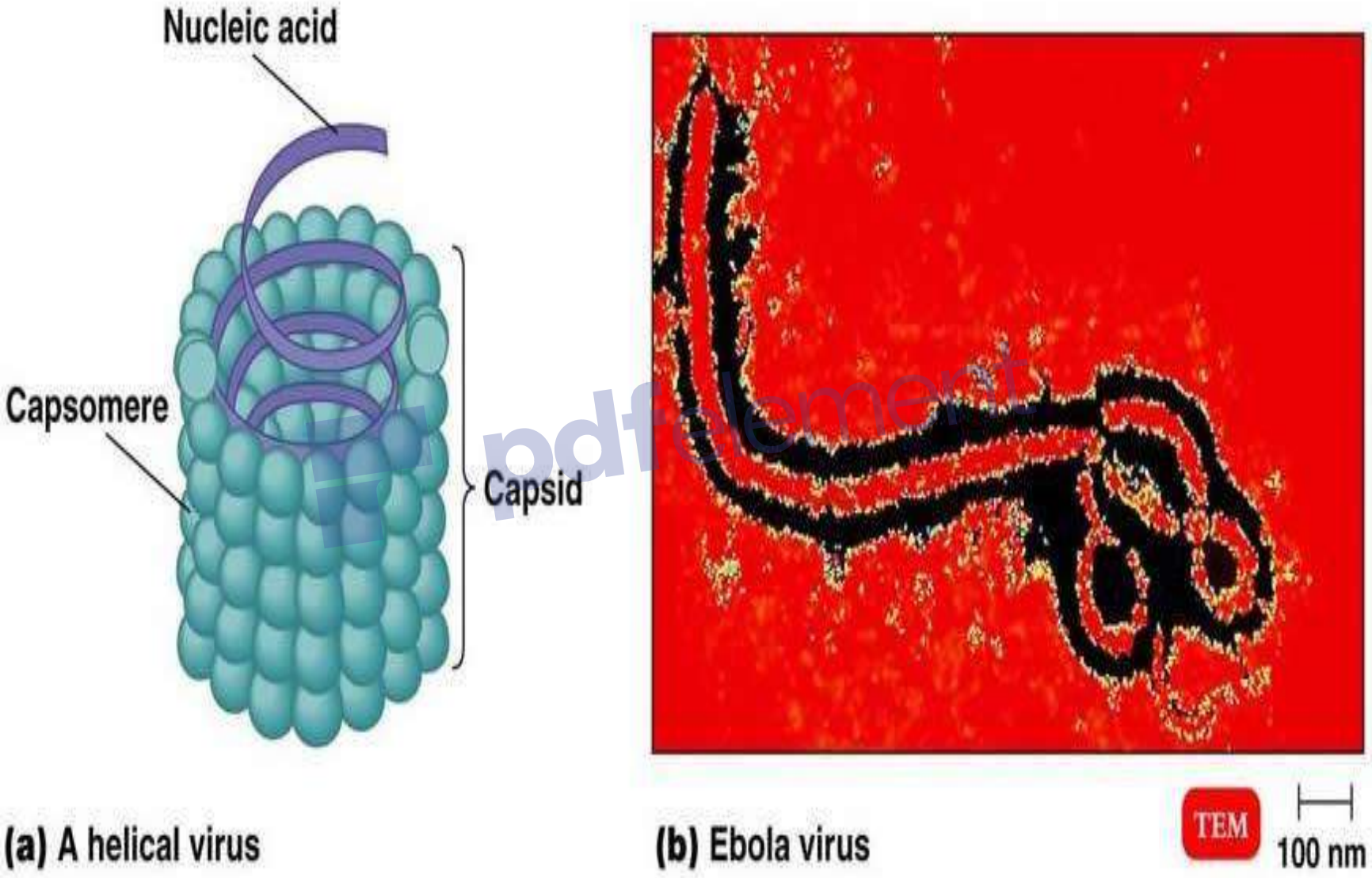
(a) An enveloped helical virus

(b) *Influenzavirus*

Morphology

Helical

These viruses are composed of a single type of capsomer stacked around a central axis to form a helical structure, which may have a central cavity, or hollow tube. This arrangement results in rod-shaped or filamentous virions: These can be short and highly rigid, or long and very flexible. The genetic material, in general, single-stranded RNA, but ssDNA in some cases, is bound into the protein helix by interactions between the negatively charged nucleic acid and positive charges on the protein. Overall, the length of a helical capsid is related to the length of the nucleic acid contained within it and the diameter is dependent on the size and arrangement of capsomers. The well-studied tobacco mosaic virus is an example of a helical virus.

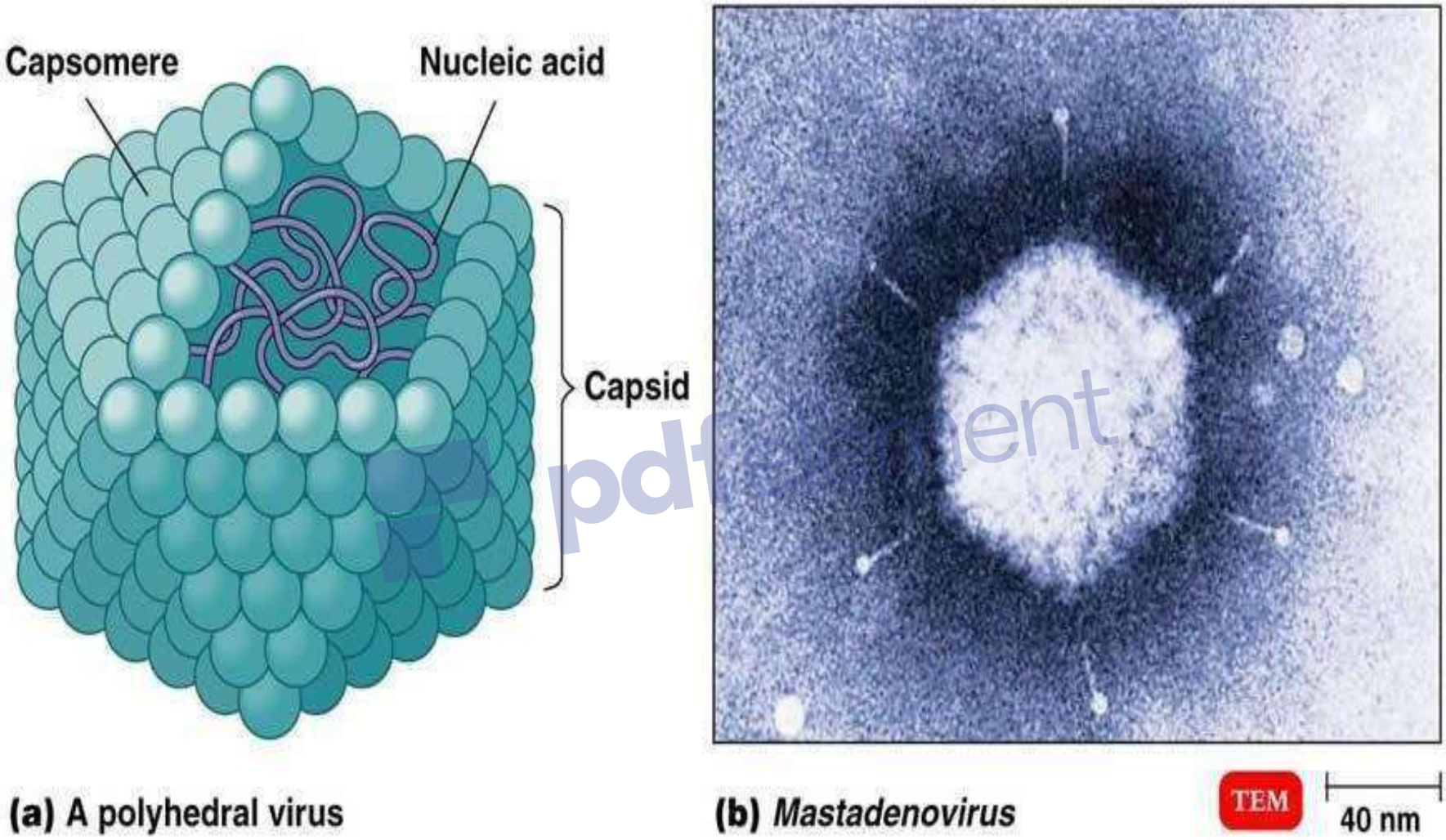


(a) A helical virus

(b) Ebola virus

Icosahedral

Most animal viruses are icosahedral or near-spherical with icosahedral symmetry. A regular icosahedron is the optimum way of forming a closed shell from identical sub-units. The minimum number of identical capsomers required is twelve, each composed of five identical sub-units. Many viruses, such as rotavirus, have more than twelve capsomers and appear spherical but they retain this symmetry. Capsomers at the apices are surrounded by five other capsomers and are called pentons. Capsomers on the triangular faces are surrounded by six others and are called hexons. Hexons are in essence flat and pentons, which form the 12 vertices, are curved. The same protein may act as the subunit of both the pentamers and hexamers or they may be composed of different proteins.



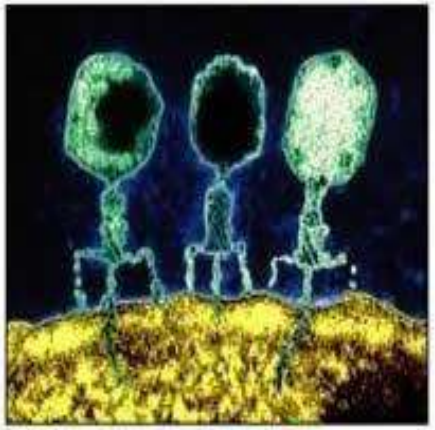
(a) A polyhedral virus

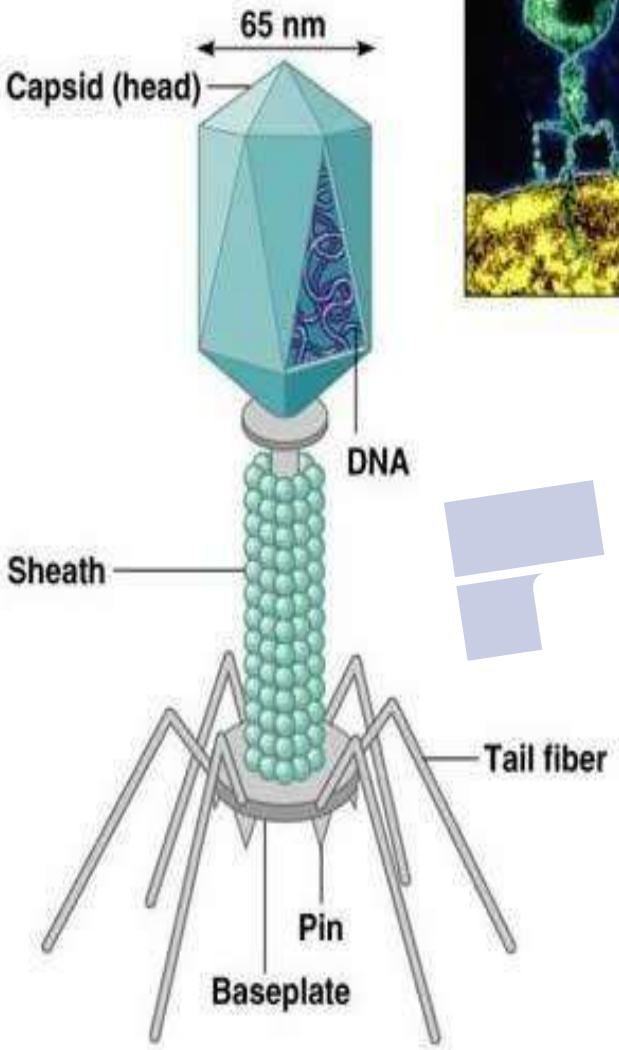
(b) *Mastadenovirus*

Complex:

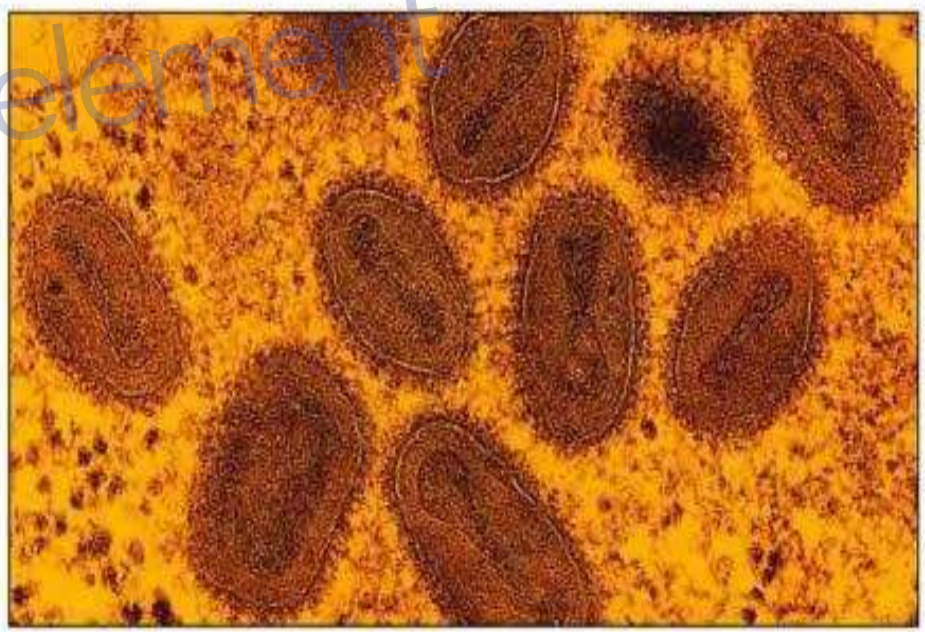
These viruses possess a capsid that is neither purely helical nor purely icosahedral, and that may possess extra structures such as protein tails or a complex outer wall. Some bacteriophages, such as Enterobacteria phage T4, have a complex structure consisting of an icosahedral head bound to a helical tail, which may have a hexagonal base plate with protruding protein tail fibres. This tail structure acts like a molecular syringe, attaching to the bacterial host and then injecting the viral genome into the cell.

The poxviruses are large, complex viruses that have an unusual morphology. The viral genome is associated with proteins within a central disk structure known as a nucleoid. The nucleoid is surrounded by a membrane and two lateral bodies of unknown function. The virus has an outer envelope with a thick layer of protein studded over its surface. The whole virion is slightly pleiomorphic, ranging from ovoid to brick shape. Mimivirus is the largest characterised virus, with a capsid diameter of 400 nm. Protein filaments measuring 100 nm project from the surface. The capsid appears hexagonal under an electron microscope, therefore the capsid is probably icosahedral. In 2011, researchers discovered a larger virus on ocean floor of the coast of Las Cruces, Chile. Provisionally named *Megavirus chilensis*, it can be seen with a basic light microscope.





(a) A T-even bacteriophage



(b) *Orthopoxvirus*

Some viruses that infect Archaea have complex structures that are unrelated to any other form of virus, with a wide variety of unusual shapes, ranging from spindle-shaped structures, to viruses that resemble hooked rods, teardrops or even bottles. Other archaeal viruses resemble the tailed bacteriophages, and can have multiple tail structures.

Genomic diversity among viruses

Property	Parameters
	▪ **DNA**
Nucleic acid	▪ **RNA** ▪ **Both DNA and RNA (at different stages in thelife cycle)**
Shape	▪ **Linear** ▪ **Circular** ▪ **Segmented**
Strandedness	▪ **Single-stranded** ▪ **Double-stranded** ▪ **Double-stranded with regions of single-strandedness**
Sense	▪ **Positive sense (+)** ▪ **Negative sense (−)** ▪ **Ambisense (+/−)**

Strategies for virus survival

- **Finding and getting into a host cell. As viruses are obligate parasites they must find the right type of cell for their replication, they must invade that cell and get their genome to the site of replication.**
- **Making virus protein. All viruses are parasites of translation. The virus must make mRNA (unless it has a + sense RNA genome already). Strategies must exist to synthesize mRNA.**
- **Making viral genomes. Many viral genomes are copied by the cell's synthetic machinery in cooperation with viral proteins.**

- **Forming progeny virions. The virus genome, capsid (and envelope) proteins must be transported through the cell to the assembly site, and the correct information for assembly must be pre-programmed.**
- **Spread within and between hosts. Toensure survival the virus must propagate itself in new cells.**
- **Overcoming host defences.The host defends itself against "nonself". Viruses have evolved ways to fight back.**

Methods of viruses"purification

It is not an easy matter to free virions of associated cell debris or even from viral proteins synthesized in excess in the infected cell, furthermore the infectivity of virions is very sensitive to inactivation by heat, acid, alkali and sometimes lipids solvents or osmotic shock, throughout all purification protocols the virus is maintained at near neutral pH and 4^0 C.

1- Liberation of virus from cells: **The first step in the purification consists of obtaining virions free from the cells in which they were grown, viruses are released from cells by methods of sonic vibration, homogenization, or repeated freeze thawing, however the supernatant, fluid in an infected cell culture provides source of virions.**

2- Chemical methods of purification: **The chemical methods of purification must be adsorbed to column of DEAE cellulose or sephadex or to erythrocytes or ion exchange resins and then eluted with buffers of different pH and ionic strength or at different temp.**

3- Physical methods of purification:

Viruses can be separated from soluble contaminants by centrifugation. Differential centrifugation consists of alternate cycles of low and high speed centrifugation to deposit first contaminating particles then virions. Rate zonal centrifugation through performed gradient of a dense solute such as sucrose forces virions to sediment through the gradient at a rate determined by their sedimentation coefficient after prolong ultracentrifugation at very high forces, the virions will come to rest in a sharp band in that part of the tube where the solution has the same density as the virions, usually within the range 1.15-1.4.

4- Concentration of the virion: **Viruses can be Concentrated in to small volume by ultracentrifugation, freeze-drying, or dialysis against hydrophilic as gents such as polyethylene glycol**

Inactivation and removal of viruses

In general viruses are more sensitive than bacteria or fungi to inactivation by physical and chemical agents.

Viral inactivation

Viral inactivation renders viruses *inactive*, or unable to infect. Many viruses contain lipid or protein coats that can be inactivated by chemical alteration. Viral inactivation is different from viral removal because, in the former process, the surface chemistry of the virus is altered and in many cases the (now non-infective) viral particles remain in the final product. Rather than simply rendering the virus inactive, some viral inactivation processes actually denature the virus completely. Viral inactivation is used widely in the blood plasma industry.

In order to achieve inactivation of the viruses in the sample, it is necessary to perform "special" purification processes that will chemically alter the virus in some way. Some of the more widely used processes are as follows:

- **Solvent/detergent inactivation**
- **Pasteurization (heating)**
- **Acidic pH inactivation**

In some cases viral inactivation is not a viable removal alternative because even the denatured or otherwise inactivated viral particles can have deleterious effects on the process stream or the product itself.

This process is only effective for viruses enveloped in a lipid coat, however. The detergents used in this method interrupt the interactions between the molecules in the virus's lipid coating. Most enveloped viruses cannot exist without their lipid coating so is destroyed when exposed to these detergents. Other viruses may not be destroyed but they are unable to reproduce rendering them non- infective. The solvent creates an environment in which the aggregation reaction between the lipid coat and the detergent happen more rapidly. The detergent typically used is Triton-X 100

This process has many of the advantages of the "traditional" removal techniques. This process does not denature proteins, because the detergents only affect lipids and lipid derivatives. There is a 100% viral death achieved by this process and the equipment is relatively simple and easy to use. Equipment designed to purify post-virus inactivated material would be necessary to guard against contamination of subsequent process streams

Pasteurization

Inactivation of viruses by means of pasteurization can be very effective if the proteins that you are trying to protect are more thermally resistant than the viral impurities with which they are in solution. Some of the more prominent advantages of these types of processes are that they require simple equipment and they are effective for both enveloped *and* non-enveloped viruses. Because pasteurization involves increasing the temperature of solution to a value that will sufficiently denature the virus, it does not matter whether the virus has an envelope or not because the envelope alone cannot protect the virus from such high temperatures. However, there are some proteins which have been found to act as thermal stabilizers for viruses. Of course, if the target protein is not heat-resistant, using this technique could denature that target protein as well as the viral impurity. Typical incubation lasts for 10 hours and is performed at 60°C.

Acidic pH inactivation

Some viruses, when exposed to a low pH, will denature spontaneously. Similar to pasteurization, this technique for viral inactivation is useful if the target protein is more resistant to low pHs than the viral impurity. This technique is effective against enveloped viruses, and the equipment typically used is simple and easy to operate. This type of inactivation method is not as effective for non- enveloped viruses however, and also requires elevated temperatures. So in order to use this method, the target protein must be resistant to low pHs and high temperatures which is unfortunately not the case for many biological proteins. Incubation for this process typically occurs at a pH of 4 and lasts anywhere between 6 hours and 21 days.

Ultraviolet (UV) inactivation

UV rays can damage the DNA of living organisms by creating nucleic acid dimers. However, the damages are usually not important due to low penetration of UVs through living tissues. UV rays can be used, however, to inactivate viruses since virus particules are small and the UV rays can reach the genetic material, inducing the dimerisation of nucleic acids. Once the DNA dimerised, the virus particules cannot replicate their genetic material which prevent them from spreading.

UV light in combination with riboflavin has been shown to be effective in reducing pathogens in blood transfusion products. Riboflavin and UV light damages the nucleic acids in viruses, bacteria, parasites, and donor white blood cells rendering them unable to replicate and cause disease.

Virus removal

This overarching process, which has come to be known simply as virus removal, is one in which all of the viruses in a given sample are removed by traditional extraction or filtration methods. Some of the more prominent methods include:

- **Nanofiltration**
- **Chromatography**

These extraction processes are considered "traditional processes" because they do not chemically affect the virus in any way; they simply remove it physically from the sample.

Nanofiltration

Virus removal processes using nanofiltration techniques remove viruses specifically by size exclusion. This type of process is typically used for parvoviruses and other viruses containing a protein coat. A typical HIV virion is 180 nm and a typical parvovirus can vary between 15 and 24 nm, which is very small. One great advantage of filtration, as opposed to methods involving extremes of temperature or acidity, is that filtration will not denature the proteins in the sample. Nanofiltration is also effective for most types of proteins. Since it is not chemically selective, no matter what the surface chemistry of the viral particle is, viral removal processes using nanofiltration techniques will still be effective. Another great advantage of this technique is its ability to be performed on a lab scale and then effectively scaled up to production standards. It is important to consider, however, the fact that the level of removal of the viruses is dependent on the size of the pores of the nanofilter. In some cases, very small viruses will not be filtered out. It is also necessary to consider the possible effects of pressure and flow rate variation.

Some of the filters used for to perform these types of processes are Planova 15N, Planova 20N, BioEX, VAG - 300, Viresolve 180, and Viresolve 70TM.

Chromatography

Chromatographic methods of removing viruses are great for purifying the protein and are also effective against all types of viruses, but the level of virus removal is dependent on the column composition and the reagents that are used in the process. It is also worthy to note that the effectiveness of this process can vary greatly between viruses and that the efficiency of the process can change based on the buffer that is used. Sanitation between batches is also a concern when performing this procedure.

Inactivated vaccines

The traditional method of inactivating viruses for use as vaccines has been treatment with formaldehyde. Other chemicals that inactivate the nucleic acid with minimal effect on the viral proteins and therefore on immunogenicity are used such as ß propiolactone and psoralen followed by UV irradiation.

LONG-TERM AND SHORT-TERM STABILITY OF VIRUSES DEPEND ON STORAGE TEMPERATURE AND PRESERVATION METHOD

Maintaining virus viability over long periods of time with little or no change of infectivity is very valuable. Biological Resource Centers (BRCs) such as ATCC® depend on being able to store viruses under conditions that maintain viability for long periods without the need for re-growth. Methods of preservation include cryopreservation in liquid nitrogen, lyophilization (freeze-drying), and storage at low temperature (-70°C) in mechanical freezers. As a result of their preservation function, BRCs tend to collect, characterize and maintain a rich and varied collection of biological materials.

Classification of viruses

Viruses are found throughout the world and infect all known organisms. Viruses cause a range of different diseases and display a diversity of host range, morphology, and genetic makeup. Bringing order to this huge diversity requires the designation of classification groups to permit study of representative viruses that can inform us about their less well studied relatives.

Basis of Classification

The following properties have been used as a basis for the classification of viruses. The amount of information available in each category is not the same for all viruses. The way in which viruses are characterized is changing rapidly. Genome sequencing is now often performed early in virus identification, and genomic sequence data are advancing taxonomic criteria (eg, gene order) and may provide the basis for the identification of new virus families.

(1) Virion morphology, including size, shape, type of symmetry, presence or absence of peplomers, and presence or absence of membranes.

(2) Virus genome properties, including type of nucleic acid (DNA or RNA), size of genome in kilobases (kb) or kilobase pairs (kbp), strandedness (single or double), whether linear or circular, sense (positive, negative, ambisense), segments (number, size), nucleotide sequence, G + C content, and presence of other special features.

(3) Physicochemical properties of the virion, including molecular mass, buoyant density, pH stability, thermal stability, and susceptibility to physical and chemical agents, especially ether and detergents.

(4) Virus protein properties, including number, size, and functional activities of structural and nonstructural proteins, amino acid sequence, modifications (glycosylation, phosphorylation, myristylation), and special functional activities (transcriptase, reverse transcriptase, neuraminidase, fusion activities).

(5) Genome organization and replication, including gene order, number and position of open reading frames, strategy of replication (patterns of transcription, translation), and cellular sites (accumulation of proteins, virion assembly, virion release).

(6) Antigenic properties.

(7) Biologic properties, including natural host range, mode of transmission, vector relationships, pathogenicity, tissue tropisms, and pathology.

The oldest classification of viruses is based on the disease they produce called (symptomatology), but this system is not satisfactory for the biologist because the same virus may appear in several groups if it cause more than one disease depending upon the organ they attach.

ICTV classification:

The International Committee on Taxonomy of Viruses began to devise and implement rules for the naming and classification of viruses early in the 1990s, an effort that continues to the present day. The ICTV is the only body charged by the International Union of Microbiological Societies (IUMS) with the task of developing, refining, and maintaining universal virus taxonomy. The system shares many features with the classification system of cellular organisms, such as taxon structure. Viral classification starts at the level of realm and continues as follows, with the taxon suffixes given in italics:

Realm (*-viria*)

Subrealm (*-vira*)

Kingdom (*-viriae*)

Subkingdom (*-virites*)

Phylum (*-viricota*)

Subphylum (*-viricotina*)

Class (*-viricetes*)

Subclass (*-viricetidae*)

Order (*-virales*)

Suborder (*-virineae*)

Family (*-viridae*)

Subfamily (*-virinae*)

Genus (*-virus*)

Subgenus (*-virus*)

Species

Species names often take the form of *[Disease] virus*, particularly for higher plants and animals. As of November 2018, only phylum, subphylum, class, family, order, suborder, family, subfamily, genus, and species are used.

The establishment of an order is based on the inference that the virus families it contains have most likely evolved from a common ancestor. The majority of virus families remain unplaced.

As of 2018, just one single phylum, two subphyla, six classes, 14 orders, five suborders, 143 families, 64 subfamilies, 846 genera, and 4,958 species of viruses have been defined by the ICTV

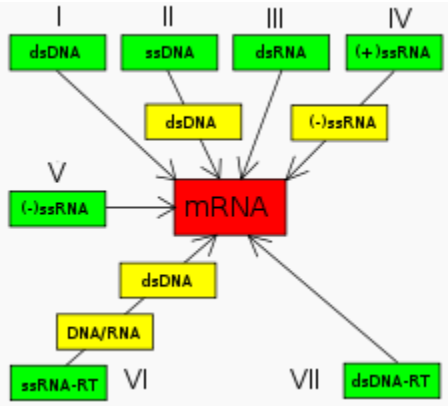
As of 2019 there were 4 realms, 9 kingdoms, 16 phyla, 2 subphyla, 36 classes, 55 orders, 8 suborders, 168 families, 103 subfamilies, 1421 genera, 68 subgenera, 6590 species.

Virus Taxonomy: 2020 Release

6 realms, 10 kingdoms, 17 phyla, 2 subphyla, 39 classes, 59 orders, 8 suborders, 189 families, 136 subfamilies, 2224 genera, 70 subgenera, 9110 species.

Species names generally take the form of *[Disease] virus*.

Baltimore classification



The Baltimore Classification of viruses is based on the method of viral mRNAsynthesis.

The Nobel Prize-winning biologist David Baltimore devised the Baltimore classification system. The ICTV classification system is used in conjunction with the Baltimore classification system in modern virus classification.

The Baltimore classification of viruses is based on the mechanism of mRNA production. Viruses must generate mRNAs from their genomes to produce proteins and replicate themselves, but different mechanisms are used to achieve this in each virus family. Viral genomes may be single-stranded (ss) or double-stranded (ds), RNA or DNA, and may or may not use reverse transcriptase (RT). In addition, ssRNA viruses may be either sense (+) or antisense (−). This classification places viruses into seven groups:

- I: **dsDNA viruses** (e.g. Adenoviruses, Herpesviruses, Poxviruses)
- II: **ssDNA viruses** (+)sense DNA (e.g. Parvoviruses)
- III: **dsRNA viruses** (e.g. Reoviruses)
- IV: **(+)ssRNA viruses** (+)sense RNA (e.g. Picornaviruses, Togaviruses)
- V: **(−)ssRNA viruses** (−)sense RNA (e.g. Orthomyxoviruses, Rhabdoviruses)
- VI: **ssRNA-RT viruses** (+)sense RNA with DNA intermediate in life-cycle (e.g. Retroviruses)
- VII: **dsDNA-RT viruses** (e.g. Hepadnaviruses)

As an example of viral classification, the chicken pox virus, varicella zoster (VZV), belongs to the order Herpesvirales, family *Herpesviridae*, subfamily*Alphaherpesvirinae*, and genus *Varicellovirus*. VZV is in Group I of the Baltimore Classification because it is a dsDNA virus that does not use reverse transcriptase.

For some RNA viruses, the infecting RNA produces messenger RNA (mRNA). This is translation of the genome into protein produces. For others with negative stranded RNA and DNA, viruses are produced by transcription then translation.

The mRNA is used to instruct the host cell to make virus components. The virus takes advantage of the existing cell structures to replicate itself.

This classification depend on the virus replication

Group I: Double-stranded DNA viruses

dsDNA virus

These types of viruses usually must enter the host nucleus before it is able to replicate. Furthermore, these viruses require host cell polymerases to replicate the viral genome and, hence, are highly dependent on the cell cycle. Proper infection and production of progeny requires that the cell be in replication, as it is during replication that the cell's polymerases are active. The virus may induce the cell to forcefully undergo cell division, which may lead to transformation of the cell and, ultimately, cancer. Examples include Herpesviridae, Adenoviridae, and Papovaviridae.

There is only one well-studied example in which a class 1 virus is not replicating within the nucleus: the Poxvirus family, a highly pathogenic virus that infects vertebrates and includes the smallpox virus.

The mRNA is transcribed in the normal way from viral DNA using the host transcriptase enzymes, into two types of mRNA's: 1) early mRNA, transcribed prior to the synthesis of viral DNA, and 2) late mRNA, transcribed from progeny DNA.

Group II: Single-stranded DNA viruses

ssDNA virus

Viruses in this category include the Anelloviridae, Circoviridae, and Parvoviridae (which infect vertebrates), the Geminiviridae and Nanoviridae (which infect plants), and the Microviridae (which infect prokaryotes). Most of them have circular genomes (the parvoviruses are the only known exception). Eukaryote-infecting viruses replicate mostly within the nucleus - usually via a rolling circle mechanism, forming double-stranded DNA intermediate in the process. A prevalent but asymptomatic human Anellovirus, called Transfusion Transmitted Virus (TTV), is included within this classification.

Group III: Double-stranded RNA viruses

dsRNA virus

As with most RNA viruses, this class replicates in the "Core" capsid that is in cytoplasm, not having to use the host replication polymerases to as much a degree as DNA viruses. This family is also not as well-studied as the rest and includes 2 major families, the Reoviridae and Birnaviridae. Replication is monocistronic and includes individual, segmented genomes, meaning that each of the genes codes for only one protein, unlike other viruses that exhibit more complex translation.

Group IV & V: Single-stranded RNA viruses

The ssRNA viruses belong to Class IV or V of the Baltimore classification. They could be grouped into negative sense or positive sense according to the sense of polarity of RNA. The single stranded RNA is the common feature of these viruses. The replication of viruses happens in the cytoplasm. Class IV and V ssRNA viruses do not depend as heavily as DNA viruses on the cell cycle.

Group IV: Single-stranded RNA viruses - Positive-sense

positive-sense ssRNA virus

The positive-sense RNA viruses and indeed all RNA defined as **positive-sense** can be directly accessed by host ribosomes to immediately form proteins. These can be divided into two groups, both of which reproduce in the cytoplasm:

- Viruses with polycistronic mRNA where the genome RNA forms the mRNA and is translated into a polyprotein product that is subsequently cleaved to form the mature proteins. This means that the gene can utilize a few methods in which to produce proteins from the same strand of RNA, all in the sake of reducing the size of its gene.
- Viruses with complex transcription, for which subgenomic mRNAs, ribosomal frameshifting, and proteolytic processing of polyproteins may be used. All of which are different mechanisms with which to produce proteins from the same strand of RNA.

Examples of this class include the families Astroviridae, Caliciviridae, Coronaviridae, Flaviviridae, Picornaviridae, Arteriviridae, and Togaviridae.

Group V: Single-stranded RNA viruses - Negative-sense
negative-sense ssRNA virus

The negative-sense RNA viruses and indeed all genes defined as **negative-sense** cannot be directly accessed by host ribosomes to immediately form proteins. Instead, they must be transcribed by viral polymerases into a "readable" form, which is the positive-sense reciprocal. These can also be divided into two groups:

- Viruses containing nonsegmented genomes for which the first step in replication is transcription from the (-)-stranded genome by the viral RNA-dependent RNA polymerase to yield monocistronic mRNAs that code for the various viral proteins. A positive-sense genome copy is then produced that serves as template for production of the (-)-strand genome. Replication is within the cytoplasm.
- Viruses with segmented genomes for which replication occurs in the nucleus and for which the viral RNA-dependent RNA polymerase produces monocistronic mRNAs from each genome segment. The largest difference between the two is the location of replication.

Examples in this class include the families Arenaviridae, Orthomyxoviridae, Paramyxoviridae, Bunyaviridae, Filoviridae, and Rhabdoviridae (the latter which includes rabies).

Group VI: Positive-sense single-stranded RNA viruses that replicate through a DNA intermediate
ssRNA-RT virus

A well-studied family of this class of viruses include the retroviruses. One defining feature is the use of reverse transcriptase to convert the positive-sense RNA into DNA. Instead of using the RNA for templates of proteins, they use DNA to create the templates, which is spliced into the host genome using integrase. Replication can then commence with the help of the host cell's polymerases.

Group VII: Double-stranded DNA viruses that replicate through a single-stranded RNA intermediate
dsDNA-RT virus

This small group of viruses, exemplified by the Hepatitis B virus (which is in the Hepadnaviridae family), have a double-stranded, gapped genome that is subsequently filled in to form a covalently closed circle (cccDNA) that serves as a template for production of viral mRNAs and a subgenomic RNA. The pregenome RNA serves as template for the viral reverse transcriptase for production of the DNA genome.

Holmes classification

Holmes (1948) used Carl Linnaeus's system of binomial nomenclature to classify viruses into 3 groups under one order, Virales. They are placed as follows:

- **Group I:** *Phaginae* (attacks bacteria)
- **Group II:** *Phytophaginae* (attacks plants)
- **Group III:** *Zoophaginae* (attacks animals)

Viral Multiplication

Viruses do not contain enzymes for energy production or protein synthesis.

For a virus to multiply, it must invade a host cell and direct the host's metabolic machinery to produce viral enzymes, viral proteins, and copies of its nucleic acid, using the host cell's ATP to power the reactions (fig).

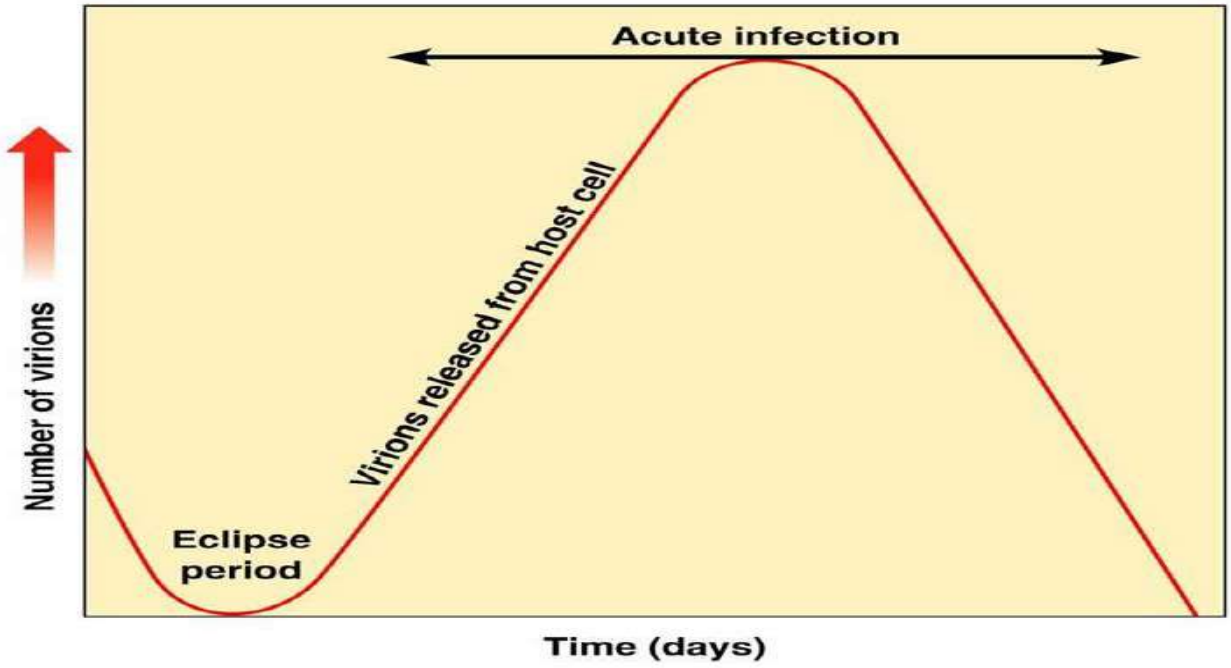


Fig.: Stages of viral multiplication in relation to the number of released virions.

Viral particles disappear upon penetration, none are seen during biosynthesis and assembly, and eventually all cells die so no new virions can be produced.

The **eclipse period** is the period when all viral particles are present but before they are assembled.

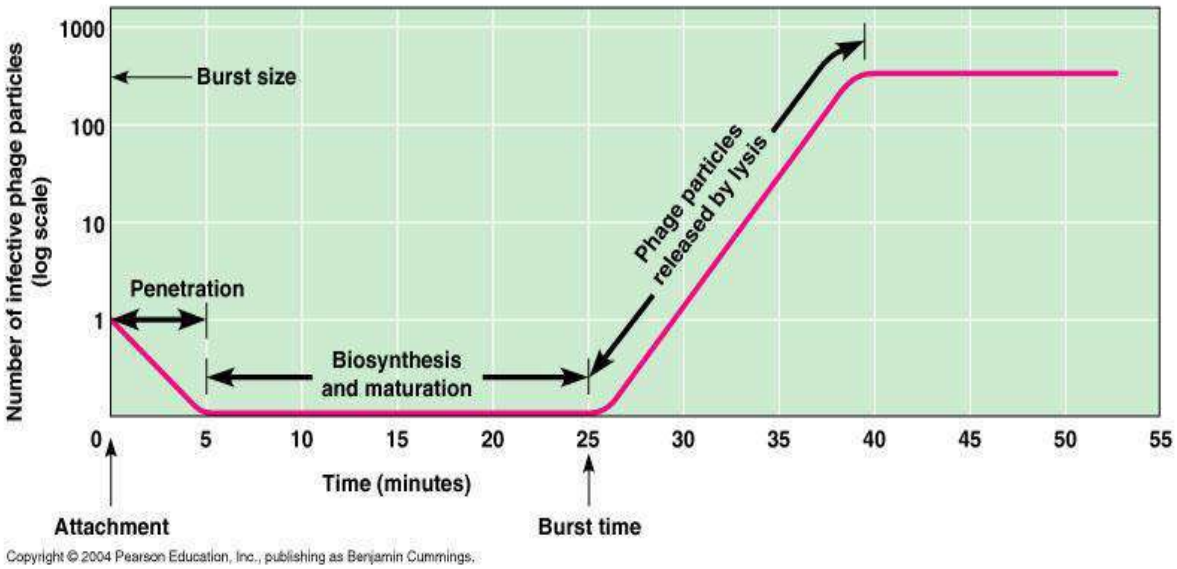


Fig.: The number of infective particles in relation to time

Burst time is the time from phage adsorption to release.

Burst size is the number of newly synthesized phages produced from one infected cell.

Multiplication of Bacteriophages

The virus may cause lysis or lysogeny.

Lytic cycle:

1- Attachment or adsorption: Requires a receptor.
2- Penetration: release lysozyme to break down a portion of the cell wall. The tail sheath contracts and the tail core is driven through the hole in the wall to the plasma membrane. The viral genome is then injected into the bacterium.
3- Biosynthesis: Viral DNA and proteins are synthesized. Host protein synthesis is stopped by degradation of host DNA, interference with transcription, or repression of translation.
4- Maturation: During maturation or assembly phage DNA and capsids are assembled into complete viruses.
5- Release: Release occurs when phage lysozyme breaks down the cell wall and newly synthesized phage particles are released.

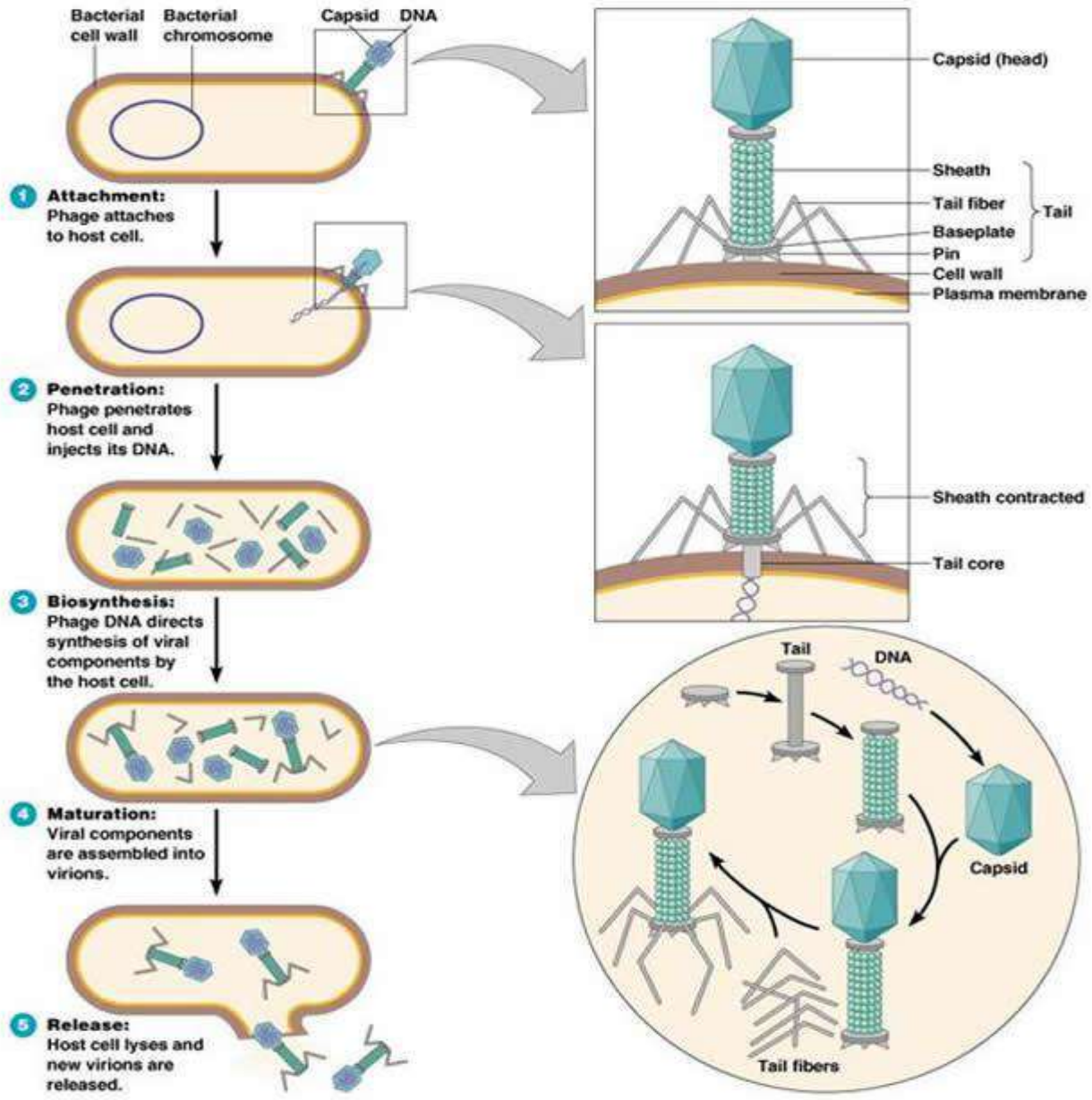
Lysogeny:

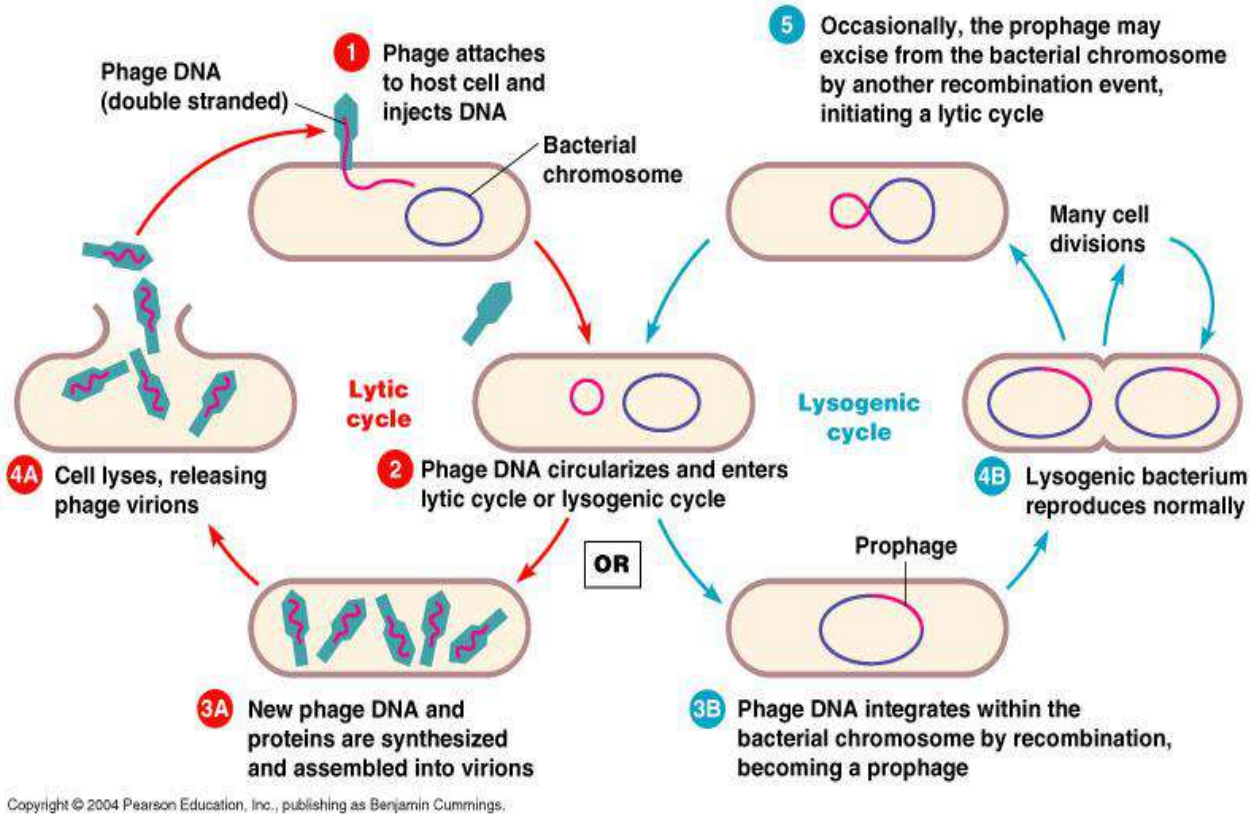
is a cycle in which the phage DNA recombines with the bacterial chromosome.

The incorporated viral DNA is now a prophage.

The prophage genes are regulated by a repressor coded for by the prophage, the prophage is replicated each time the host DNA is replicated.

Exposure to mutagens can lead to excision of the prophage and initiation of the lytic cycle.





Outcomes of lysogeny

Bacterium can't be reinfected by the same kind of phage.

Host cell may exhibit new properties due to viral genes carried on the prophage

Specialized transduction - host cell may gain new bacterial genes packaged with the phage

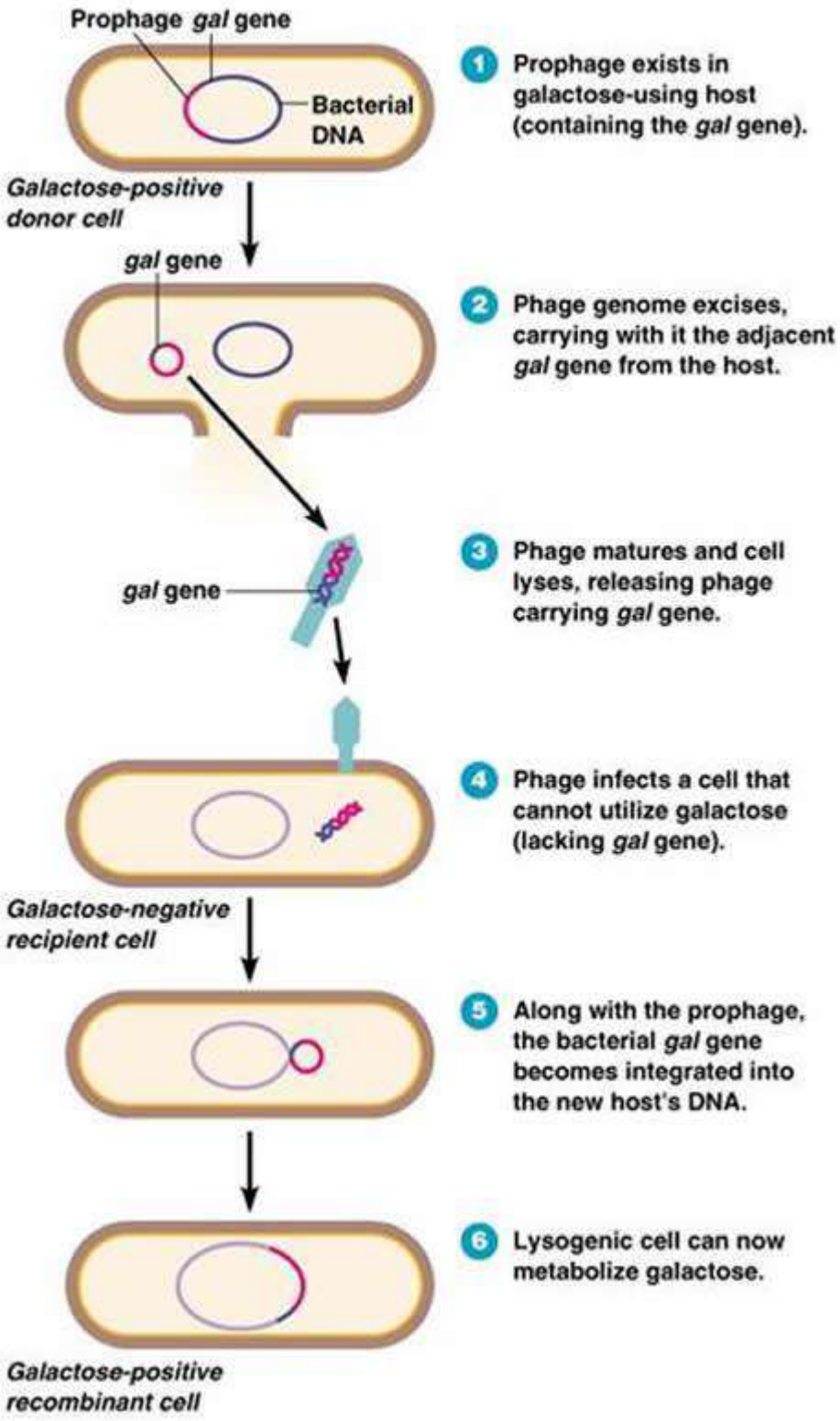

Prophage gal gene
Bacterial DNA
Galactose-positive donor cell
gal gene
gal gene
Galactose-negative recipient cell
Galactose-positive recombinant cell
1 Prophage exists in galactose-using host (containing the gal gene).
2 Phage genome excises, carrying with it the adjacent gal gene from the host.
3 Phage matures and cell lyses, releasing phage carrying gal gene.
4 Phage infects a cell that cannot utilize galactose (lacking gal gene).
5 Along with the prophage, the bacterial gal gene becomes integrated into the new host's DNA.
6 Lysogenic cell can now metabolize galactose.

TABLE 13.3 Bacteriophage and Viral Multiplication Compared

Stage	Bacteriophages	Animal Viruses
Attachment	Tail fibers attach to cell wall proteins	Attachment sites are plasma membrane proteins and glycoproteins
Entry	Viral DNA injected into host cell	Capsid enters by endocytosis or fusion
Uncoating	Not required	Enzymatic removal of capsid proteins
Biosynthesis	In cytoplasm	In nucleus (DNA viruses) or cytoplasm (RNA viruses)
Chronic infection	Lysogeny	Latency; slow viral infections; cancer
Release	Host cell lysed	Enveloped viruses bud out; nonenveloped viruses rupture plasma membrane

Multiplication of Animal Viruses

Multiplication process that is shared by both DNA and RNA containing animal cell, viral genome contain information that: ensure the replication of the genome, ensure packing of genomes in to virion, and alter the structure and or function of the host cell to greater or lesser degree.

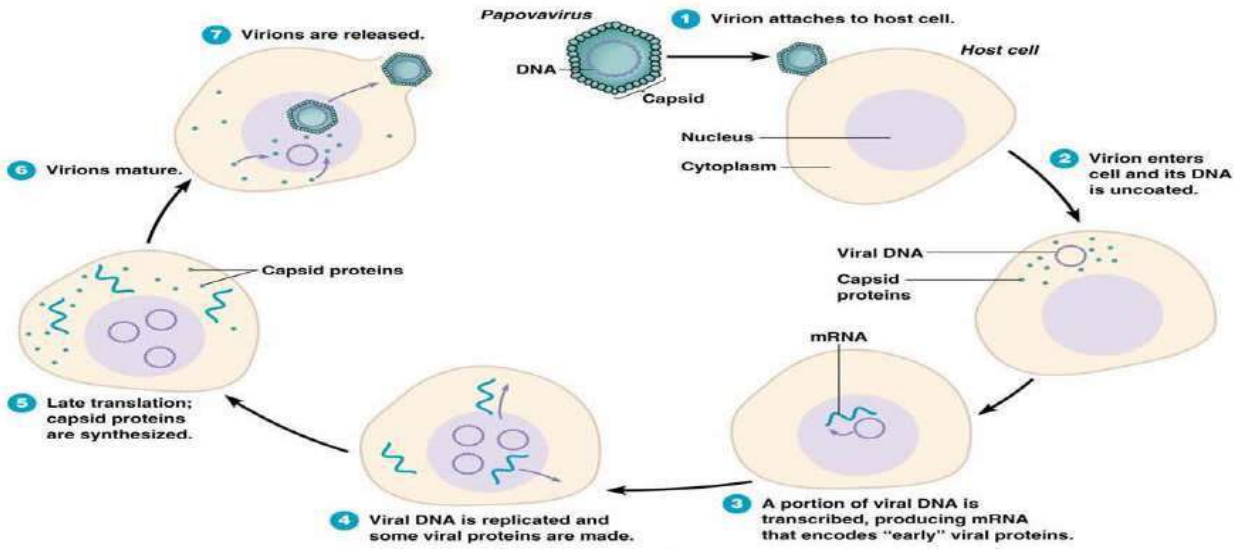


Fig.: Main steps in viral multiplication cycle.

1- Attachment or adsorption

The virus becomes attached to the cells, and at this stage, it can be recovered in the infectious form without cell lysis by procedures that either destroy the receptors or weaken their bonds to the virions. Animal viruses have specialized attachment sites distributed over the surface of the virion e.g. orthomyxoviruses and paramyxoviruses attach through glycoprotein spikes, and adenoviruses attach through the penton fibers. Adsorption occurs to specific cellular receptors. Some receptors are glycoproteins, others are phospholipids or glycolipids. These are usually macromolecules with specific physiological functions, such as complement receptors for EBV. Whether or not receptors for a certain virus are present on a cell depends on the species, the tissue and its physiological state.

Cells lacking specific receptors are resistant. Attachment is blocked by antibodies that bind to the viral or cellular sites involved.

Viral attachment protein receptors (attachment sites)

Influenza haemagglutinin sialoglycosaccharides. HIV envelope glycoprotein gp120 CD4 (T-helper). Some complex viruses (pox virus) may have more than one receptor, therefore there may be alternative routes of virus uptake in to cells.

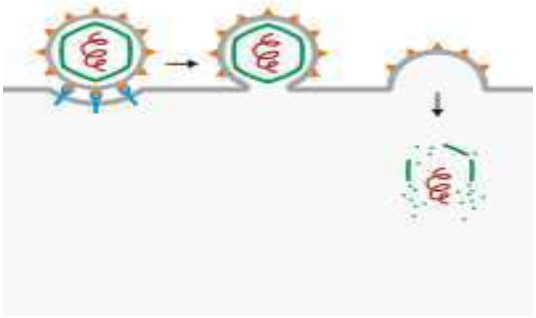
2-Penetration

a) Endocytosis (pinocytosis) - togavirus

b) Fusion - herpesvirus

c) **Entry via Genetic Injection**

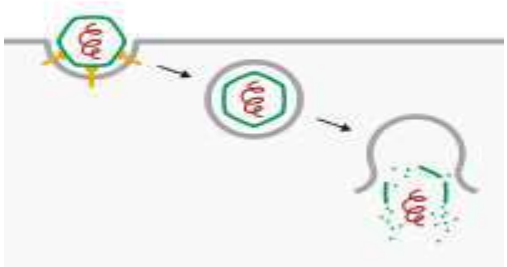
a) Entry via Membrane Fusion



Viral entry via membrane fusion.

The most well-known example is through membrane fusion. In viruses with a viral envelope, viral receptors attach to the receptors on the surface of the cell and secondary receptors may be present to initiate the puncture of the membrane or fusion with the host cell. Following attachment, the viral envelope fuses with the host cell membrane, emptying the now-bare virus into the cell. In essence, the virus's envelope "blends" with the host cell membrane, releasing its contents into the cell. Obviously, this can only be done with viruses that have an envelope (examples of such enveloped viruses include HIV and herpes simplex virus.

b) Entry via Endocytosis

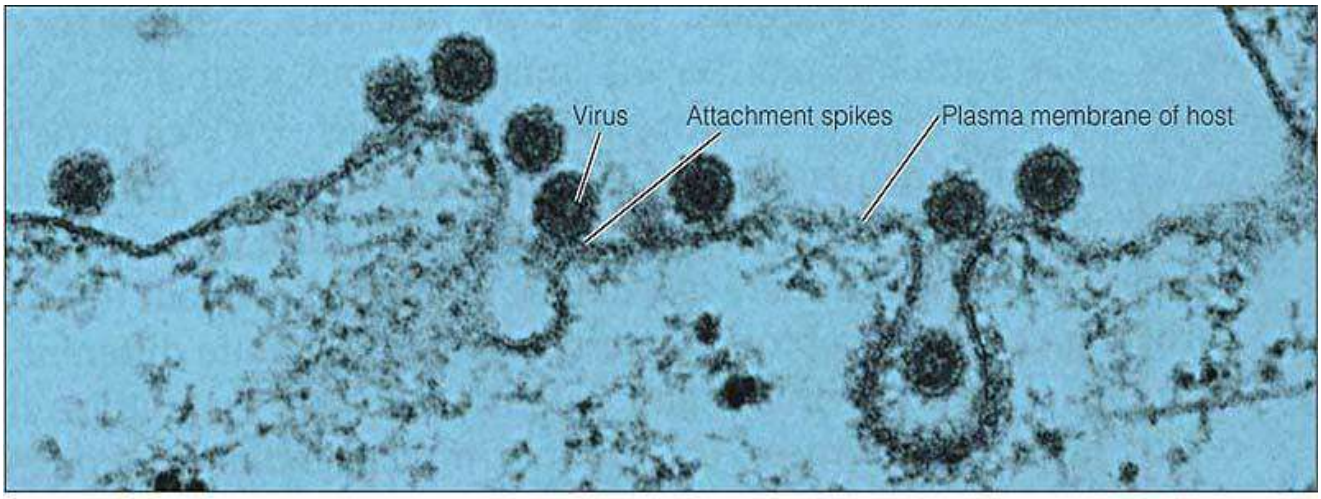


Viral entry via endocytosis.

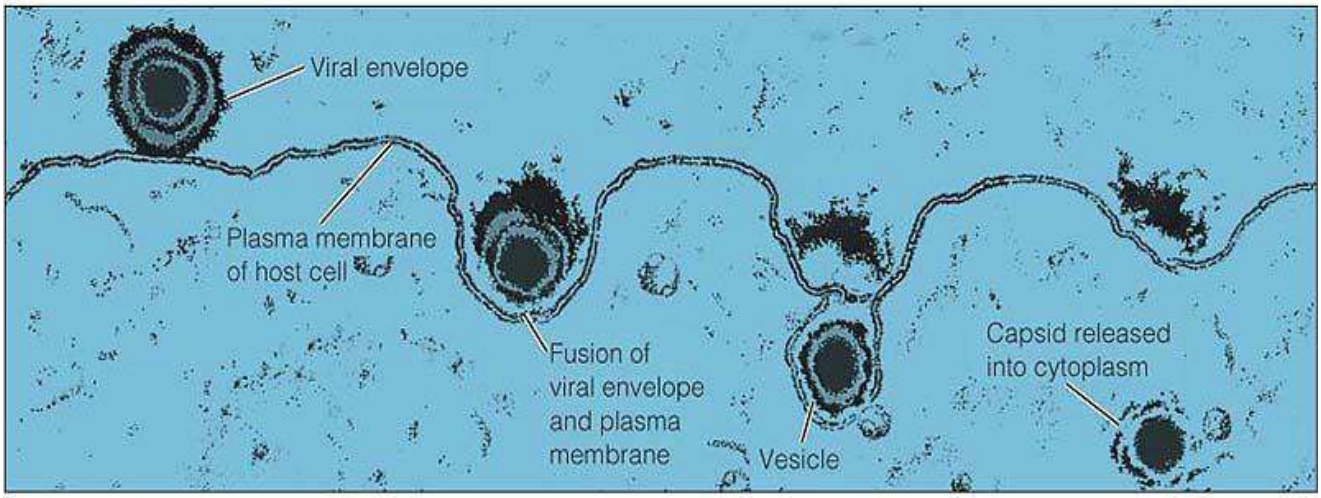
Viruses with no viral envelope enter the cell through endocytosis; they are ingested by the host cell through the cell membrane. In essence, the virus tricks the cell into thinking that the virus knocking at the door is nothing more than nutrition or harmless goods. A cell, which naturally takes in resources from the environment by attaching goods onto surface receptors and bringing them into the cell, will engulf the virus. Once inside the cell, the virus must now break out of the vesicle by which it was taken up in order to gain access to the cytoplasm. Examples include the poliovirus, Hepatitis C virus[5] and Foot-and-mouth disease virus.

c) Entry via Genetic Injection

A third and more specific example, is by simply attaching to the surface of the cell via receptors on the cell, and injecting only its genome into the cell, leaving the rest of the virus on the surface. This is restricted to viruses in which only the gene is required for infection of a cell (most positive-sense, single-stranded RNA viruses because they can be immediately translated) and further restricted to viruses that actually exhibit this behavior. The best studied example includes the bacteriophages; for example, when the tail fibers of the T2 phage land on a cell, its central sheath pierces the cell membrane and the phage injects DNA from the head capsid directly into the cell.



(a) Entry of togavirus



(b) Entry of herpesvirus

3- Uncoating

With some viruses, the genome is completely released from the capsid during or after penetration. This is known as "uncoating". In others, such as retroviruses and reoviruses, the first stages of the viral replication cycle (transcription, replication) actually occur inside the capsid. These capsids have undergone some conformational changes during infection that allow viral gene expression and/or replication to begin, and the resulting structures are sometimes known as partially uncoated particles. Since almost all DNA viruses replicate in the nucleus of infected cells, they must be targeted there. In many cases the entire nucleocapsid enters the nucleus, where uncoating then takes place.

Uncoating is a general term for the events which occur after penetration, in which the capsid is removed and the virus genome exposed, usually in the form of a nucleoprotein complex. This might be relatively simple in structure, e.g: ***Picornaviruses*** have a small basic protein of ~23 amino acids (VpG) covalently attached to the 5' end of the vRNA genome; or highly complex: ***Retrovirus*** cores are highly ordered nucleoprotein complexes which contain, in addition to the diploid RNA genome,

the reverse transcriptase enzyme responsible for converting the viral RNA genome into the DNA ***PROVIRUS***. This process occurs inside the core particle. For viruses which replicate in the cytoplasm, e.g. Picornaviruses, the genome is simply released into the cell, but for viruses which replicate in the nucleus, e.g. Herpesviruses, the genome, often with associated nucleoproteins, must be transported through the nuclear membrane. This is achieved by interactions of the nucleoproteins (or capsid) with the cytoskeleton. At the nuclear pores, the capsid is stripped off, and the genome passes into the nucleus.

Uncoating of viral nucleic acid may be accomplished by host or viral enzymes. Bacteriophages don't require uncoating because their nucleic acid is injected into the host cell.

4- Biosynthesis

Biosynthesis of DNA viruses

TABLE 13.4 The Biosynthesis of DNA and RNA Viruses Compared

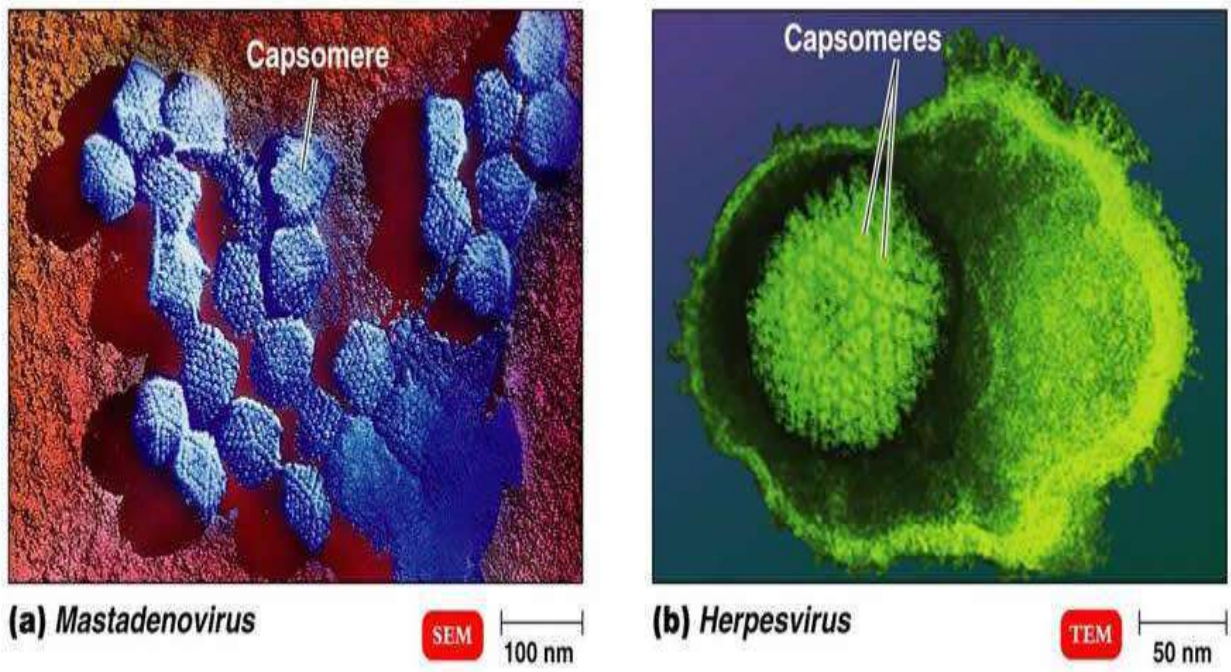
Viral Nucleic Acid	Virus Family	Special Features of Biosynthesis
DNA, single-stranded	Parvoviridae	Cellular enzyme transcribes viral DNA in nucleus
DNA, double-stranded	Herpesviridae Papovaviridae	Cellular enzyme transcribes viral DNA in nucleus
	Poxviridae	Viral enzyme transcribes viral DNA in virion, in cytoplasm
DNA, reverse transcriptase	Hepadnaviridae	Cellular enzyme transcribes viral DNA in nucleus; reverse transcriptase copies mRNA to make viral DNA
RNA, + strand	Picornaviridae Togaviridae	Viral RNA functions as a template for synthesis of RNA polymerase which copies − strand RNA to make mRNA in cytoplasm
RNA, − strand	Rhabdoviridae	Viral enzyme copies viral RNA to make mRNA in cytoplasm
RNA, double-stranded	Reoviridae	Viral enzyme copies − strand RNA to make mRNA in cytoplasm
RNA, reverse transcriptase	Retroviridae	Viral enzyme copies viral RNA to make DNA in cytoplasm; DNA moves to nucleus

DNA of most DNA viruses is released into the nucleus of the host cell.

Transcription and translation of early genes produces enzymes to reproduce viral DNA

Transcription and translation of late genes produces capsid proteins in the cytoplasm.

DNA-containing animal viruses



Some examples of DNA viruses:

Adenoviridae - from adenoids, cause respiratory diseases.

Poxviridae - pox refers to the pus-filled lesions that accompanies the diseases caused by these viruses

Herpesviridae - named after spreading (herpetic) appearance of cold sores

Papoviridae - named for ***pa***pillomas (warts), ***po***lyomas (tumors), and ***va***cuolation (cytoplasmic vacuoles)

Hepadnaviridae - name comes from the fact that they cause ***hepa***titis and contain ***DNA***.

Biosynthesis of RNA Viruses

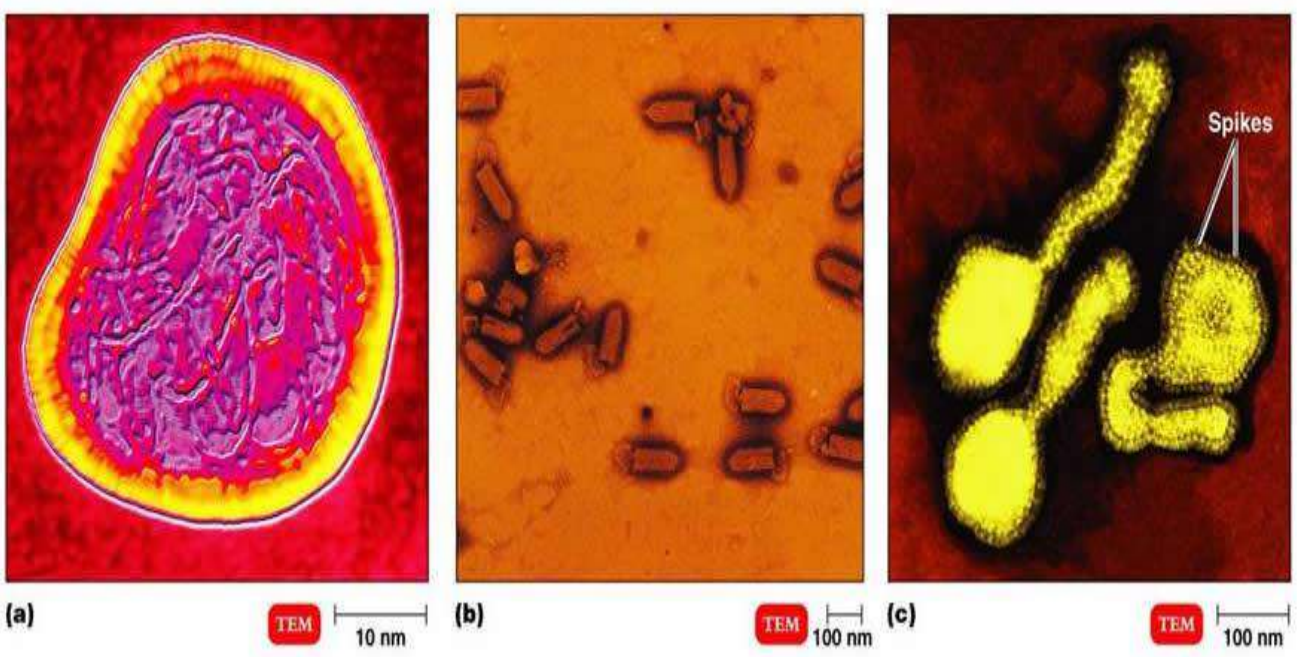
RNA viruses multiply in the cytoplasm. RNA-dependent RNA polymerase synthesizes a double-stranded RNA.

Sense strand (+ strand) can act as mRNA directly and as a template for antisense strand (- strand) synthesis.

ss + strand RNA viral replication: The viral genome, a single stranded sense strand, is transcribed to make antisense (-) strands. The antisense strands serve as the template for making mRNA (sense, or + strands), which code for viral proteins and serve as the viral genome that is packaged inside the capsid during assembly.

ss - strand RNA viral replication: The viral genome, a single stranded antisense strand, is transcribed to make sense (+) strands, which serve as mRNA to code for viral proteins and also as a template to make more copies of the viral genome, single stranded antisense (-) strands, which will be packaged inside the capsid during assembly.

ds +/- RNA viral replication: transcription of - strand makes more copies of the + strand, which serves as mRNA. Transcription of the + strand provides viral protiens (including RNA-directed RNA polymerase) and more copies of - strand, which is packaged along with the complementary + strands in the capsid during assembly.



Picornaviridae - some of the smallest viruses (pico-); contain RNA, name comes from pico + RNA. Single stranded + strand viruses.

Example: poliovirus

Togaviridae - enveloped, name comes from toga (covering). Single stranded + strand viruses - transcription of a - strand serves as a template, the + strands transcribed from the - strand template are produced as a short strand mRNA that codes for envelope proteins and a long strand mRNA that codes for capsid proteins.

Examples: *Art*hropod-*bo*rne *arbo*viruses or alphaviruses which cause viral encephalitis.

Rhabdoviridae - Rhabdo- is from the Greek for rod (they're really more bullet shaped). Single stranded - strand viruses.

Example: *Lyssavirus* (rabiesvirus)

Reoviridae - named for habitat, respiratory and enteric tract. Before they were associated with disease they were considered orphan viruses, name comes from ***r***espiratory, ***e***nteric, and ***o***rphan. Double stranded RNA viruses.

Example: Rotavirus

Retroviridae - *Lentivirus* (HIV-1, HIV-2, HTLV-1, HTLV-2)

Multiplication of Retroviruses

Retroviruses use reverse transcriptase (RNA-dependent DNA polymerase) to transcribe DNA from RNA.

Both viral RNA strands are + strands (making the virus diploid, how about that?) which are transcribed by reverse transcriptase to make complementary DNA strands.

The original viral RNA is degraded and the DNA copies integrate into the host cell's genome.

5-Maturation or Assembly: **Release**

A) Rupture – naked viruses
B) Budding – enveloped viruses

ASSEMBLY

Involves the assembly of all the components necessary for the formation of the mature virion at a particular site in the cell. During this process, the basic structure of the virus is formed. The site of assembly varies for different viruses, e.g: Picornaviruses, Poxviruses, Reoviruses - In the ***cytoplasm***. Adenoviruses, Papovaviruses, Parvoviruses - In the ***nucleus***. Retroviruses - On the ***inner surface of the cell membrane***.

RELEASE

For lytic viruses (most ***non-enveloped*** viruses), release is a simple process - the cell breaks open and releases the virus. ***Enveloped*** viruses acquire the lipid membrane as the virus buds out through the cell membrane. Virion envelope proteins are picked up during this process as the virus is extruded. Budding may or may not kill the cell, but is controlled by the virus - the physical interaction of the capsid proteins on the inner surface of the cell membrane forces the particle out through the membrane:

MATURATION

The stage of the life-cycle at which the virus becomes infectious. Usually involves structural changes in the particle, often resulting from specific cleavage of capsid proteins to form the mature products, which frequently leads to a conformational change in the capsid, or the condensation of nucleoproteins with the genome. For some viruses, assembly and maturation are inseparable, whereas for others, maturation may occur after the virus particle has left the cell

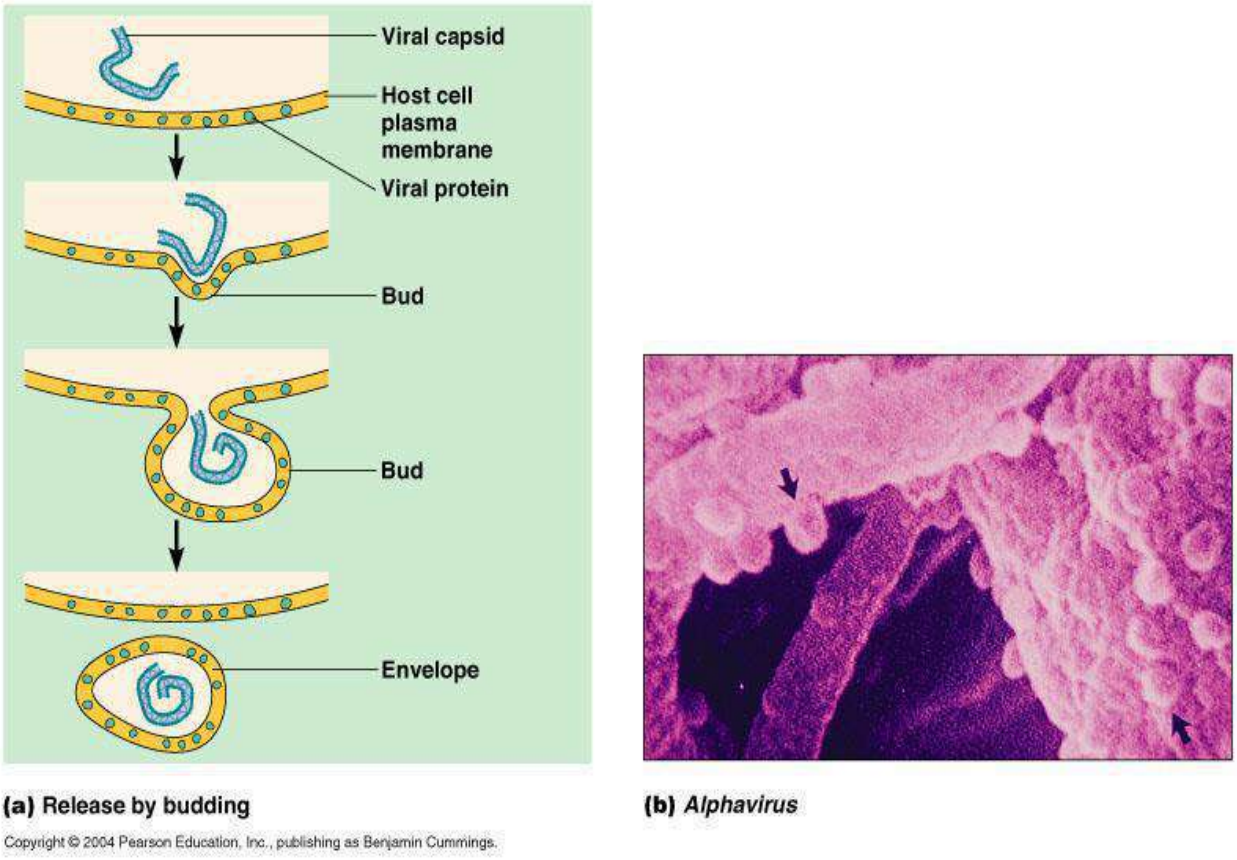


Fig.: The budding process to release virus after cell infection.

Gene expression:

Gene expression is divided in to 2 phases:

1- The **early genes** that code **for early proteins as** enzymes and regulatory proteins needed to start viral replication as well as proteins that regulate host cell RNA and protein synthesis, these early proteins have important regulatory functions in controlling the transcription.
   a- Segmented genome transcribes to produce **monocistronic mRNA** which codes for 1 protein, so it can produce various proteins in different amounts as in paramyxoviridae and rhabdoviridae.
   b- Non segmented genome produce **polycistronic mRNA**, which is transcribed from the whole of viral genome and translated to polyprotein then cleaved by proteolytic cleavage

to form mature product. In other cases the polycistronic mRNA may be subsequently cleaved enzymatically in to monocistronic mRNA.

2- **Late genes** code for structural proteins (**Late proteins**), that are needed for assembly of the mature virus; some of these proteins may be as regulatory protein that modulate the transcription and translation of cellular or early viral genes.

Transfection

Transfection is the process of inserting genetic material, such as DNA and double stranded RNA, into mammalian cells. The insertion of DNA into a cell enables the expression, or production, of proteins using the cells own machinery, whereas insertion of RNA into a cell is used to down-regulate the production of a specific protein by stopping translation. While the site of action for transfected RNA is the cytoplasm, DNA must be transported to the nucleus for effective transfection. There, the DNA can be transiently expressed for a short period of time, or become incorporated into the genomic DNA, where the change is passed on from cell to cell as it divides.

Interaction among viruses

When two or more viral particles are infecting the same host cell, they may interact in a variety of ways. For more type of interaction to occur, viruses must be sufficiently closely related, usually within the same family.

A) Genetic interaction: result in some progeny that are heritably (genetically) different from either parents. The actual nucleic acid molecules interact.

1- Recombination: Is a physical interaction of viral genome in the mixed infected cell. The results of recombination are progeny genomes that contain genetic information in non- parental combination. The classic mechanism is that the nucleic acid strand break and part of the genome of one parent is joined to part of the second parent. The recombinant virus is genetically stable yielding progeny like itself upon replication. This kind of break/ join recombination is common in DNA viruses or those RNA viruses which have a DNA phase (retroviruses). Viruses with non- segmented ssRNA genome don't recombinant.

2- Genetic reactivation: This phenomenon represents a special case of recombinant.

a- Marker rescue: This occurs between the genome of an active virion and the genome of inactivated viral particle, so that certain markers of inactivated parents

are rescue and appear in the viable progeny. None of the progeny produced are identical to the inactivated parents.

b- Multiplicity reactivation: Occurs when many in active viral particles interact in the same cell to generate a viable virus. Recombination occurs between that damaged nucleic acid of the parents, producing a viable genome that can replicate.

B) Non- genetic interaction: In non genetic interaction the product of the gene are involved and this can be happened by:

1- Complementation: This refers to the interaction of viral gene products in cells infected with two viruses, one or both of which may be defective. It result in the replication of one or both under conditions in which replication would not ordinarily occur. In this case one virus provides a gene product in which the second is defective allowing the second virus to grow and the gene types of the tow viruses remain unchanged.

2- Phenotype mixing: If tow 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. It involves no alteration in genetic material, then progeny of such virion will be determined by which parental genome is packaged and not by the nature of the envelope. Phenotypic mixing may occur between related viruses or between genetically unrelated viruses. We can also get the situation where a coat is entirely that of another virus. This kind of phenotypic mixing is sometimes reffered to as pseudotype (pseudovirion) formation.

3- Interference: Infection of either cultures or animals with tow viruses often leads to an inhibition of multiplication of one of the viruses. Interference does not occur withal viral combination. Several mechanisms have been suggested as causes of interference:

a- One virus may inhibit the ability of the second virus to adsorb to the cell either by blocking its receptors for ex. Enteroviruses or by destroying its receptors such as in Orthomyxoviruses.

b- One virus may compete with the second virus for components of the replication apparatus (ex. Polymerase).

c- The first virus may induce the infected cell to produce an inhibitor such as Interferon which prevents replication of the second virus.

When interference occurs between unrelated viruses it's called (Heterologous interference). But when it occurs between related viruses it's called (Homologous interference), and when virus interference with their own replication due to defective interfering particle this called (Autointerference).

Defective viruses

A defective virus is one that lack one or more functional genes required for viral replication. Defective viruses required helper activity from another virus for some step in replication or maturation.

Types of defective viruses:

1- Deletion mutant: viruses lack a portion of its genome.

Spontaneous deletion mutants may interfere with the replication of homologous virus and are called defective interfering virus particles (DIP) have lost essential segments of genome but contain normal capsid protein; they required virus as helper for replication.

2- Viruses that required an unrelated replication competent viruses as helper ex. Hepatitis D viruses' replication only in the presence of HBV.

3- Pseudovirions: A different type of defective particle, contain host DNA rather than the viral genome.

Viral mutations

Types of viral mutations:

Mutants can be **point mutants** (one base replace by another) or **insertion/ deletion mutants.** Good mutants resulting from single mutation that are easily scored and reasonably stable.

A- Spontaneous mutation: These arise naturally during viral replication (ex. Due to errors by the genome- replicating polymerase).

B- Induced mutation: Mutations that are induced by certain mutagen (physical agents such as UV- light or X-ray or chemical agents ex. Nitrous acid acting directly on the bases).

C- Engineered mutation: Molecular techniques now make it possible to introduce nearly any type of mutation into many viruses.

Examples of kind of phenotypic changes seen in viruses mutants:

1- **Conditional lethal mutants:** these mutants multiply under some conditions but not others whereas the wild- type virus grows under both sets of conditions (ex. Temperature sensitive mutants; these will grow at low temperature 31^{o}C but not at 39^{o} C wild- type grows at 31 and 39 ^{o}C. The reason for this is altered protein cannot maintain a function at elevated temp.).

2- **Drug resistance,** enzyme- deficient, plaque size, hot, attenuated mutants.

Pathogenesis

Pathogenesis is the process by which an infection leads to disease. Pathogenic mechanisms of viral disease include (1) implantation of virus at the portal of entry, (2) local replication, (3) spread to target organs (disease sites), and (4) spread to sites of shedding of virus into the environment.

Most viral infections are subclinical, suggesting that body defences against viruses arrest most infections before disease symptoms become manifest. Knowledge of subclinical infections comes from serologic studies showing that sizeable portions of the population have specific antibodies to viruses even though the individuals have no history of disease. These unapparent infections have great epidemiologic importance: they constitute major sources for dissemination of virus through the population, and they confer immunity.

Many factors affect pathogenic mechanisms.

1-An early determinant is the extent to which body tissues and organs are accessible to the virus. Accessibility is influenced by physical barriers (such as mucus and tissue barriers), by the distance to be traversed within the body, and by natural defense mechanisms. If the virus reaches an organ, infection occurs only if cells capable of supporting virus replication are present.

2-Cellular susceptibility requires a cell surface attachment site (receptor) for the virions and also an intracellular environment that permits virus replication and release. Even if virus initiates infection in a susceptible organ, replication of sufficient virus to cause disease may be prevented by host defenses.

3- Other factors that determine whether infection and disease occur are the many virulence characteristics of the infecting virus. To cause disease, the infecting virus must be able to overcome the inhibitory effects of physical barriers, distance, host defences, and differing cellular susceptibilities to infection. The inhibitory effects are genetically controlled and therefore may vary among individuals and races. Virulence characteristics enable the virus to initiate infection, spread in the body, and replicate to large enough numbers to impair the target organ. These factors include the ability to replicate under certain circumstances during inflammation, during the febrile response, in migratory cells, and in the presence of natural body inhibitors and interferon. Extremely virulent strains often occur within virus populations. Occasionally, these strains become dominant as a result of unusual

selective pressures. The viral proteins and genes responsible for specific virulence functions are only just beginning to be identified.

Fortunately for the survival of humans and animals (and hence for the infecting virus), most natural selective pressures favour the dominance of less virulent strains. Because these strains do not cause severe disease or death, their replication and transmission are not impaired by an incapacitated host. Mild or unapparent infections can result from absence of one or more virulence factors. For example, a virus that has all the virulence characteristics except the ability to multiply at elevated temperatures is arrested at the febrile stage of infection and causes a milder disease than its totally virulent counterpart. Live virus vaccines are composed of viruses deficient in one or more virulence factors; they cause only unapparent infections and yet are able to replicate sufficiently to induce immunity.

The occurrence of spontaneous or induced mutations in viral genetic material may alter the pathogenesis of the induced disease, e.g. HIV. These mutations can be of particular importance with the development of drug resistant strains of virus.

Disease does not always follow successful virus replication in the target organ. Disease occurs only if the virus replicates sufficiently to damage essential cells directly, to cause the release of toxic substances from infected tissues, to damage cellular genes or to damage organ function indirectly as a result of the host immune response to the presence of virus antigens.

As a group, viruses use all conceivable portals of entry, mechanisms of spread, target organs, and sites of excretion. This abundance of possibilities is not surprising considering the astronomic numbers of viruses and their variants

Cellular Pathogenesis

Direct cell damage and death from viral infection may result from (1) diversion of the cell's energy, (2) shutoff of cell macromolecular synthesis, (3) competition of viral mRNA for cellular ribosome, (4) competition of viral promoters and transcriptional enhancers for cellular transcriptional factors such as RNA polymerases, and inhibition of the interferon defence mechanisms. Indirect cell damage can result from integration of the viral genome, induction of mutations in the host genome, inflammation, and the host immune response.

Also, viruses cannot synthesize their genetic and structural components, and so they rely almost exclusively on the host cell for these functions. Their parasitic replication therefore robs the host cell of energy and macromolecular components,

severely impairing the host's ability to function and often resulting in cell death and disease.

Pathogenesis at the cellular level can be viewed as a process that occurs in progressive stages leading to cellular disease. As noted above, an essential aspect of viral pathogenesis at the cellular level is the competition between the synthetic needs of the virus and those of the host cell. Since viruses must use the cell's machinery to synthesize their own nucleic acids and proteins, they have evolved various mechanisms to subvert the cell's normal functions to those required for production of viral macromolecules and eventually viral progeny. The function of some of the viral genetic elements associated with virulence may be related to providing conditions in which the synthetic needs of the virus compete effectively for a limited supply of cellular macromolecule components and synthetic machinery, such as ribosome.

Tissue Tropism

Viral affinity for specific body tissues (tropism) is determined by (1) cell receptors for virus, (2) cell transcription factors that recognize viral promoters and enhancer sequences, (3) ability of the cell to support virus replication, (4) physical barriers, (5) local temperature, pH, and oxygen tension enzymes and non-specific factors in body secretions, and (6) digestive enzymes and bile in the gastrointestinal tract that may inactivate some viruses.

Many examples of viral tissue tropism are known. Polioviruses selectively infect and destroy certain nerve cells, which have a higher concentration of surface receptors for polioviruses than do virus-resistant cells. Rhinoviruses multiply exclusively in the upper respiratory tract because they are adapted to multiply best at low temperature and pH and high oxygen tension. Enteroviruses can multiply in the intestine, partly because they resist inactivation by digestive enzymes, bile, and acid. The cell receptors for some viruses have been identified. Rabies virus uses the acetylcholine receptor present on neurons as a receptor, and hepatitis B virus binds to polymerized albumin receptors found on liver cells. Similarly, Epstein-Barr virus uses complement CD21 receptors on B lymphocytes, and human immunodeficiency virus uses the CD4 molecules present on T lymphocytes as specific receptors.

To produce disease, virus most enter a host come in contact with susceptible cells, replicate and produce cell injury, host immune response, viral clearance or establishment of persistent infection and viral shedding.

Acute infection

A-Implantation at the Portal of Entry

Virions implant onto living cells mainly via the respiratory, gastrointestinal, skin-penetrating, and genital routes although other routes can be used. The final outcome of infection may be determined by the dose and location of the virus as well as its infectivity and virulence.

B- Local Replication and Local Spread

Successful implantation may be followed by local replication and local spread of virus .Virus that replicates within the initially infected cell may spread to adjacent cells extracellularly or intracellularly. Extracellular spread occurs by release of virus into the extracellular fluid and subsequent infection of the adjacent cell. Intracellular spread occurs by fusion of infected cells with adjacent, uninfected cells or by way of cytoplasmic bridges between cells. Most viruses spread extracellularly, but herpesviruses, paramyxoviruses, and poxviruses may spread through both intracellular and extra cellular routes. Intracellular spread provides virus with a partially protected environment because the antibody defense does not penetrate cell membranes.

Spread to cells beyond adjacent cells may occur through the liquid spaces within the local site (e.g., lymphatics) or by diffusion through surface fluids such as the mucous layer of the respiratory tract. Also, infected migratory cells such as lymphocytes and macrophages may spread the virus within local tissue.

Establishment of infection at the portal of entry may be followed by continued local virus multiplication, leading to localized virus shedding and localized disease. In this way, local sites of implantation also are target organs and sites of shedding in many infections. Respiratory tract infections that fall into this category include influenza, the common cold, and parainfluenza virus infections. Alimentary tract infections caused by several gastroenteritis viruses (e.g., rotaviruses and picornaviruses) also may fall into this category. Localized skin infections of this type include warts, cowpox, and molluscum contagious. Localized infections may spread over body surfaces to infect distant surfaces. An example of this is the picornavirus epidemic conjunctivitis; virus spreads directly from the eye (site of implantation) to the pharynx and intestine. Other viruses may spread internally to distant target organs and sites of excretion (disseminated infection). A third category of viruses may cause both local and disseminated disease, as in herpes simplex and measles.

C-Dissemination from the Portal of Entry

Viremic: The most common route of systemic spread from the portal of entry is the circulation, which the virus reaches via the lymphatics. Virus may enter the target organs from the capillaries by (1) multiplying in endothelial cells or fixed macrophages, (2) diffusing through gaps, and (3) being carried in a migrating leukocyte.

Neural: Dissemination via nerves usually occurs with rabies virus and sometimes with herpesvirus and poliovirus infections.

Incubation Period

The incubation period is the time between exposure to virus and onset of disease. During this usually asymptomatic period, implantation, local multiplication, and spread (for disseminated infections) occur.

During most virus infections, no signs or symptoms of disease occur through the stage of virus dissemination. Thus, the incubation period (the time between exposure to virus and onset of disease) extends from the time of implantation through the phase of dissemination, ending when virus replication in the target organs causes disease. Occasionally, mild fever and malaise occur during viremia, but they often are transient and have little diagnostic value.

The incubation period tends to be brief (1 to 3 days) in infections in which virus travels only a short distance to reach the target organ (i.e., in infections in which disease is due to virus replication at the portal of entry). Conversely, incubation periods in generalized infections are longer because of the stepwise fashion by which the virus moves through the body before reaching the target organs. Other factors also may influence the incubation period. Generalized infections produced by togaviruses may have an unexpectedly short incubation period because of direct intravascular injection (insect bite) of a rapidly multiplying virus. The mechanisms governing the long incubation period (months to years) of persistent infections are poorly understood. The persistently infected cell is often not lysed, or lysis is delayed. In addition, disease may result from a late immune reaction to viral antigen (e.g., arenaviruses in rodents), from unknown mechanisms in slow viral infections during which no immune response has been detected (as in the scrapie-kuru group), or mutation in the host genetic material resulting in cellular transformation and cancer.

D-Multiplication in Target Organs

Depending on the balance between virus and host defenses, virus multiplication in the target organ may be sufficient to cause disease and death.

Virus replication in the target organ resembles replication at other body sites except that (1) the target organ in systemic infections is usually reached late during the stepwise progression of virus through the body, and (2) clinical disease originates there. At each step of virus progression through the body, the local recovery mechanisms (local body defenses, including interferon, local inflammation, and local immunity) are activated. Thus, when the target organ is infected, the previously infected sites may have reached various stages of recovery.

Circulating interferon and immune responses probably account for the termination of viremia, but these responses may be too late to prevent seeding of virus into the target organ and into sites of shedding. Nevertheless, these systemic defenses can diffuse in various degrees into target organs and thereby help retard virus replication and disease.

Depending on the balance between virus and host defenses, virus multiplication in the target organ may be sufficient to produce dysfunction manifested by disease or death. Additional constitutional disease such as fever and malaise may result from diffusion of toxic products of virus replication and cell necrosis, as well as from release of lymphokines and other inflammatory mediators. Release of leukotriene C4 during respiratory infection may cause bronchospasm. Viral antigens also may participate in immune reactions, leading to disease manifestations. In addition, impairment of leukocytes and immunosuppression by some viruses may cause secondary bacterial infection.

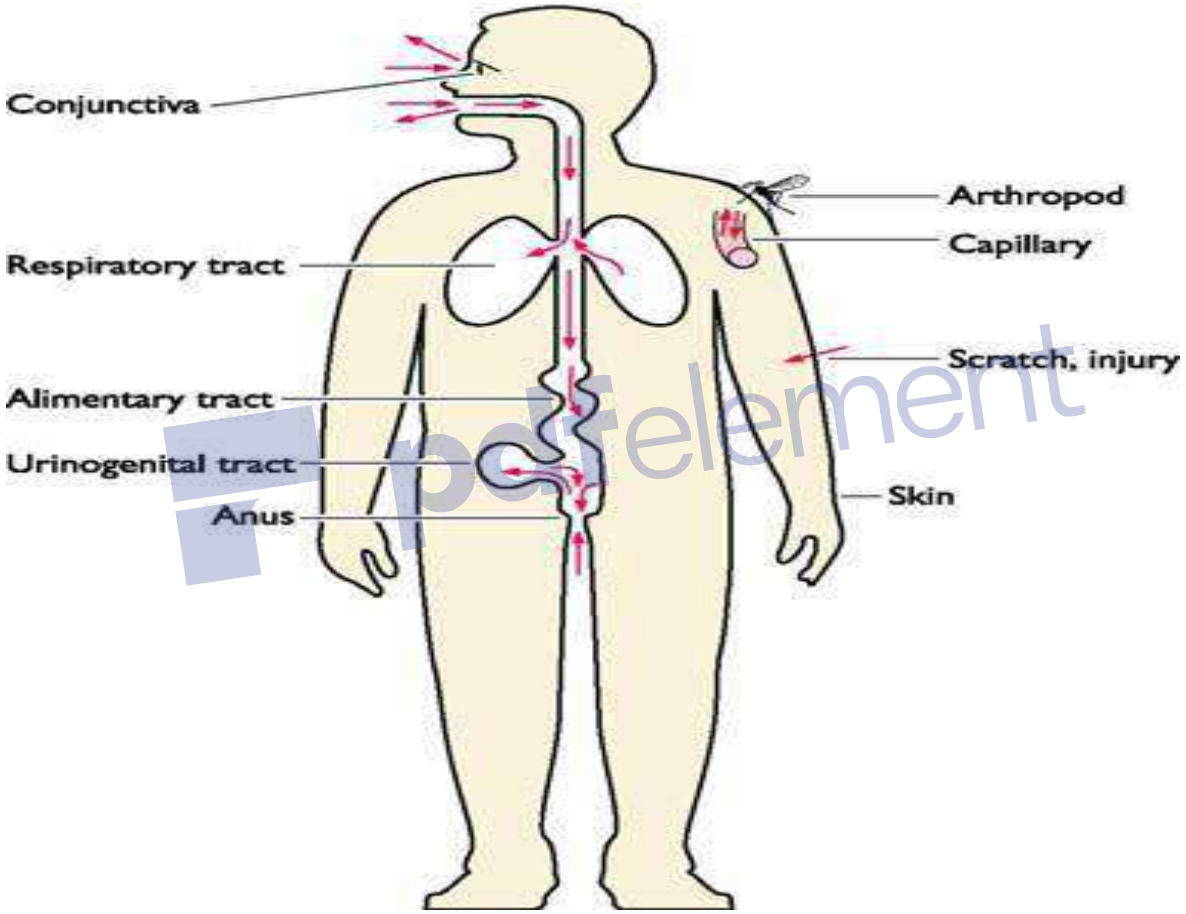
E-Shedding of Virus

Although the respiratory tract, alimentary tract, urogenital tract and blood are the most frequent sites of shedding, diverse viruses may be shed at virtually every site.

Because of the diversity of viruses, virtually every possible site of shedding is utilized; however, the most frequent sites are the respiratory and alimentary tracts. Blood and lymph are sites of shedding for the arboviruses, since biting insects become infected by this route. HIV is shed in blood and semen. Milk is a site of shedding for viruses such as some RNA tumor viruses (retroviruses) and cytomegalovirus (a herpesvirus). Several viruses (e.g., cytomegaloviruses) are shed simultaneously from the urinary tract and other sites more commonly associated with shedding. The genital tract is a common site of shedding for herpesvirus type 2 and may be the route through which the virus is transmitted to sexual partners or

the fetus. Saliva is the primary source of shedding for rabies virus. Cytomegalovirus is also shed from these last two sites. Finally, viruses such as tumor viruses that are integrated into the DNA of host cells can be shed through germ cells.

Dissemination from the Portal of Entry



1- Respiratory Tract

The most common route of viral entry is through the respiratory tract. The combined absorptive area of the human lung is almost 140 m2. Humans have a resting ventilation rate of 6 litters of air per minute, which introduces large numbers of foreign particles and aerosolized droplets into the lungs with every

breath. Many of these particles and droplets contain viruses. Fortunately, there are

numerous host defense mechanisms to block respiratory tract infection. Mechanical barriers play a significant role in anti-viral defense. For example, the tract is lined with a mucociliary blanket consisting of ciliated cells, mucous secreting goblet cells, and sub-epithelial mucous-secreting glands. Foreign particles deposited in the nasal cavity or upper respiratory tract are trapped in mucus, carried to the back of the throat, and swallowed. In the lower respiratory tract, particles trapped in mucus are brought up from the lungs to the throat by ciliary action. The lowest portions of the tract, the alveoli, lack cilia or mucus, but macrophages lining the alveoli ingest and destroy particles. Other cellular and humoral immune responses also intervene.

Viruses may enter the respiratory tract in the form of aerosolized droplets expelled by an infected individual by coughing or sneezing, or through contact with saliva from an infected individual. Larger virus-containing droplets are deposited in the nose, while smaller droplets find their way into the airways or the alveoli. To infect the respiratory tract successfully, viruses must not be swept away by mucus, neutralized by antibody, or destroyed by alveolar macrophages.

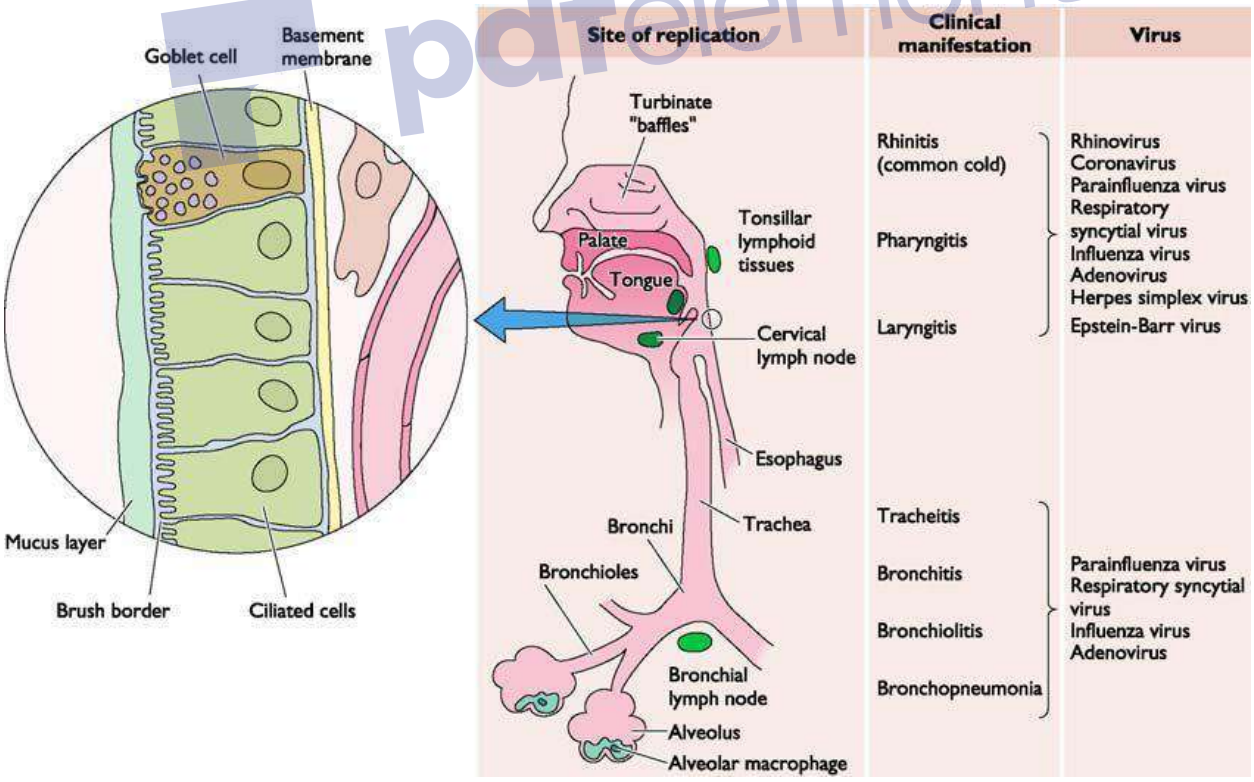


Figure 2. Sites of viral entry in the respiratory tract.

2- Gastrointestinal Tract

The gastrointestinal tract (GI) or alimentary tract is a common route of infection and dispersal. Eating, drinking, and some social activities routinely place viruses in the alimentary tract. It is designed to mix, digest, and absorb food, providing a good opportunity for viruses to encounter a susceptible cell and to interact with cells of the circulatory, lymphatic, and immune systems. Viruses may initiate local infection (e.g**., rotaviruses, coronaviruses, adenoviruses**) or invade the host to produce systemic illness (e.g., **enteroviruses, hepatitis A**). Local infections can be defined as those confined to epithelial cells adjacent to the intestinal lumen, whereas systemic infection occurs when viruses cross the mucosal layer to invade underlying tissues and spread within the host. The stomach is acidic, the intestine is alkaline, digestive enzymes and bile detergents abound, mucus lines the epithelium, and the luminal surfaces of intestines contain antibodies and phagocytic cells. Viruses that infect by the intestinal route must, at a minimum, be resistant to extremes of pH, proteases, and bile detergents. Indeed, viruses that lack these features are destroyed when exposed to the alimentary tract, and must infect at other sites. The hostile environment of the alimentary tract actually facilitates infection by some viruses. For example**, reovirus** particles are converted by host proteases in the intestinal lumen into infectious subviral particles, the forms that subsequently infect intestinal cells. As might be expected, most enveloped viruses do not initiate infection in the alimentary tract, because viral envelopes are susceptible to dissociation by detergents such as bile salts.

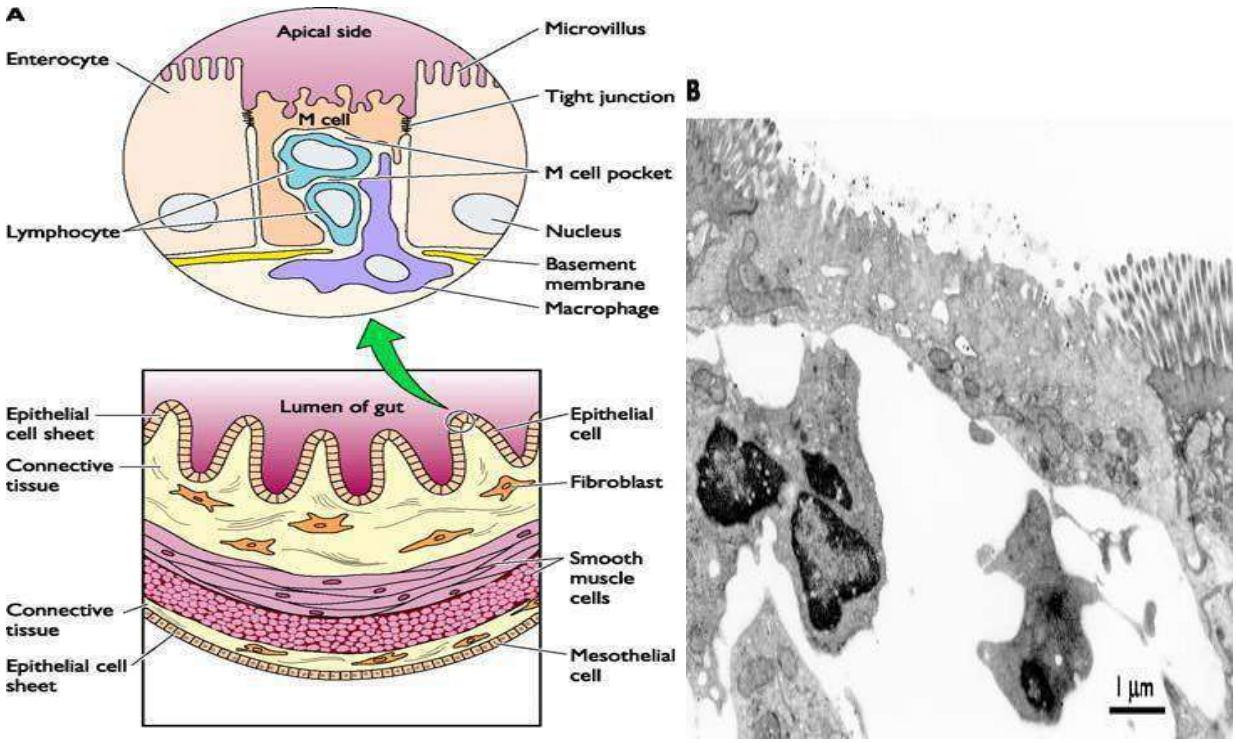


Figure 3. Viral entry in the intestine cells.

3- Genitourinary Tract

Some viruses enter the genitourinary tract as a result of sexual activities. The genitourinary tract is well protected by physical barriers, including mucus and low pH (in the case of the vagina).Some viruses infect the epithelium and produce local lesions (e.g., certain human **papillomaviruses**, which cause genital warts). Other viruses gain access to cells in the underlying tissues and infect cells of the immune system (e.g., **human immunodeficiency virus type 1**), or sensory and autonomic neurons (in the case of **herpes simplex viruses**). The nature of the cervical mucus, pH, and the secretion and the chemical composition of urine may all play a role in host defense against infection.

Congenital Infections

Infection of the fetus is a special case of infection in a target organ. The factors that determine whether a target organ is infected also apply to the fetus, but the fetus presents additional variables. The immune and interferon systems of the very young fetus are immature. This immaturity, coupled with the partial placental

barrier to transfer of maternal immunity and interferon, deprive the very young fetus of important defense mechanisms. Another variable is the high vulnerability to disruption of the rapidly developing fetal organs, especially during the first trimester of pregnancy. Furthermore, susceptibility to virus replication may be modulated by the undifferentiated state of the fetal cells and by hormonal changes during pregnancy. Although virus multiplication in the fetus may lead to congenital anomalies or fetal death, the mother may have only a mild or unapparent infection.

To cause congenital anomalies, virus must reach the fetus and multiply in it, thereby causing maldeveloped organs. Generally, virus reaches the fetus during maternal viremia by infecting or passing through the placenta to the fetal circulation and then to fetal target organs. Sufficient virus multiplication may disrupt development of fetal organs, especially during their rapid development (the first trimester of pregnancy). Although many viruses occasionally cause congenital anomalies, cytomegalovirus and rubella virus are the most common offenders. Virus shedding by the congenitally infected newborn infant may occur as a result of persistence of the virus infection at sites of shedding.

4- Eyes

The epithelium covering the exposed part of the sclera and the conjunctivae is the route of entry for several viruses. Every few seconds the eyelid passes over the sclera, bathing it in secretions that wash away foreign particles. There is usually little opportunity for viral infection of the eye, unless it is injured by abrasion. Direct inoculation into the eye may occur during ophthalmologic procedures or from environmental contamination (e.g., improperly sanitized swimming pools). In most cases, replication is localized and results in inflammation of the conjunctiva (conjunctivitis). Systemic spread of the virus from the eye is rare, although it does occur (e.g., paralytic illness after **enterovirus 70** conjunctivitis).

Herpesviruses can also infect the cornea at the site of a scratch or other injury. This infection may lead to immune destruction of the cornea and blindness.

5- Skin

The skin of most animals is an effective barrier against viral infections, as the dead outer layer cannot support viral growth (Fig. 4). Entry through this organ

occurs primarily when its integrity is breached by breaks or punctures. Replication is usually limited to the site of entry because the epidermis is devoid of blood or lymphatic vessels that could provide pathways for further spread. Other viruses can gain entry to the vascularized dermis through the bites of arthropod vectors such as mosquitoes, mites, ticks, and sandflies. Even deeper inoculation, into the tissue and muscle below the dermis, can occur by hypodermic needle punctures, body piercing or tattooing, animal bites, or sexual contact when body fluids are mingled through skin abrasions or ulcerations. In contrast to the strictly localized replication of viruses in the epidermis, viruses that initiate infection in dermal or sub-dermal tissues can reach nearby blood vessels, lymphatic tissues, and cells of the nervous system. As a consequence, they may spread to other sites in the body.

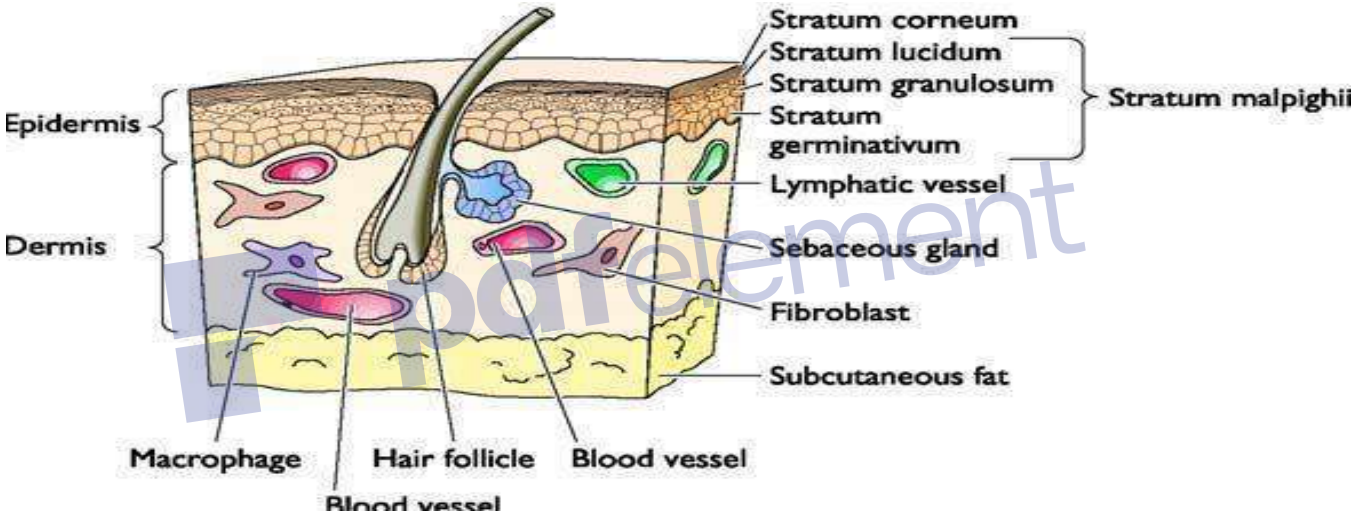


Figure 4. Diagram of the skin.

Table 1. Routes of virus entry into the host

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

6- Dissemination in the Bloodstream

At the portal of entry, multiplying virus contacts pathways to the blood and peripheral nerves, the principal routes of widespread dissemination through the body. The most common route of systemic spread of virus involves the circulation. Viruses such as those causing poliomyelitis, smallpox, and measles disseminate through the blood after an initial period of replication at the portal of entry (the alimentary and respiratory tracts), where the infection often causes no significant symptoms or signs of illness because the virus kills cells that are expendable and easily replaced. Virus progeny diffuse through the afferent lymphatics to the lymphoid tissue and then through the efferent lymphatics to infect cells in close contact with the bloodstream (e.g., endothelial cells, especially those of the lymphoreticular organs). This initial spread may result in a brief primary viremia. Subsequent release of virus directly into the bloodstream induces a secondary viremia, which usually lasts several days and puts the virus in contact with the

capillary system of all body tissues. Virus may enter the target organ from the capillaries by replicating within a capillary endothelial cell or fixed macrophage and then being released on the target organ side of the capillary. Virus may also diffuse through small gaps in the capillary endothelium or penetrate the capillary wall through an infected, migrating leukocyte. The virus may then replicate and spread within the target organ or site of excretion by the same mechanisms as for local dissemination at the portal of entry. Disease occurs if the virus replicates in a sufficient number of essential cells and destroys them. For example, in poliomyelitis the central nervous system is the target organ, whereas the alimentary tract is both the portal of entry and the site of shedding. In some situations, the target organ and site of shedding may be the same.

7- Dissemination in Nerves

Dissemination through the nerves is less common than bloodstream dissemination, but is the means of spread in a number of important diseases. This mechanism occurs in rabies virus, herpesvirus, and, occasionally, poliomyelitis virus infections. For example, rabies virus implanted by a bite from a rabid animal replicates subcutaneously and within muscular tissue to reach nerve endings. Evidence indicates that the virus spreads centrally in the neuritis (axons and dendrites) and perineural cells, where virus is shielded from antibody. This nerve route leads rabies virus to the central nervous system, where disease originates. Rabies virus then spreads centrifugally through the nerves to reach the salivary glands, the site of shedding.

Viral Persistence: Chronic, Latent, and Slow virus infection:

Persistent infections are characterized as those in which the virus is not cleared but remains in specific cells of infected individuals. Persistent infections may involve stages of both silent and productive infection without rapidly killing or even producing excessive damage of the host cells. There are three types of overlapping persistent virus-host interaction that may be defined as latent, chronic and slow infection.

Chronic infections are those in which virus can be continuously detected; mild or no clinical symptoms may be evident. Ex: infants infected with hepatitis B virus frequently become persistently infected (chronic carriers): most carriers are asymptomatic.

Latent infections are those in which the virus persists in an occult or cryptic form most of time (lack of demonstrable infectious virus between episodes of recurrent disease). Ex: Herpesviruses; Chickenpox virus (varicella- zoster) also become latent in sensory ganglia. Also EBV and CMV.

Slow infection is characterized by a prolonged incubation period (lasting months or years) followed by progressive disease. Unlike latent and chronic infections, slow infection may not begin with an acute period of viral multiplication. During persistent infections, the viral genome may be either stably integrated into the cellular DNA or maintained episomally. Ex: Leukemia, Scrapie in sheep, and Creutzfeldt- Jakob disease occur in humans.

No measures to eradicate persistent viruses have been developed. Vaccination, interferon and antiviral drugs can reduce the frequency of clinical recurrence and ameliorate clinical symptom, yet the virus continues to remain associated with the host.

Medical science has begun to control a number of acute virus infections, many by drug treatment and/or immunization, but persistent virus infections are largely uncontrolled. Diseases caused by persistent virus infections include acquired immune deficiency syndrome (AIDS), AIDS-related complexes, chronic hepatitis, subacute sclerosing panencephalitis (chronic measles encephalitis), chronic papovavirus encephalitis (progressive multifocal leukoencephalopathy), spongioform encephalopathies (caused by prions), several herpesvirus-induced diseases, and some neoplasias. The pathogenic mechanisms by which these viruses cause disease include disorders of biochemical, cellular, immune, and physiologic processes. Ongoing studies are rapidly advancing our understanding of many persistent infections. Viruses have evolved a wide variety of strategies by which they maintain long-term infection of populations, individuals, and tissue cultures.

IMMUNE RESPONSE TO VIRAL INFECTION

Virus infection in vertebrates results in two general types of immune response. The first is a rapid-onset "innate" response against the virus, which involves the synthesis of proteins called interferon and the stimulation of "natural killer" lymphocytes. In some cases, the innate response may be enough to prevent a large scale infection. However, if the infection proceeds beyond the first few rounds of viral replication, the "adaptive immune response", will be began. The adaptive immune response itself has two components, the humoral response (the synthesis of virus-specific antibodies by B lymphocytes) and the cell-mediated response (the synthesis of specific cytotoxic T lymphocytes that kill infected cells). Both of these components of the adaptive immune response result also in the production of long-lived "memory cells" that allow for a much more rapid response (i.e., immunity) to a subsequent infection with the same virus.

Interferons :

- These cytokines are the most important to virologists.
- They discovered by Isaacs and Lindenmann in 1957.
- They are host – coded proteins that inhibit viral replication and are produced by intact animals or cultured cells in response to viral infection or other inducers.
- They are produced by all vertebrate species.
- They are believed to be the body's first line of defence against viral infection.
- They are modulating humoral and cellular immunity and have broad cell-growth regulatory activities.
- There are 3-general groups of interferon (IFN)

- **Interferon type I.** IFN-α , IFN-β (beta), IFN-κ (kappa), IFN-δ (delta), IFN-ε (epsilon), IFN-τ (tau), IFN-ω (omega) and IFN-ζ (zeta, also known as limitin).
- **Interferon type II.** A sole member makes up interferon type II and is called IFN-γ (gamma).
- **Interferon type III.** The recently classified type Interferon type III group consists of three IFN-λ (lambda) molecules called IFN-λ1, IFN-λ2 and IFN-λ3.

(a) I FN -α: synthesized mainly by leucocytes

(b) IFN -β: synthesized mainly by fibroblasts

(c) IFN -γ (immune IFN): synthesized mainly by lymphocytes.

❖ The different IFNs are similar in size, but the three classes are antigenically distinct.

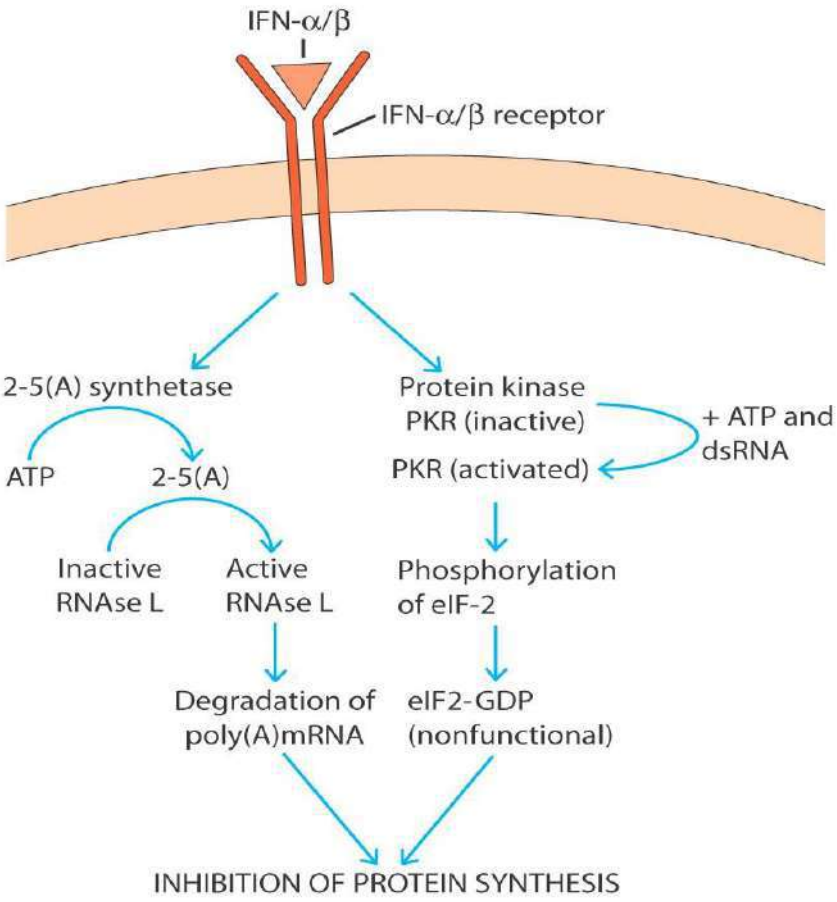
- ❖ RNA viruses are stronger inducers of interferon than DNA viruses.
- ❖ IFNs also can be induced by double – stranded RNA, bacterial endotoxin, and small molecules.
- ❖ IFN-γ: is not produced in response to most viruses but is induced by mitogern stimulation.
- ❖ The different types of IFN are roughly equivalent in antiviral activity.

- IFN- γ (type I) is produced soon (within a day) after infection.
- The cell regulatory activity of IFN –γ is much greater than that of IFN – α or β.
- IFNs- are almost always a host species- specific in function, by contrast, IFN activity is not specific for a given virus: the replication of a wide variety of viruses can be inhibited.

Mechanism of action :- After a cell has come in contact with a virus or some other IFN induced , IFN released from virus infected cells binds to receptors on neighboring cells and induces an antiviral state , the mechanism may involve inhibition of viral protein or nucleic acid synthesis or may be virus assembly , it also may inhibit cell growth .

The mechanism in which IFN –inhibit protein synthesis , is by induce the synthesis of two enzymes , protein kinase and oligoadinylate synthetase and these two enzymes subsequently block viral reproduction .

IFN also may increase recognition of viral antigens by the immune system and it may activate the NK cell, macrophages, B-cell and cytotoxic cells.



1. How can we classify viral infections by 'outcome of the immune response'?

We can roughly establish four categories of virus infection based on what happens to the host (not including sub-clinical, unapparent infections).

Category	Examples
1. Acute (only)	Smallpox, Influenza, Rhinovirus, Rotavirus, Ebola, SARS
2. Latent	Herpesviruses
3. Chronic/Persistent	Hepatitis B & C
4. Progressive	HIV

Category 1 includes all cases for which the person gets sick and then either dies or recovers completely, with the elimination of all viruses from the body. For categories 2-4, the initial infection may be acute or unapparent, but the body's immune response does not clear the virus completely, and things proceed to one of these situations, where there may

be very little ("latent"), some ("chronic/persistent"), or abundant ("progressive") virus replication going on during the rest of the person's life. There is some overlap and ambiguity in these terms, but they are useful categorizations nonetheless.

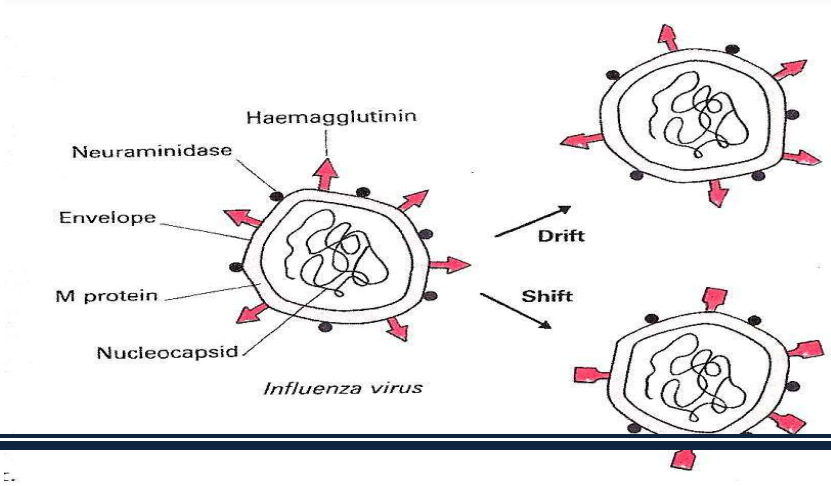
2. After recovery from an acute viral infection, a person is usually "immune" to getting the same viral disease for years (perhaps a lifetime). What, then, is the explanation for the fact that we remain susceptible to getting a rhinovirus-caused "cold" throughout our lifetime?

There are over 100 different "serotypes" of rhinoviruses (and a smaller number of coronaviruses and adenoviruses) that all cause upper respiratory infections. When we get infected with one of these, our immune system responds and clears the virus from the body within a couple of weeks. The immune response also leaves us with a supply of specific "memory cells" that prevent that same virus from causing a clinically significant infection in our bodies for years. But....., there are all of those other related viruses that can still infect us. For example, although all of the rhinoviruses are similar, their surface proteins are different enough such that the memory cells made during a previous immune response against, say, rhinovirus will most likely not provide immunity to rhinovirus. So, at any time we are somewhat susceptible to whatever rhinoviruses we have not been infected by previously. (And, for older people, it's probably possible to get a cold from the same rhinovirus a couple decades apart.)

Antigenic shift and drift:

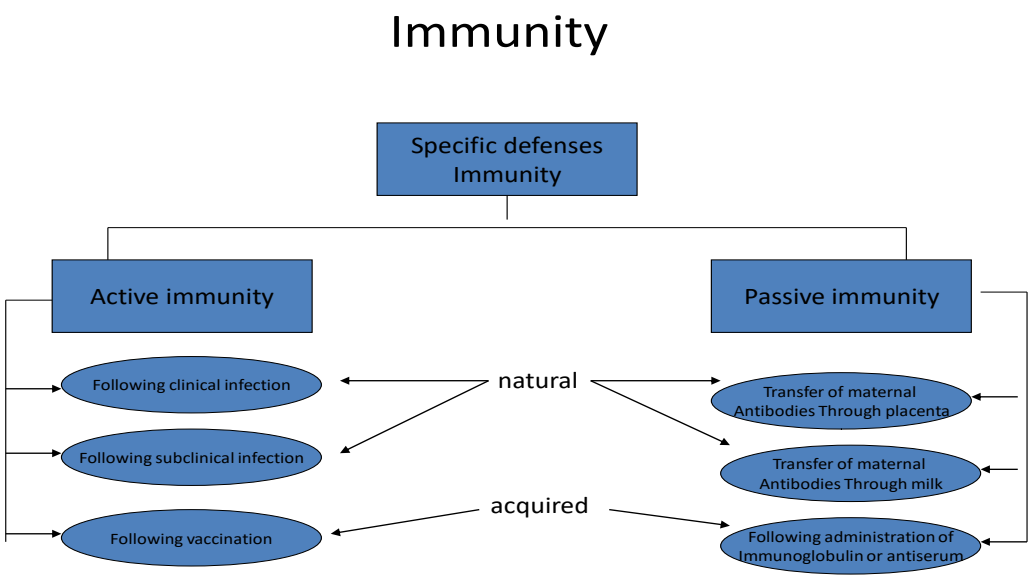
Viruses have evolved various strategies to evade recognition by Ab, this occurs by antigenic variation. E.g. HIV and foot and mouth disease virus and influenza virus that is responsible for the antigenic shift and drift.

In influenza virus the major surface antigens are haemagglutinine and neuraminidase. This virus can change its surface slightly (antigenic drift) or radically (antigenic shift).



Vaccines

Immunity



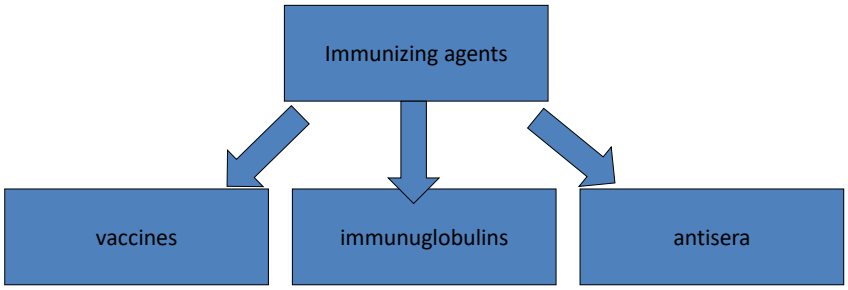
Active immunity

- Resistance developed in response to stimulus by an antigen (infecting agent or vaccine) and is characterized by the production of antibodies by the host.

Passive immunity

- Immunity conferred by an antibody produced in another host. It may be acquired naturally or artificially (through an antibody-containing preparation).

Immunizing agents



Vaccination

- Vaccination is a method of giving antigen to stimulate the immune response through active immunization.
- A vaccine is an immuno-biological substance designed to produce specific protection against a given disease.
- A vaccine is “antigenic” but not “pathogenic”.

Even 2,500 Years Ago,
People Knew Immunity Worked.

- Greek physicians noticed that people who survived smallpox never got it again.
- The insight: Becoming infected by certain diseases gives immunity.

Vaccination

- Charles Jenner 1796 : Cowpox/Swinepox
- 1800’s Compulsory childhood vaccination

Smallpox

- 25% mortality
- Life-long immunity
- UK: 1700’s
- China 1950
- Pakistan/Afghanistan/ Ethiopia 1970

As a result, after a world-wide effort
Smallpox was eliminated as a human disease in 1978

Types of vaccines

- Live vaccines
- Attenuated live vaccines
- Inactivated (killed vaccines)
- Toxoids
- Polysaccharide and polypeptide (cellular fraction) vaccines
- Surface antigen (recombinant) vaccines.

Live vaccines

- Live vaccines are made from live infectious agents without any amendment.
- The only live vaccine is “Variola” small pox vaccine, made of live vaccinia cow-pox virus (not variola virus) which is not pathogenic but antigenic, giving cross immunity for variola.
- Live attenuated (avirulent) vaccines
- Virulent pathogenic organisms are treated to become attenuated and avirulent but antigenic. They have lost their capacity to induce full-blown disease but retain their immunogenicity.
- Live attenuated vaccines should not be administered to persons with suppressed immune response due to:
  - Leukemia and lymphoma
  - Other malignancies
  - Receiving corticosteroids and anti-metabolic agents
  - Radiation
  - pregnancy

Live Attenuated Vaccines have several advantages

- Attenuated (weakened) form of the "wild" virus or bacterium
- Can replicate themselves so the immune response is more similar to natural infection
- Usually effective with one dose

Live Attenuated Vaccines also have several disadvantages

- Severe reactions possible especially in immune compromised patients
- Worry about recreating a wild-type pathogen that can cause disease
- Fragile – must be stored carefully

A number of the vaccines you received were live Attenuated Vaccines

- Viral measles, mumps, rubella, vaccinia, varicella/zoster, yellow fever, rotavirus, intranasal influenza, oral polio, Bacteria BCG (TB), oral typhoid.

Inactivated (killed) vaccines

- Organisms are killed or inactivated by heat or chemicals but remain antigenic.

- They are usually safe but less effective than live attenuated vaccines.
- The only absolute contraindication to their administration is a severe local or general reaction to a previous dose.

Inactivated Vaccines

- Cannot replicate and thus generally not as effective as live vaccines
- Usually require 3-5 doses
- Immune response mostly antibody based.

- No chance of recreating live pathogen
- Less interference from circulating antibody than live vaccines

Inactivated Vaccines are also a common approach today

- Viral polio, hepatitis A, rabies, influenza*
- Bacterial pertussis*, typhoid*cholera*, plague*

Other Inactivated Vaccines now contain purified proteins rather than whole bacteria/viruses

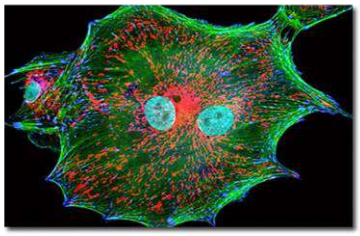
- Proteins hepatitis B, influenza, acellular pertussis,human papillomavirus, anthrax.
- Toxins diphtheria, tetanus

Sabin Polio Vaccine

Attenuated by passage in foreign host (monkey kidney cells)

Selection to grow in new host makes virus

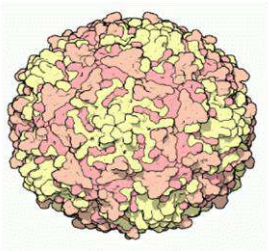
less suited to original host



- Grows in epithelial cells
- Does not grow in nerves
- No paralysis
- Local gut immunity (IgA)

Salk Polio Vaccine

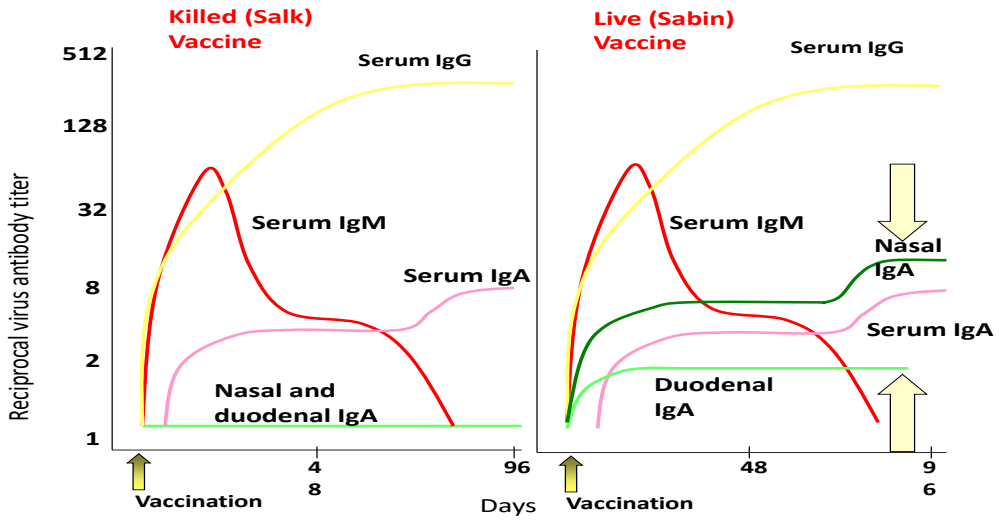
- **Formaldehyde-fixed**
- **No reversion**



Polio Vaccine illustrates the pluses and minuses of live vaccines
US: Sabin attenuated vaccine
~ 10 cases vaccine-associated polio per year =
1 in 4,000,000 vaccine infections
Scandinavia: Salk dead vaccine

- No gut immunity
- Cannot wipe out wt virus

Live virus generates a more complete immune response



- Killed- virus vaccines (inactivated vaccine): They are made by purifying viral preparation to a certain extent and then inactivating viral infectivity in a way that does minimal damage to the viral structural proteins: mild formalin treatment is frequently used. They generally stimulate the development of circulating Ab against the coat proteins of the virus.

♦The immunity is often brief and must be boosted.
♦ Extreme care should be made that no residual live virulent virus is present in the vaccines.
♦ The cell-mediated response to inactivated vaccines is generally poor.
♦ Some killed- virus vaccines have induced hypersensitivity to subsequent infection.
♦ It could be either whole virus vaccine or subunit vaccine.
Ex. Hepatitis A, Poliomyelitis (subcutaneous), Influenza, Rabies

- Attenuated live- virus vaccines: It is utilizing viral mutants that antigenically overlap with wild-type virus.

They selected naturally attenuated strains or by cultivating the virus serially in various hosts and cultures.

- They acting like natural infection.
- They multiply in the host and tend to stimulate longer- lasting Ab production to induce a good cell- mediated response, and to induce Ab production. But there is a risk of reversion to greater virulence during multiplication within the vaccine.

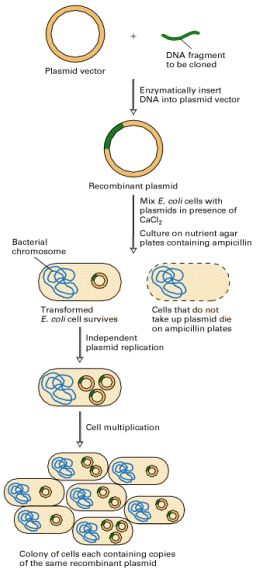
Ex. Varicella, measles, mumps, Rubella, poliomyelitis (oral).

COVID-19 Vaccines Trails

Platform	Candidates in clinical trials and phase[a]	Type of candidate vaccine	Target antigen	Single/ multiple dose	Speed[b]	Immune response	Advantages	Disadvantages
DNA	Inovio Pharmaceuticals - phase 1/2	DNA plasmid vaccine with electroporation	Spike protein	Multiple	Fast	Both humoral and cellular	-Electroporation generates a robust immune response -Made using genetic sequence and does not need to be cultured	-Although deemed to be safe, electroporation can be complicated and potentially problematic. -No DNA based vaccine has been previously produced
RNA	Moderna/NIAID - phase 3	Lipid nanoparticle [LNP]-encapsulated mRNA	Spike protein	Multiple	Fast	Both humoral and cellular	-Made using genetic sequence and does not need to be cultured	-LNP is temperature sensitive -Ability to manufacture large scale unknown -No RNA based vaccine has been produced before
	BioNTech/Fosun Pharma/Pfizer - phase 3	3 LNP-mRNAs	Spike protein					
Non-replicating viral vector	AstraZeneca/ University of Oxford - phase 3	AZD1222	Spike protein	Single	Medium	Both humoral and cellular	-Can be manufactured large scale -Safe and effective immunologically as shown with Ebola	-Pre-existing immunity could hamper clinical use and reduce immune response
	CanSino Biological Inc./Beijing Institute of Biotechnology - phase 2	Adenovirus type 5 vector	Spike protein					
Inactivated	Wuhan Institute of Biological Products/Sinopharm - phase 3	Inactivated	Whole virus	Multiple	Medium	Mostly humoral	-Pathogen is killed and hence, no risk of reversion	-Risk of vaccine-enhanced disease -Usually produce a weak immune response
	Beijing Institute of Biological Products/Sinopharm - phase 3		Whole virus					
	Sinovac - phase 3	Inactivated + aluminum adjuvant	Whole virus			Mostly humoral - aluminum adjuvant enhances response more robust		

Modern molecular biology has offered new approaches to make vaccines

1. Clone gene from virus or bacteria and express this protein antigen in yeast, bacteria or mammalian cells in culture



Polysaccharide and polypeptide (cellular fraction) vaccines

- **They are prepared from extracted cellular fractions e.g. meningococcal vaccine from the polysaccharide antigen of the cell wall, the pneumococcal vaccine from the polysaccharide contained in the capsule of the organism, and hepatitis B polypeptide vaccine.**
- **Their efficacy and safety appear to be high.**

Surface antigen (recombinant) vaccines.

- It is prepared by cloning HBsAg gene in yeast cells where it is expressed. HBsAg produced is then used for vaccine preparations.
- Their efficacy and safety also appear to be high.

Routes of administration

- Deep subcutaneous or intramuscular route (most vaccines)
- Oral route (sabine vaccine, oral BCG vaccine)
- Intradermal route (BCG vaccine)
- Scarification (small pox vaccine)
- Intranasal route (live attenuated influenza vaccine)

Scheme of immunization

- **Primary vaccination**
  - One dose vaccines (BCG, variola, measles, mumps, rubella, yellow fever)
  - Multiple dose vaccines (polio, DPT, hepatitis B)
- Booster vaccination

  To maintain immunity level after it declines after some time has elapsed (DT, MMR).

Periods of maintained immunity due to vaccines

- Short period (months): cholera vaccine
- Three to five years: DPT vaccine
- Five or more years: BCG vaccine
- Ten years: yellow fever vaccine
- Solid immunity: measles, mumps, and rubella vaccines.

Levels of effectiveness

- Absolutely protective(100%): yellow fever vaccine
- Almost absolutely protective (99%): Variola, measles, mumps, rubella vaccines, and diphtheria and tetanus toxoids.
- Highly protective (80-95%): polio, BCG, Hepatitis B, and pertussis vaccines.
- Moderately protective (40-60%) cholera vaccine, and influenza killed vaccine.

Cultivation of viruses:

Viruses are obligate intracellular parasites. They depend totally on their host cells for their existence. Their total host dependence makes it extremely difficult to get good insight of them natural conditions, because the internal characteristics of the host cells are likely to interfere with the observations. Due to these reasons, it has been found desirable that viruses are cultivated or grown in the laboratory itself.

It has two advantages:
a) This enables to get sufficient amount of virus particles at any given time.
b) This would mean lesser degree of contamination from the host cell material, ensuring a purer virus sample.

Since viruses are host dependent, it is not possible to cultivate them solely in presence of organic or inorganic nutrient medium. They can be grown only if living cells and tissues are used as culture medium. These tissues and cells would act as the host for the virus in laboratory conditions. For this purpose, the relevant cells or tissues must be cultivated first.

There are three methods employed for virus cultivation:

A) Inoculation into animals:

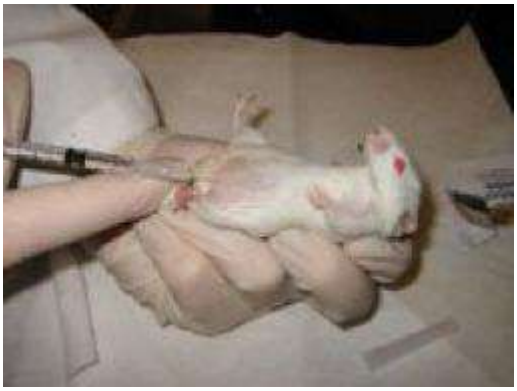
The earliest method for cultivation of viruses causing human diseases was inoculation into human volunteers. Reed and his colleagues (1900) used human volunteers for their work on yellow fever. Due to serious risk involved, human volunteers are involved only when no other method is available and the virus is relatively harmless.

Monkeys were used for the isolation of Poliovirus by Handsteiner and popper (1909). Due to their cost, and risk to handlers, they have limitations. The use of white mice by Theiler extended the scope of animal inoculation greatly. Mice are still most widely used animals in virology. Infant mice are very susceptible to Coxsackie's and arboviruses. Mice can be inoculated through several routes i.e. intracerebral, subcutaneous, intraperiotonial, intranasal.

Other animals such as guinea rabbits and ferrets are also used. The growth of virus in inoculated animals is indicated by death, disease or visible lesions. Animal inoculation has a disadvantage that immunity may interfere with viral growth and that animals often harbor latent viruses.

Monkeys

Mouse



Mouse infection

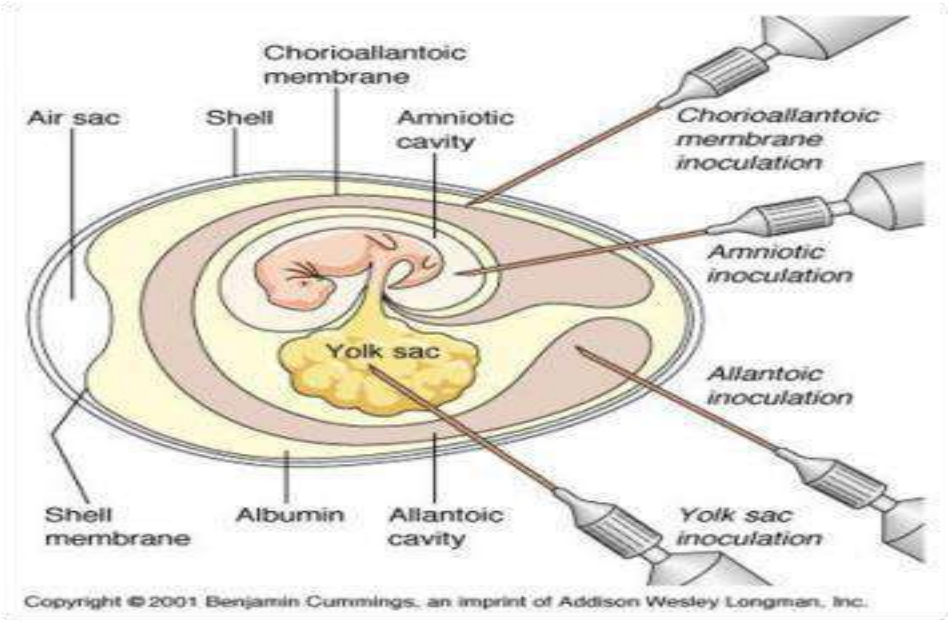
B) Embryonated eggs

The Embryonated hen's egg was first used for cultivation of viruses by Good Pasteur and Burnet (1931). Cultivation of viruses in organized tissues like chick embryo necessitates a different type of approach. These are live embryos so very beautifully packed in their shells. For all practical purposes they all themselves behave as tissue cultures. The process of cultivation of viruses in embryonated eggs depends on the type of egg which is used. The egg used for cultivation must be sterile and the shell should be intact and healthy.

Process for cultivation of virus in embryonated egg:

In order to cultivate viruses in eggs, the procedure adopted should be very simple. The eggs are kept in incubator and embryos of 7-12 days old are used. The egg containing embryo usually has an air apace at the larger end. The position of this sac is first determined. The shell over the air sac is then cut off and removed. The membrane adjacent

to the shell is then pierced. Usually the hypodermic syringe is used for piercing the shell. At this stage, the embryonic fluid may ooze out. The shell membrane is then removed. The rest of the embryo then gets exposed and ready for use. Virus suspension to be cultivated is taken in dropper and gently spread over the exposed embryo. After inoculation is thus completed, the open area of the shell is sealed eggs are incubated for one week as in hatching. The virus particles infect the membrane at random and create pock marked appearance against the transparent background. This indicate viral basis.



The embryonated offers several sites for the cultivation of viruses:

1) Chorioallantoic membrane (CAM):

CAM is inoculated mainly for growing poxvirus. Herpes simplex virus is also grown. Virus replication produces visible lesions, grey white area in transparent CAM. Each pock

is derived from a single virion. Pocks produced by different virus have different morphology. Under optimal conditions, each infectious virus particle can form one pock. Pock counting, therefore can be used for the assay of pock forming virus such as vaccinia.

2) Allantoic cavity:

Inoculation into the allantoic cavity provides a rich yield of influenza and some paramyxoviruses. Allantoic inoculation is employed for growing the influenza virus for vaccine production. Other allantoic vaccines include Yellow fever (17D strain), and rabies vaccines. Duck eggs are bigger and have a longer incubation period then hen's egg. They therefore provide a better yield of rabies virus and were used for the preparation of the inactivated non-neural rabies vaccines.

3) Amniotic cavity:

The amniotic sac is mainly inoculated for primary isolation of influenza A virus and the mumps virus.

4) Yolk sac:

It is inoculated for the cultivation of some viruses as well as for some bacteria like Chlamydiae and Rickettsiae.

Pocks/Lesions

C) Tissue culture:

Cultivation of bits of tissues and organs *in vitro* had been used by physiologists and surgeons for the study of morphogenesis and wound healing. The first application of tissue culture in virology was by Steinhardt and colleagues (1913), who maintained the vaccinia

virus in fragments in rabbit cornea. Maitland (1928) used chopped tissue in nutrient media for cultivation of vaccinia viruses. The turning point which made tissue culture the most important method for cultivation of virus was the demonstration by Enders, Weller and Robins (1949), that poliovirus can be grown in tissue culture in non-neural origin.

There are three types of tissue cultures:

1) Organ culture:

Small bits of organs can be maintained in vitro for days and weeks, preserving their original architecture and function. Formalin is used for the preservation. Organ culture is useful for the isolation of some viruses which appear to be highly specialized parasites of certain organs.

Example: Tracheal ring organ culture is employed for the isolation of cornovirus, a respiratory pathogen.

2) Explant culture:

Fragments of minced tissues can be grown as 'explants' embedded in plasma clots. They may also be cultivated in suspension. This was originally called as tissue culture. This method is now seldom employed in virology.

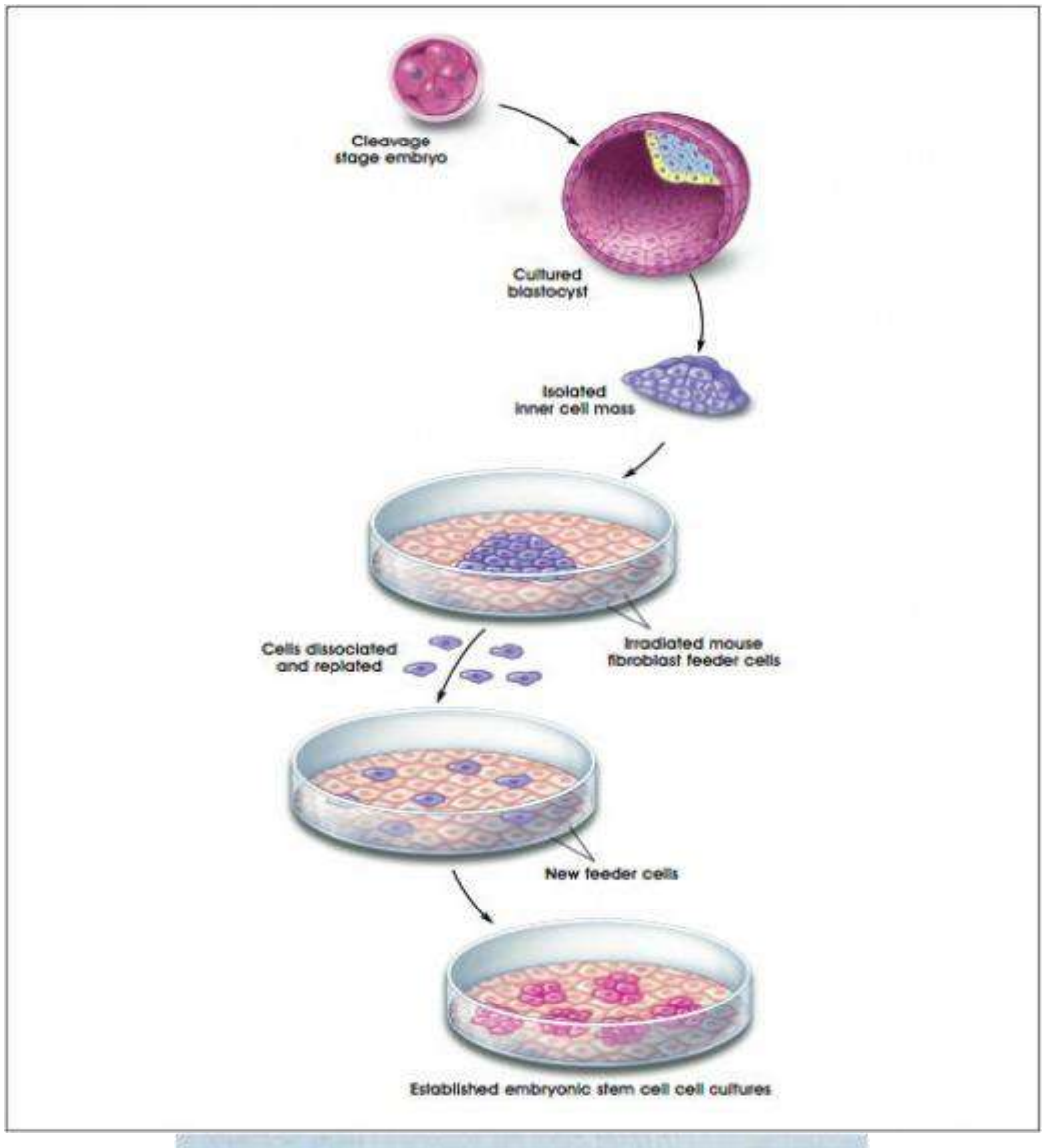
Example: Adenoid tissue explant culture was used for the isolation of adenovirus.

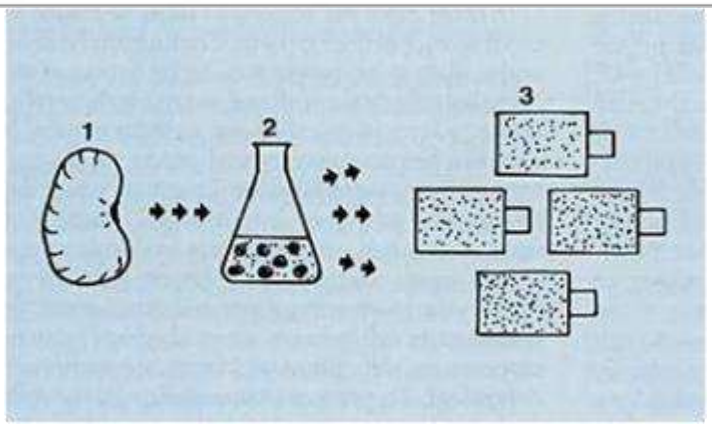
3) Cell culture:

The cell culture is the method routinely employed nowadays for identification and cultivation of viruses. Cells of various types of tissues of animals may be cultivated. But more commonly, fibroblast and muscle epithelial cells are used for the propagation of virus.

The tissue is first removed from the organism concerned. This tissue is then broken down into its constituent cells by utilizing suitable physical means. Homogenization in a homogenizer is common method utilized. The complete tissue is then converted into many small pieces. The tissue fragments are washed with salt solutions. Sterile physiological saline or other types of solution like saline or other types of solution like hank's solution or eagle's solution are used. The pieces are converted into their constituent cells by a process called dispersion of the cells from tissue. It is done by breaking down the proteinaceous cementing material (i.e. Haluronic acid), joining the cells with the help of proteolytic enzymes like trypsin and mechanical shaking. This step is called as

trypsinization.





Piece of tissue (eg. Monkey kidney) used to produce tissue culture

The washed tissue fragments are then placed in a flask with sterile trypsin solution at 4°c for about 18 hours. During this period, the tissue fragments area gradually dispersed into their cellular components. Presence of chemicals like EDTA helps in dispersion of cells. The cells are then centrifuged and resuspended in washing medium. It is done repeatedly.

The washed suspended cells are then cultivated in a suitable growth medium. The essential constituents of growth medium are physiological amounts of essential amino acids, and vitamins, salts and glucose and a buffering system generally consisting of bicarbonate in equilibrium with atmosphere containing about 5% calf or fetal calf serum. Antibiotics are added to prevent bacterial contaminants and phenol red as indicator. Such media will allow most cell types to multiply with a division time of 24-48 hrs.

Cultivation is done after adjustment of the number of cells per unit volume. The required number of cells is suspended in the growth medium taken in a tube or flask. The entire culture is then incubated at 36°c for 72hrs. The cells in culture multiply and cover the bottom of the glass container with a thin but continuous layer, which is often one cell thick. Such cell layers are called as monolayer. This technique was improved by Dr. Dulbecco and his associates in the U.S.A. They found that the monolayer can be developed on agar containing necessary nutrients. Virus particles grown on such monolayer are extremely uniform in growth.

Sometimes the dispersed cells are not allowed to settle down at the bottom of the container. Rather they are kept floating by shaking the flasks continuously on the mechanical shaker. This type of culture is called as suspension culture. A vigorously growing monolayer or suspension culture is then inoculated with the types of viruses to which it is susceptible. The inoculation is done by mixing or spreading the viral suspension with the cultivated host cells. The virus particle infects the host cells in due course. They multiply in number within the host cell and eventually come out by destroying the host cell. They are thus liberated into the surrounding medium and infect the neighboring cells. The cell culture looks disintegrated. The initially formed virus particles soon lead to the production of many more viruses. These areas appear to be completely disintegrated and take shapes of white patches called as plaques.

Types of cell cultures:

On the basis of origin, chromosomal characters, and the number of generations through which they canbe maintained, cell cultures are classified in three types.

1) Primary cell culture:

These are normal cells obtained from fresh organs of animals and cultured. Once the cells

get attached to the vessel surface, they undergo mitosis until a confluent monolayer of cells covers the surface. These layers are capable of limited growth in culture and cannot be maintained in serial culture. They are commonly employed for primary isolation of viruses and in preparation of vaccine. Primary cell cultures are generally best for viral isolation and Rhesus monkey kidney cells cultures are widely used, which are sensitive to a wide range of viruses.

Examples: Rhesus monkey kidney cell culture, Human amnion cell culture.

2) Diploid cell culture:

It is also called as semi continuous cell lines. These are subsequent cultures derived from primary cell cultures. These are cells of single type that retrain the original diploid chromosome number and karyotype during serial sub cultivation for a limited period of time. There is rapid growth rate and after 50 serial subcultures, they undergo senescence and the cell strain is lost. The diploid cell strains are susceptible to a wide range of human viruses. They are also used for isolation of some fastidious viruses and production of virus vaccines,

Examples: Human embryonic lung strain (WI-38) and Rhesus embryo cell strain (HL-8)

3) Continuous cell culture:

These are cells of a single type, usually derived from the cancer cells that are capable of continuous serial cultivations indefinitely. These cells grow faster and their chromosomes are haploid. They are also called as permanent cell lines. Permanent cell lines derived from a single separated cell are called as clones. One common example of such clone is Hela strain derived from cervical cancer of lady Hela, by name. Continuous cell lines are maintained either by serial subculture or by storing in deep freeze at -70°c so that these can be used when necessary. Some cell lines are now permitted to be used for vaccine manufacture.

Examples: Vero i.e. Vervet monkey kidney cell line, BHK, i.e. Baby Hamster kidney cell line.

Methods for Viral Growth

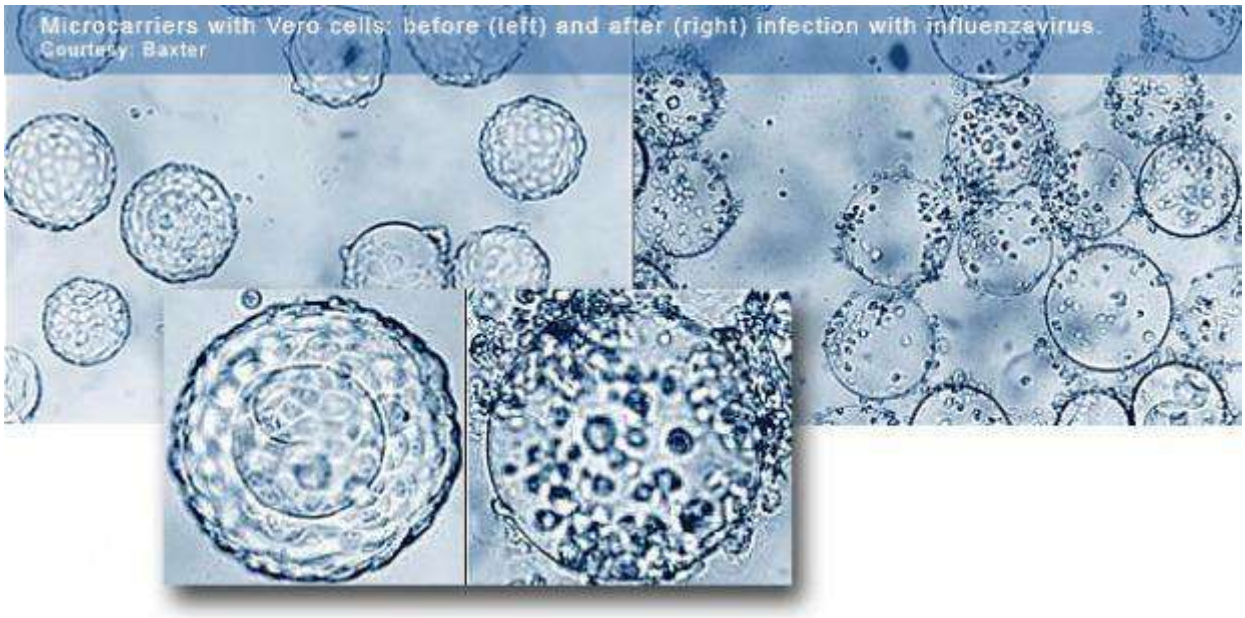
1. Cytopathic Effect (CPE)

Cytopathic effect (CPE) refers to deterioration in cells (especially in tissue

culture) associated with the multiplication of certain viruses. When in tissue culture, the spread of virus is restricted by an overlay of agar (or other suitable substance) and thus the cytopathic effect may lead to formation of plaque.

The changes in appearance depending on the particular virus and type of cell culture includes:

- Altered Shape
- Detachment from substrate
- Lysis
- Membrane fusion
- Altered membrane permeability
- Inclusion bodies
- Apoptosis (process of programmed cell death that may occur in multicellular organisms)



2. Plaque Assay

Direct counting is the actual counting of microorganisms. The basis of direct count is the actual counting of every organism present in a sub-sample of a population. However, microorganisms such as viruses are too small to be enumerated by direct counting. In order to enumerate viruses, many indirect methods have been developed. The most basic,

simple and commonly used method is the plaque assay.

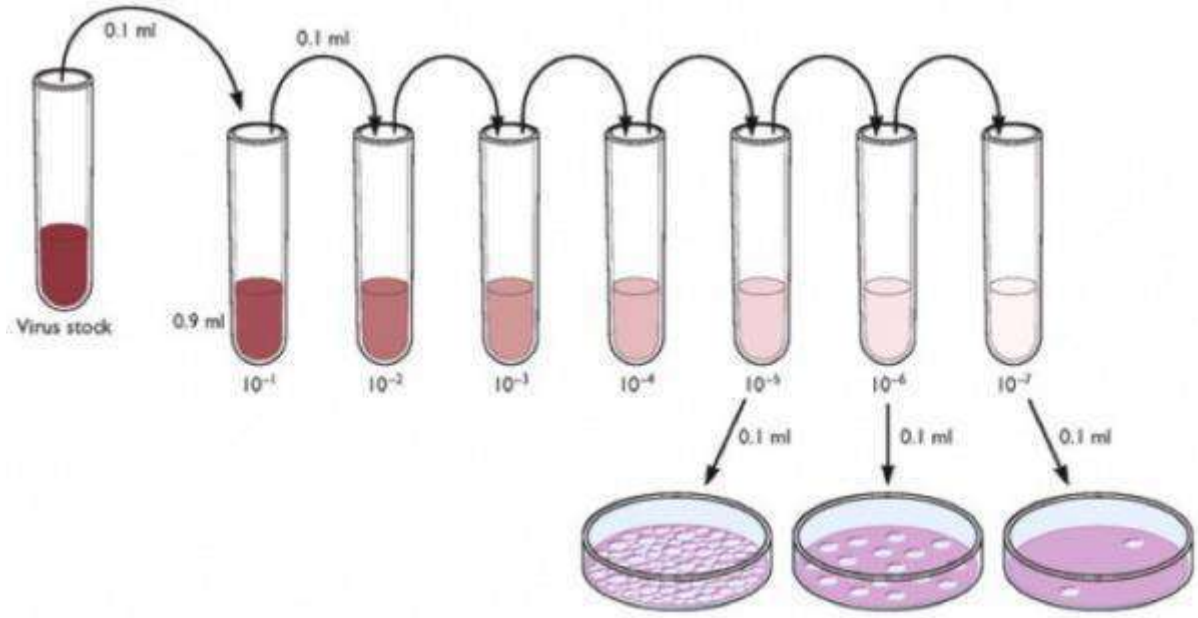
The theory of plaque assay is based on the original plaque assay for bacteriophages. Plaque assay helps to determine the quantity of infectious virus. This method requires the diluted virus to infect a monolayer cell line. The diluted virus is obtained by conducting serial dilution of the given virus. The concentration of the diluted virus is low hence one virus infects one cell and spreads to the surrounding cells. The infected monolayer is then grown under a gel medium (i.e. gelling medium like molten agar), after a virus burst only adjacent cells can be infected resulting in the formation of a visible circular plaque which indicate cell death as more cells are infected.

Principles of plaque assay

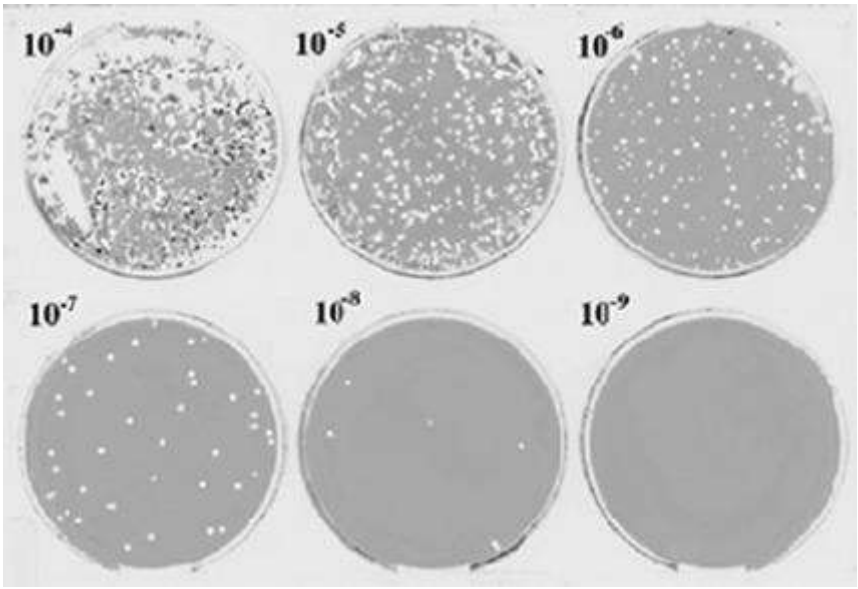
1. The concentration of virus is low enough that 1 virus infects 1 cell.
2. Virus infects monolayer cell line
3. The gelling medium restrict the movements of virus hence only adjacent cells can be infected.

Features of plaque assay:
Features:

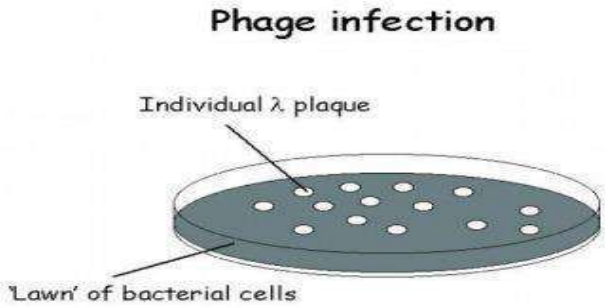
Very time-consuming
Very simple method
Only works for viruses that infect monolayer cells
Only works for viruses that causes cell lysis
Uses principle of one virus on the monolayer produces one plaque



Serial dilution of virus



Formation of plaques



Virus count

- Counts only viable virus which are virus capable of multiplying.
- Must know the culture conditions for the virus studied.
- This method is useful for samples with very low virus count. (eg. food and water sample)
- Require time for incubation

Features

Virus count is a simple method which only works for viruses that infect monolayer cells and viruses that cause cell lysis. It is based on the principle that one virus on the monolayer produces one plaque.

Antiviral chemotherapy

- Virus Structure and Replication

Viruses are the smallest infective agent, effectively consisting of nucleic acid (DNA or RNA) enclosed in a protein coat.

Viruses are intracellular parasites with no, or little, metabolic machinery of their own.

They have to borrow the biochemistry of the host cell to succeed and grow (this is what makes selective antiviral therapy so difficult).

Basic Mechanisms

Specificity against virus replication is the key issue in chemotherapy. Because of the close interaction between virus replication and normal cellular metabolism, it was originally thought too difficult to interrupt the virus replicative cycle without adversely affecting the host cell metabolism. It is now clear, however, that several events in the virus replicative cycle either do not occur in normal uninfected cells or are controlled by virus-specified enzymes that differ structurally and functionally from the corresponding host cell enzymes.

Quite schematically, the virus replicative cycle can be divided into 10 steps (Fig. 1): (1) adsorption, (2) penetration, (3) uncoating, (4) early transcription, (5) early translation, (6) replication of the viral genome, (7) late transcription, (8) late translation, (9) assembly, and (10) release of new virus particles.

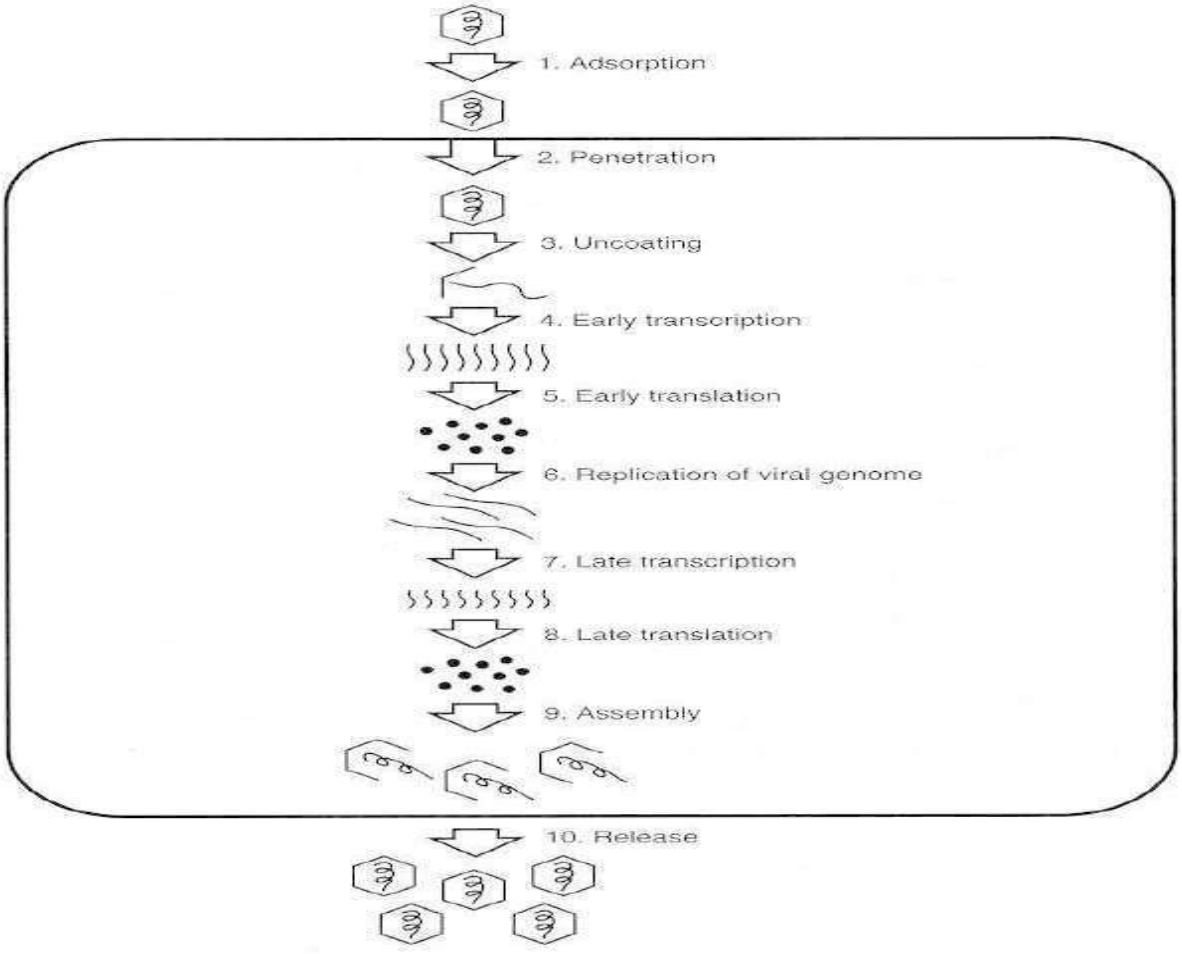


Fig (1): Steps of viral replication

Antiviral chemotherapy

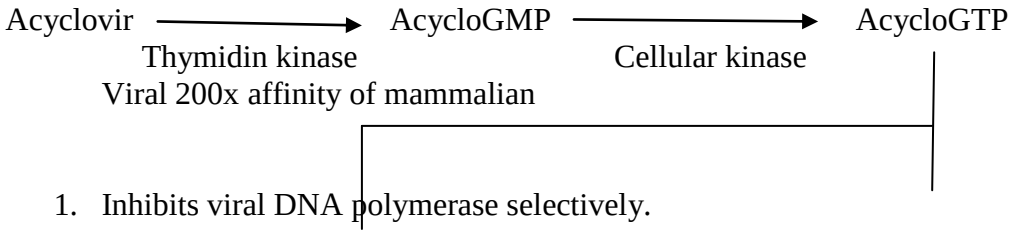
- The virus attaches to specific receptors on the host cell surface which are normal membrane components. Usually ion channels, neurotransmitter receptors.
- The receptor/virus complex enters the cell by receptor-mediated endocytosis during which the virus coat may be removed.
- The nucleic acid of the virus then hijacks the cellular machinery for replicating viral nucleic acids and proteins for the manufacture of new virus particles.

Treatment of Herpesviruses:
(Varicella-zoster,Cytomegalavirus, Herpes simplex)
Acyclovir

- A virally coded thymidine kinase (specific to H.simplex and varicella-zoster virus) performs the initial phosphorylation step; the remaining two phosphate residues are attached by cellular kinases.
- Acyclovir triphosphate inhibits viral DNA polymerase resulting in chain termination.

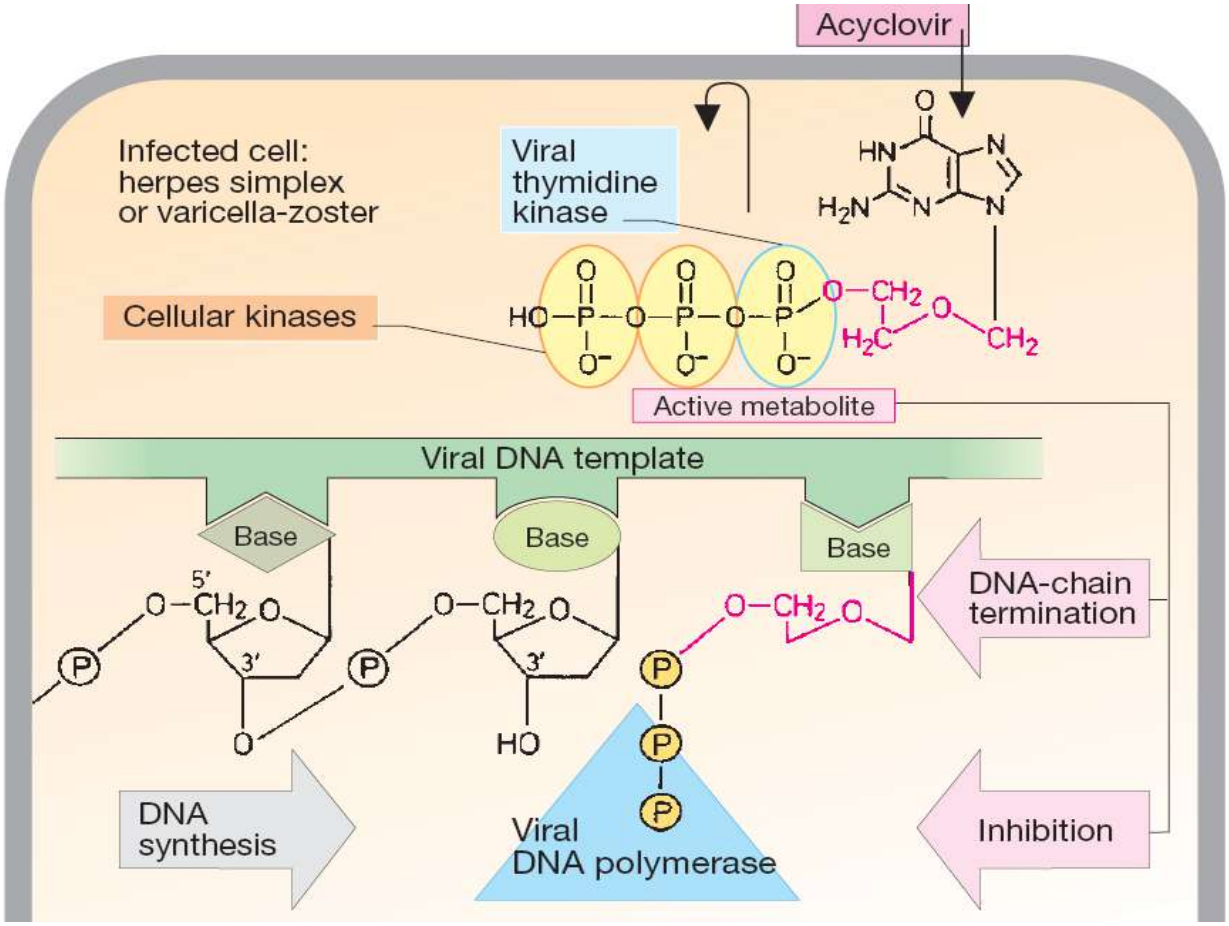
It is 30-fold more potent against the virus enzyme than the host enzyme.

Acyclovir is active against herpes simplex and varicellar-zoster virus.
It is rapidly broken down in cells, is orally active and is relatively non-toxic systemically.
Acyclovir and Valacyclovir (pro-drug, better availability)
A Guanine analogue with antiviral for Herpes group only



1. Inhibits viral DNA polymerase selectively.
2. Incorporated into DNA and terminates synthesis.

Resistance:
1. ↓ activity of thymidine kinase
2. altered DNA polymerase



Acyclovir is used to treat:

- Herpes simplex infections (genital herpes, and herpes encephalitis).
- Chickenpox in immuno-compromised patients.
- Prophylactically in patients treated with immunosuppressant drugs or radiotherapy who are in danger of infection by reactivation of latent virus.
- Prophylactically in patients with frequent recurrences of genital herpes.
- Oral acyclovir has multiple uses. In first episodes of genital herpes, oral acyclovir shortens the duration of symptoms by approximately 2 days, the time to lesion healing by 4 days, and the duration of viral shedding by 7 days. In recurrent genital herpes, the time course is shortened by 1–2 days.
- Oral acyclovir is only modestly beneficial in recurrent herpes labialis.
- Topical acyclovir cream is substantially less effective than oral therapy for primary HSV infection. It is of no benefit in treating recurrent genital herpes.
- Common adverse drug reactions are nausea, vomiting, diarrhea and headache.
- Additional common adverse effects, when acyclovir is administered IV, include :

  Renal insufficiency and neurologic toxicity.

Docosanol

- Docosanol is a saturated 22-carbon aliphatic alcohol that inhibits fusion between the plasma membrane and the HSV envelope, thereby preventing viral entry into cells and subsequent viral replication.
- Topical docosanol 10% cream is available without a prescription; application site reactions occur in approximately 2% of patients.
- When applied within 12 hours of the onset of prodromal symptoms, five times daily, median healing time was shortened by 18 hours compared with placebo in recurrent orolabial herpes.

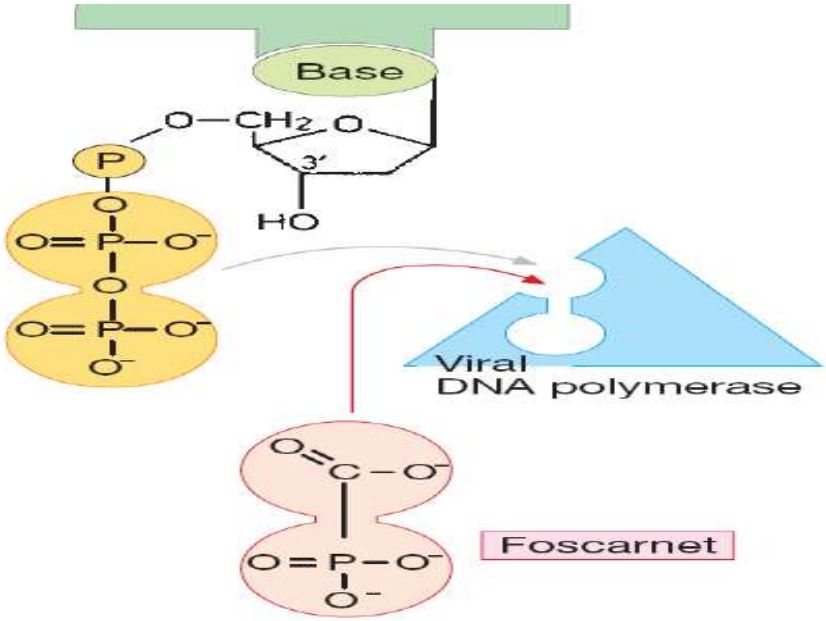
Ganciclovir

- Mechanism like Acyclovir
- Active against all Herpes viruses including CMV (100 time than acyclovir)
- Low oral bioavailability given I.V.
- Most common adverse effect: bone marrow suppression (leukopenia 40%, thrombocytopenia 20%) and CNS effects (headache, behavioral, psychosis, coma, cnvulsions).
- 1/3 of patients have to stop because of adverse effects
- Drug of choice for CMV infections: retinitis, pneumonia, colitis.

Foscarnet

- An inorganic pyrophosphate analog
- Active against Herpes (I, II, Varicella , CMV), including those resistant to Acyclovir and Ganciclovir.
- Direct inhibition of DNA polymerase and Reverse Transcriptase
- Nephrotoxicity (25%) most common side effect
- Use: (1) CMV retinitis and other CMV infections instead of ganciclovir.

  (2) H. simplex resistant to Acyclovir.
  (3) HIV.



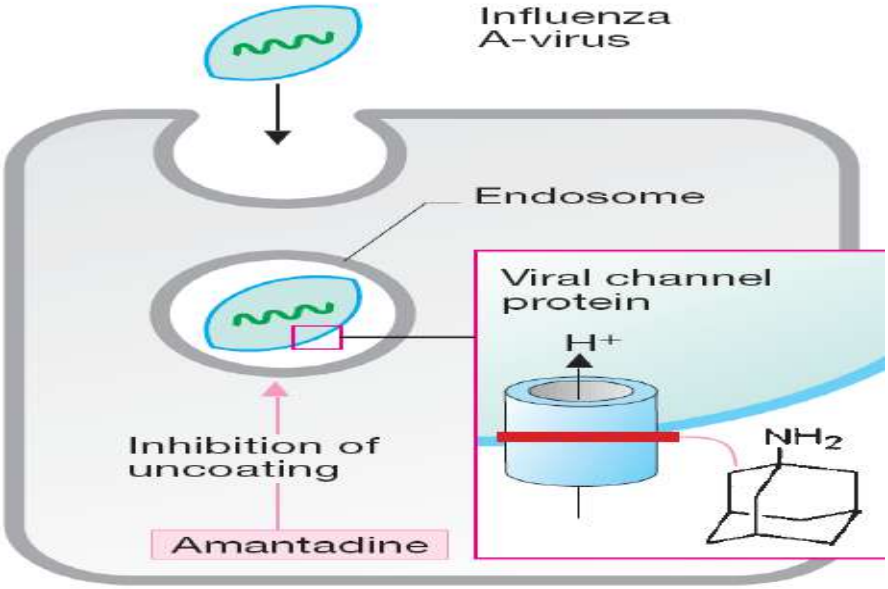
Vidarabine

- Inhibits virally induced DNA polymerase more strongly than it does the endogenous enzyme.
- Vidarabine is a chain terminator and is active against herpes simplex, varicella zoster, and vaccinia are especially sensitive.
- Its use is now limited to topical treatment of severe herpes simplex infection. Before the introduction of the better tolerated acyclovir, vidarabine played a major part in the treatment of herpes simplex encephalitis.
- Its clinically used in treatment of immunocompromised patients with herpetic and vaccinia keratitis and in keratoconjunctivitis.

Treatment of respiratory virus infection

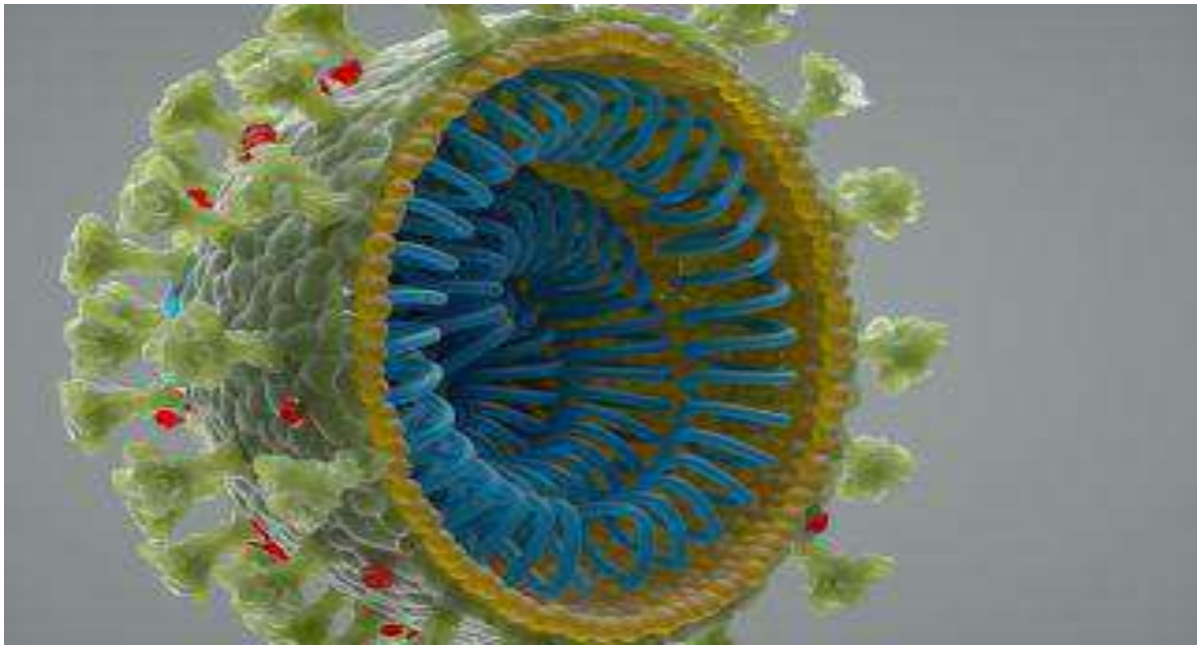
Influenza A & B , and Respiratory suncytial virus (RSV)
Attachment Inhibitors

- The primary antiviral mechanism of Amantadine and Rimantadine is to block the viral membrane matrix protein, which function as an ion channel that is required for the fusion of the viral membrane with the cell membrane.
- Their clinical use is limited to Influenza A infection.
- They are very effective in preventing infection if the treatment is begun at the time of-or prior to-exposure to the virus.



- Side effects of Amantadine are mainly associated with the CNS, such as ataxia and dizziness.
- While Rimantadine produce little CNS effect because it does not penetrate the blood brain barrier.
- Both should be used with caution in pregnant and nursing women.

Treatments for COVID-19: Drugs being tested against the coronavirus



Currently, multiple protease inhibitors like darunavir and atazanavir could prevent viral replication of SARS-CoV-2 which had used for HIV medication.The proteases, which are essential medication for inactivated replication of the virus. The key medicines used in the

national emergency is Lopinavir/ritonavir which included in the COVID-19 management strategy, which is mainly used in patients with COVID-19 infection and patients with less serious symptoms Managed at home as well as in the hospital in the early stages of the disease.

Remdesivir, as well as Immunomodulatory drugs like anti-IL-6 and anti-IL-1 and antimalarials, could be used for treatment covid-19. Tocilizumab was the most used drug in COVID-19 therapy, drugs with antiviral activity, chloroquine, and hydroxychloroquine, are both also have an immunomodulatory activity that could synergistically be able to enhance the *in vivo* antiviral effect. Therapeutic antibodies as a therapeutic option, antibodies are taken from the blood of recovered patients are currently under investigation.

Neuroaminidase inhibitors

Oseltamivir and Zanamavir

Mechanism of action

- Viral neuraminidase catalyzes cleavage of terminal sialic acid residues attached to glycoproteins and glycolipids, a process necessary for release of virus from host cell surfaces.
- Neuraminidase inhibitors thus prevent release of virions from infected cell
- Administration of neuraminidase inhibitors is a treatment that limits the severity and spread of viral infections.
- Neuraminidase inhibitors are useful for combating influenza infection:

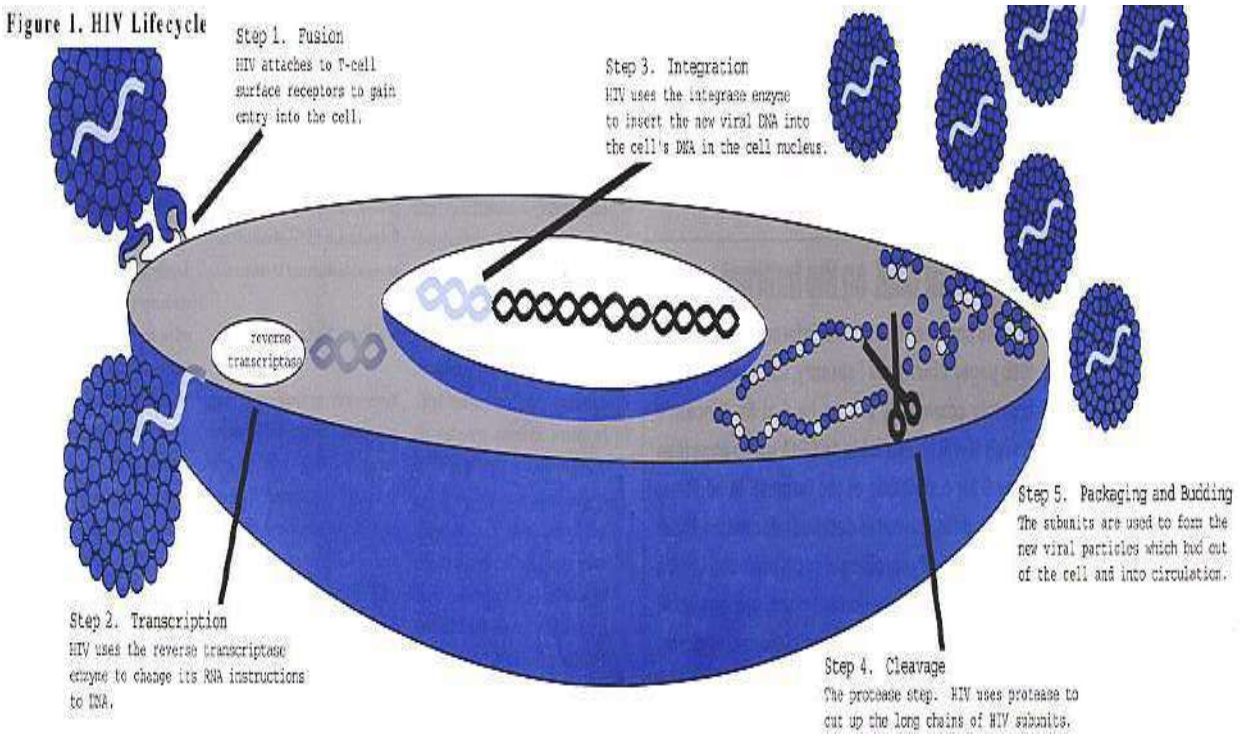
  zanamivir, administered by inhalation;
  oseltamivir, administered orally.
- Toxicities

  – Exacerbation of reactive airway disease by zanamavir
  – Nausea and vomiting for oseltamivir

Ribavirin

- It is an antimetabolite that inhibits influenza RNA polymerase .
- An aerosol form is used against RSV (respiratory syncytial virus) and the drug is used intravenously against Lassa fever.
- Adverse reactions includes: Anemia due to hemolysis and bone marrow suppression

Antiretroviral agents



Azidothymidine (Zidovudin(AZT))

- It is a potent antagonist of reverse transcriptase, It is a chain terminator.
- Cellular enzyme phosphorylate AZT to the triphosphate form which inhibits RT and causes chain termination
- It is widely use in the treatment of AIDS (The only clinical use).
- AZT is toxic to bone marrow, for example, it cause severe anaemia and leukopenia In patient receiving high dose. Headache is also common

Didanosine (Dideoxyinosine)

- Didanosine act as chain terminators and inhibitors of reverse transcriptase because they lack a hydroxyl group.
- is phosphorylated to the active metabolite of dideoxyadenosine triphosphate
- It is used in the treatment of AIDS (second drug approved to treat HIV-1 infection).
- They are given orally,
- and their main toxicities are pancreatitis, peripheral neuropathy, GI disturbance, bone marrow depression.

Abacavir

- Abacavir is a guanosine analog that is well absorbed following oral administration (83%) and is unaffected by food. The serum half-life is 1.5 hours. The drug undergoes hepatic glucuronidation and carboxylation. Cerebrospinal fluid levels are approximately one third those of plasma.

- Abacavir is often co-administered with lamivudine, and a once-daily, fixed-dose combination formulation is available. Abacavir is also available in a fixed-dose combination with lamivudine and zidovudine.
- High-level resistance to abacavir appears to require at least two or three concomitant mutations and thus tends to develop slowly.
- Hypersensitivity reactions, occasionally fatal, have been reported in up to 8% of patients receiving abacavir and may be more severe in association with once-daily dosing.
- All NRTIs may be associated with mitochondrial toxicity, probably owing to inhibition of mitochondrial DNA polymerase gamma. Less commonly, lactic acidosis with hepatic steatosis may occur, which can be fatal.

Rash

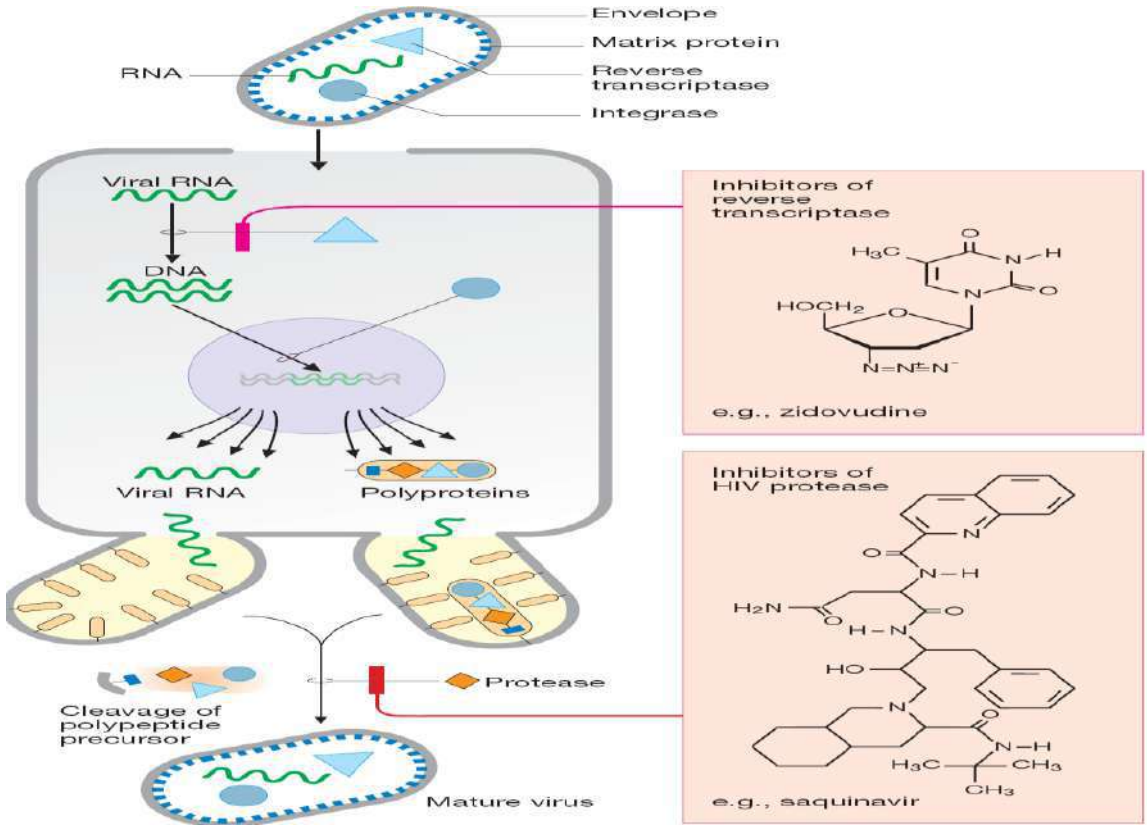
Rash, usually a maculopapular eruption that spares the palms and soles, occurs in up to 20% of patients, usually in the first 4–6 weeks of therapy.

Although typically mild and self-limited, rash is dose-limiting in about 7% of patients. Women appear to have an increased incidence of rash.

When initiating therapy, gradual dose escalation over 14 days is recommended to decrease the incidence of rash.

Protease Inhibitors

- HIV Protease Inhibitors; have significantly alter the course of the HIV disease.
- All are reversible inhibitors of HIV Protease-the viral enzyme responsible for cleavage of viral polyprotein into number of essential enzymes (reverse transcription, polymerase).
- Examples are : Saquinavir, and Ritonavir.
- They are orally active, side effects include GI disturbances and hyperglycemia, interact with cytochrome.



(HAART)

- Highly active anti-retroviral therapies
- Combination therapies (triple drug cocktail, HAART) are very effective and can reduce viral load in the patient below detectable levels implying that HIV replication has ceased.
- The trouble with all of these complicated drug regimens is compliance. The components of HAART must be taken at different times.
- Non-compliance with protease inhibitor therapy is of serious concern as the new virus that emerges is resistant to the inhibitor being taken and also resistant to other protease inhibitors.

Anti-Hepatitis B Virus Agents

Treatment for acute hepatitis B infection

If hepatitis B virus infection is acute — meaning it is short-lived and will go away on its own — you may not need treatment. Instead, your doctor might recommend rest, proper nutrition and plenty of fluids while your body fights the infection. In severe cases, antiviral drugs or a hospital stay is needed to prevent complications.

Treatment for chronic hepatitis B infection

Most people diagnosed with chronic hepatitis B infection need treatment for the rest of their lives. Treatment helps reduce the risk of liver disease and prevents patients from passing the infection to others. Treatment for chronic hepatitis B may include:

- **Antiviral medications.** Several antiviral medications — including entecavir (Baraclude), tenofovir (Viread), lamivudine (Epivir), adefovir (Hepsera) and telbivudine (Tyzeka) — can help fight the virus and slow its ability to damage the liver. These drugs are taken by mouth. Talk to your doctor about which medication might be right for you.
- **Interferon injections.** Interferon alfa-2b (Intron A) is a man-made version of a substance produced by the body to fight infection. It's used mainly for young people with hepatitis B who wish to avoid long-term treatment or women who might want to get pregnant within a few years, after completing a finite course of therapy. Interferon should not be used during pregnancy. Side effects may include nausea, vomiting, difficulty breathing and depression.

Liver transplant. If the liver has been severely damaged, a liver transplant may be an option.

Main viruses' families

The International Committee on Taxonomy of Viruses began to devise and implement rules for the naming and classification of viruses early in the 1970s, an effort that continues to the present. In 2018, 6 classes, 14 orders, 5 suborders, 143 families, 64 subfamilies, 846 genera, and 4,958 species of viruses have been defined by the ICTV.

Families of Animal Viruses that Contain Members Able to Infect Humans includes:

1-DNA containing viruses 8 families (Parvoviridae, Anelloviridae, Polyomaviridae, Papillomaviridae, Adenoviridae, Hepadnaviridae, Herpesviridae, Poxviridae).

2-RNA containing viruses 17 families (Picornaviridae, Astroviridae, Caliciviridae, Hepeviridae, Picobirnaviridae, Reoviridae, Togaviridae, Flaviviridae, Arenaviridae, Coronaviridae, Retroviridae, Orthomyxoviridae, Bunyaviridae, Bornaviridae, Rhabdoviridae, Paramyxoviridae, Filoviridae).

DNA containing viruses

Herpesviridae

The herpesvirus family contains several of the most important human viral pathogens. Clinically, the herpesviruses exhibit a spectrum of diseases. Some have a wide host-cell range, and others have a narrow host-cell range. The outstanding property of herpesviruses is their ability to establish lifelong persistent infections in their hosts and to undergo periodic reactivation. Their frequent reactivation in immunosuppressed patients causes serious health complications.

Curiously, the reactivated infection may be clinically quite diferent from the disease caused by the primary infection. Herpesviruses possess a large number of genes, some of which have proved to be susceptible to antiviral chemotherapy. The herpesviruses that commonly infect humans include herpes simplex virus types 1 and 2 (HSV-1, HSV-2), varicella zoster virus, cytomegalovirus (CMV), Epstein-Barr virus (EBV), herpesviruses 6 and 7, and herpesvirus 8 (Kaposi sarcoma-associated herpesvirus [KSHV]). Herpes B virus of monkeys can also infect humans. There are nearly 100 viruses of the herpes group that infect many different animal species.

Properties of herpesviruses: Important properties of herpesviruses are summarized below.

Virion: Spherical, 150–200 nm in diameter (icosahedral)
genome: Double-stranded DNA, linear, 125–240 kbp, reiterated sequences.
Proteins: More than 35 proteins in virion
Envelope: Contains viral glycoproteins, Fc receptors
Replication: Nucleus, bud from nuclear membrane

Outstanding characteristics:
Encode many enzymes
Establish latent infections

Persist indefinitely in infected hosts
Frequently reactivated in immunosuppressed hosts
Some cause cancer

Structure and Composition

Herpesviruses are large viruses. Different members of the group share architectural details and are indistinguishable by electron microscopy. All herpesviruses have a core of double- stranded DNA, in the form of a toroid, surrounded by a protein coat that exhibits icosahedral symmetry and has 162 capsomeres. The nucleocapsid is surrounded by an envelope that is derived from the nuclear membrane of the infected cell and contains viral glycoprotein spikes about 8 nm long. An amorphous, sometimes asymmetric structure between the capsid and envelope is designated the tegument. The enveloped form measures 150–200 nm; the "naked" virion, 125 nm.

The double-stranded DNA genome (125–240 kbp) is linear. A striking feature of herpesvirus DNAs is their sequence arrangement. Herpesvirus genomes possess terminal and internal repeated sequences. Some members, such as the HSVs, undergo genome rearrangements, giving rise to different genome "isomers." The base composition of herpesvirus DNAs varies from 31% to 75% (G + C). There is little DNA homology among different herpesviruses except for HSV-1 and HSV-2, which show 50% sequence homology, and human herpesviruses 6 and 7 (HHV-6 and HHV-7), which display limited (30–50%) sequence homology. Treatment with restriction endonucleases yields characteristically different cleavage patterns for herpesviruses and even for different strains of each type. This "fingerprinting" of strains allows epidemiologic tracing of a given strain.

The herpesvirus genome is large and encodes at least 100 different proteins. Of these, more than 35 polypeptides are involved in the structure of the virus particle; at least 10 are part of the viral envelope. Herpesviruses encode an array of virus-specifi c enzymes involved in nucleic acid metabolism, DNA synthesis, gene expression, and protein regulation (DNA polymerase, helicase-primase, thymidine kinase, transcription factors, protein kinases). Many herpesvirus genes appear to be viral homologs of cellular genes.

Classification

Classification of the numerous members of the herpesvirus family is complicated. A useful division into subfamilies is based on biologic properties of the agents.

- Alpha herpesviruses are fast-growing, cytolytic viruses that tend to establish latent infections in neurons; HSV (genus *Simplexvirus*) and varicella-zoster virus (genus *Varicellovirus*) are members.

- Beta herpesviruses are slow growing and may be cytomegalic (massive enlargements of infected cells) and become latent in secretory glands and kidneys; CMV is classified in the *Cytomegalovirus* genus. Also included here, in the genus

Roseolovirus, are HHV-6 and HHV-7; by biologic criteria, they are similar to gammaherpesviruses because they infect lymphocytes (T lymphotropic), but molecular analyses of their genomes reveal that they are more closely related to the beta herpesviruses.

- Gamma herpesviruses, exemplified by EBV (genus *Lymphocryptovirus*), infect and become latent in lymphoid cells. KSHV, designated as HHV-8, is classified in the *Rhadinovirus* genus.

Herpesvirus Replication

The virus enters the cell by fusion with the cell membrane after binding to specific cellular receptors via envelope glycoproteins. Several herpesviruses bind to cell surface glycosaminoglycans, principally heparan sulfate. Virus attachment also involves binding to one of several coreceptors (eg, members of the immunoglobulin superfamily). After fusion, the capsid is transported through the cytoplasm to a nuclear pore, uncoating occurs, and the DNA becomes associated with the nucleus. The viral DNA forms a circle immediately upon release from the capsid. Expression of the viral genome is tightly regulated and sequentially ordered in a cascade fashion. VP16, a tegument protein, complexes with several cellular proteins and activates initial viral gene expression. Immediate-early genes are expressed, yielding "α" proteins. These proteins permit expression of the early set of genes, which are translated into "β" proteins. Viral DNA replication begins, and late transcripts are produced that give rise to "γ" proteins. More than 50 different proteins are synthesized in herpesvirus-infected cells. Many α and β proteins are enzymes or DNA-binding proteins; most of the γ proteins are structural components.

Viral DNA is transcribed throughout the replicative cycle by cellular RNA polymerase II but with the participation of viral factors. Viral DNA is synthesized by a rolling- circle mechanism. Herpesviruses differ from other nuclear DNA viruses in that they encode a large number of enzymes involved in DNA synthesis. (These enzymes are good targets for antiviral drugs.) Newly synthesized viral DNA is packaged into preformed empty nucleocapsids in the cell nucleus.

Maturation occurs by budding of nucleocapsids through the altered inner nuclear membrane. Enveloped virus particles are then transported by vesicular movement to the surface of the cell. The length of the replication cycle varies from about 18 hours for HSV to more than 70 hours for CMV. Cells productively infected with herpesviruses are invariably killed.

Host macromolecular synthesis is shut off early in infection; normal cellular DNA and protein synthesis virtually stop as viral replication begins. Cytopathic effects induced by human herpesviruses are quite distinct.

The number of potential protein-coding open-reading frames in herpesvirus genomes ranges from about 70 to more than 200. In the case of HSV, about half the genes are not needed for growth in cultured cells. The other genes are probably required for viral

survival in vivo in natural hosts.

Herpesviruses have recently been found to express multiple microRNAs, small (~22 nucleotides) single-stranded RNAs that function posttranscriptionally to regulate gene expression. It is predicted that these viral microRNAs are important in regulating entry into or exit from (or both) the latent phase of the virus life cycle and may be attractive targets for antiviral therapy.

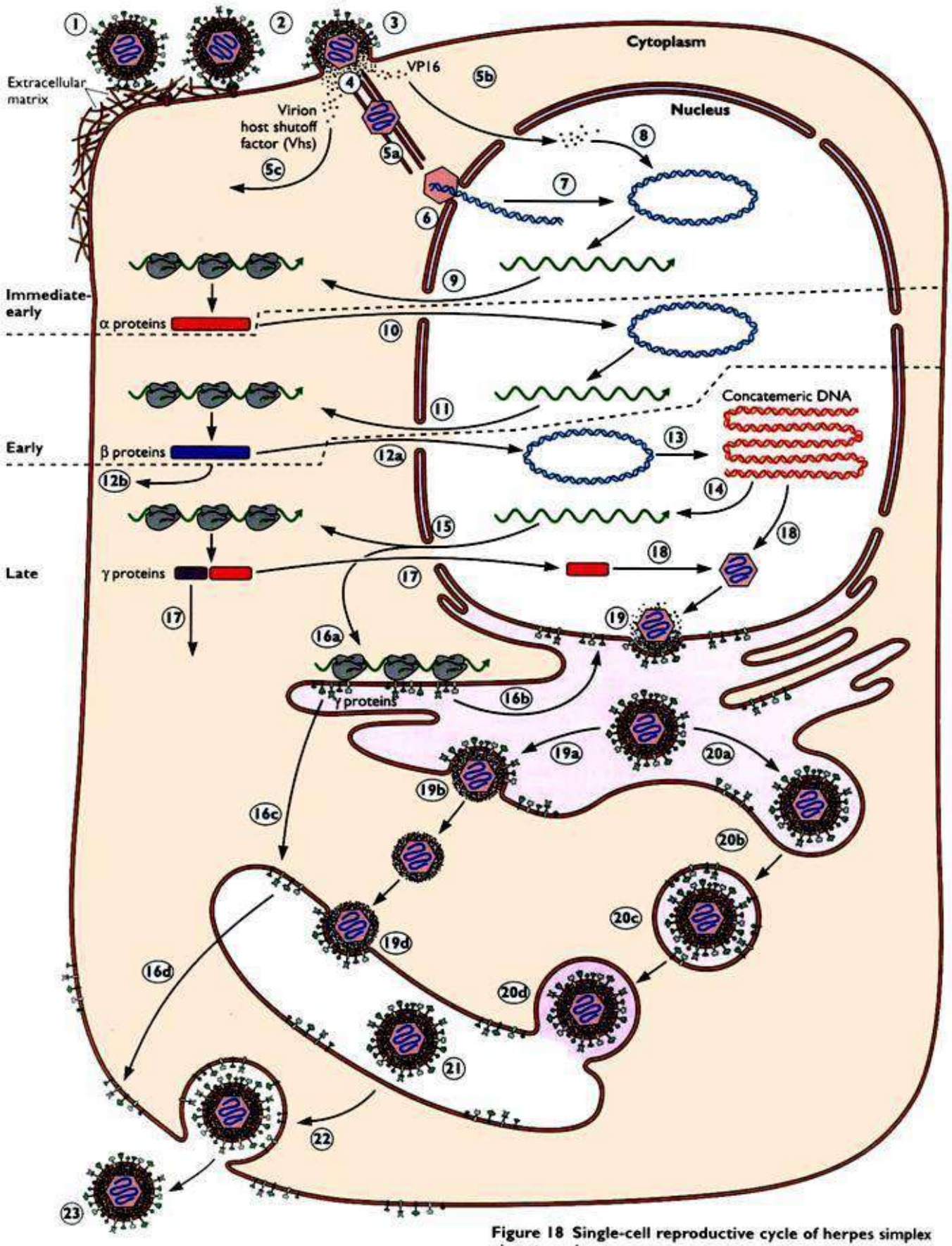


Figure 18 Single-cell reproductive cycle of herpes simplex virus type 1.

Virus latency

Virus latency (or **viral latency**) is the ability of a pathogenic virus to lie dormant (latent) within a cell, denoted as the lysogenic part of the viral life cycle. A latent viral infection is a type of persistent viral infection which is distinguished from a chronic viral infection. Latency is the phase in certain viruses' life cycles in which, after initial infection, proliferation of virus particles ceases. However, the viral genome is not fully eradicated. The result of this is that the virus can reactivate and begin producing large amounts of viral progeny without the host being infected by new outside virus, denoted as the lytic part of the viral life cycle, and stays within the host indefinitely.

Virus latency is not to be confused with clinical latency during the incubation period when a virus is *not* dormant.

Mechanisms of latency

Episomal latency

Episomal latency refers to the use of genetic episomes during latency. In this type, viral genes are stabilized floating in the cytoplasm or nucleus as distinct objects, both as linear or lariat structures. Episomal latency is more vulnerable to ribozymes or host foreign gene degradation than provirus latency.

One example is Herpes Virus family, *Herpesviridae*, all of which establish latent infection. Herpes virus include Chicken-pox virus and Herpes simplex viruses (HSV-1, HSV-2), all of which establish episomal latency in neurons and leave linear genetic material floating in the cytoplasm. The *Gamma herpesvirinae* subfamily is associated with episomal latency established in cells of the immune system, such as B-cells in the case of Epstein- Barr Virus. In the case of Herpes simplex (HSV), the virus has been shown that it fuses with DNA in neurons, such as brain cells, and HSV reactivates upon even minor chromatin loosening with stress, although the chromatin compacts (becomes latent) upon oxygen and nutrient deprivation.

Advantages of episomal latency include the fact that the virus may not need to enter the nucleus, and hence may avoid Nuclear Domain 10 (ND10) from activating interferon via that pathway. Disadvantages include more exposure to cellular defenses, leading to possible degradation of viral gene via cellular enzymes.

Reactivation may be due to stress, UV, etc.

Proviral latency

A provirus is a virus genome that is integrated into the DNA of a host cell.

A**dvantages** include automatic host cell division results in replication of the virus's genes, and the fact that it is nearly impossible to remove an integrated provirus from an infected cell without killing the cell.

A **disadvantages** of this method is the need to enter the nucleus (and the need for packaging proteins that will allow for that). However, viruses that integrate into the host cell's genome can stay there as long as the cell lives.

One of the best-studied viruses that does this is HIV. HIV uses reverse transcriptase to create a DNA copy of its RNA genome. HIV latency allows the virus to largely avoid the immune system. Like other viruses that go latent, it does not typically cause symptoms while latent. Unfortunately, HIV in proviral latency is nearly impossible to target with antiretroviral drugs.

Maintaining latency

Both proviral and episomal latency may require maintenance for continued infection and fidelity of viral genes. Latency is generally maintained by viral genes expressed primarily during latency. Expression of these *latency-associated* genes may function to keep the viral genome from being digested by cellular ribozymes or being found out by the immune system. Certain viral gene products (RNA transcripts such as non-coding RNAs and proteins) may also inhibit apoptosis or induce cell growth and division to allow more copies of the infected cell to be produced.

An example of such a gene product is the *Latency Associated Transcripts (LAT)* in Herpes simplex virus, which interfere with apoptosis by down regulating a number of host factors, including Major Histocompatibility Complex (MHC) and inhibiting the apoptotic pathway.

A certain type of latency could be ascribed to the endogenous retroviruses. These viruses have incorporated into the human genome in the distant past, and are now passed through reproduction. Generally these types of viruses have become highly evolved, and have lost the expression of many gene products. Some of the proteins expressed by these viruses have co-evolved with host cells to play important roles in normal processes.

Ramifications

While viral latency exhibits no active viral shedding nor causes any pathologies or symptoms, the virus is still able to reactivate via external activators (i.e. sunlight, stress) to cause an acute infection. In the case of Herpes simplex virus, which generally infects an individual for life, a serotype of the virus reactivates occasionally to cause cold sores. Although the sores are quickly resolved by the immune system, they may be a minor annoyance from time to time. In the case of varicella zoster virus, after an initial acute infection (chickenpox) the virus lies dormant until reactivated as herpes zoster.

More serious ramifications of a latent infection could be the possibility of transforming the cell, and forcing the cell into uncontrolled cell division. This is a result of the random insertion of the viral genome into the hosts own gene and expression of host cellular

growth factors for the benefit of the virus. A famous event of this actually happening with gene therapy through the use of retroviral vectors is the Necker Hospital in Paris, where 20 young boys received treatment for a genetic disorder, after which 5 developed leukemia-like syndromes.

This is also seen with infections of the human papilloma virus in which persistent infection may lead to cervical cancer as a result of cellular transformation.

In the field of HIV research, proviral latency in specific long-lived cell types is the basis for the concept of one or more viral reservoirs, referring to locations (cell types or tissues) characterized by persistence of latent virus. Specifically, the presence of replication-competent HIV in resting CD4-positive T cells, allows this virus to persist for years without evolving despite prolonged exposure to antiretroviral drugs. This latent reservoir of HIV may explain the inability of antiretroviral treatment to cure HIV infection.

Overview of Herpesvirus Diseases

A wide variety of diseases are associated with infection by herpesviruses. Primary infection and reactivated disease by a given virus may involve different cell types and present different clinical pictures.

HSV-1 and HSV-2 infect epithelial cells and establish latent infections in neurons. Type 1 is classically associated with oropharyngeal lesions and causes recurrent attacks of "fever blisters." Type 2 primarily infects the genital mucosa and is mainly responsible for genital herpes. Both viruses also cause neurologic disease. HSV-1 is the leading cause of sporadicencephalitis in the United States. Both types 1 and 2 can cause neonatal infections that are often severe.

Varicella-zoster virus causes chickenpox (varicella) on primary infection and establishes latent infection in neurons. Upon reactivation, the virus causes zoster (shingles). Adults who are infected for the first time with varicella-zoster virus are apt to develop serious viral pneumonia.

CMV replicates in epithelial cells of the respiratory tract, salivary glands, and kidneys and persists in lymphocytes. It causes an infectious mononucleosis (heterophil-negative).

In newborns, cytomegalic inclusion disease may occur. CMV is an important cause of congenital defects and mental retardation.

HHV-6 infects T lymphocytes. It is typically acquired in early infancy and causes exanthem subitum (roseola infantum).HHV-7, also a T-lymphotropic virus, has not yet been linked to any specific disease.

EBV replicates in epithelial cells of the oropharynx and parotid gland and establishes latent infections in lymphocytes. It causes infectious mononucleosis and is the cause of

human lymphoproliferative disorders, especially in immunocompromised patients.

HHV-8 appears to be associated withthe development of Kaposi sarcoma, a vascular

tumor that is common in patients with AIDS.

Human herpesviruses are frequently reactivated in immunosuppressed patients (eg, transplant recipients, cancer patients) and may cause severe disease, such as pneumonia or lymphomas.

Herpesviruses have been linked with malignant diseases in humans and lower animals: EBV with Burkitt lymphoma of African children, with nasopharyngeal carcinoma, and with other lymphomas; KSHV with Kaposi sarcoma.

HERPES SIMPLEX VIRUSES

HSV are extremely widespread in the human population. They exhibit a broad host range, being able to replicate in many types of cells and to infect many different animals. They grow rapidly and are highly cytolytic. The HSVs are responsible for a spectrum of diseases, ranging from gingivostomatitis to keratoconjunctivitis, encephalitis, genital disease, and infections of newborns. The HSVs establish latent infections in nerve cells; recurrences are common.

Properties of the Viruses

There are two distinct HSV, types 1 and 2 (HSV-1 and HSV-2)

Their **genomes are similar in organization** and exhibit substantial sequence homology. However, they can be distinguished by sequence analysis or by restriction enzyme analysis of viral DNA. The two viruses cross-react serologically, but some unique proteins exist for each type. They differ in **their mode of transmission**. Whereas HSV-1 is spread by contact, usually involving infected saliva, HSV-2 is transmitted sexually or from a maternal genital infection to a newborn. This results in different clinical features of human infections. The HSV growth cycle proceeds rapidly, requiring 8–16 hours for completion. The HSV genome is large (~150 kbp) and can encode at least 70 polypeptides; the functions of many of the proteins in replication or latency are not known.

At least eight viral glycoproteins are among the viral late gene products. One (gD) is the most potent inducer of neutralizing antibodies. Glycoprotein C is a complement (C3b)-binding protein, and gE is an Fc receptor, binding to the Fc portion of immunoglobulin G (IgG). Glycoprotein G is type specific and allows for antigenic discrimination between HSV-1 (gG-1) and HSV-2 (gG-2).

Pathogenesis and Pathology

A. Pathology

Because HSV causes cytolytic infections, pathologic changes are due to necrosis of infected cells together with the inflammatory response. Lesions induced in the skin and mucous membranes by HSV-1 and HSV-2 are the same and resemble those of varicella-zoster virus. Changes induced by HSV are similar for primary and recurrent infections but vary in degree, reflecting the extent of viral cytopathology.

Characteristic histopathologic changes include ballooning of infected cells, production of Cowdry type A intranuclear inclusion bodies, margination of chromatin, and formation of multinucleated giant cells. Cell fusion provides an efficient method for cell-to-cell spread of HSV, even in the presence of neutralizing antibody.

B. Primary Infection

HSV is transmitted by contact of a susceptible person with an individual excreting virus.

The virus must encounter mucosal surfaces or broken skin for an infection to be initiated (unbroken skin is resistant). HSV-1 infections are usually limited to the oropharynx, and the virus is spread by respiratory droplets or by direct contact with infected saliva. HSV-2 is usually transmitted by genital routes. Viral replication occurs first at the site of infection. Virus then invades local nerve endings and is transported by retrograde axonal flow to dorsal root ganglia, where, after further replication, latency is established. Whereas oropharyngeal HSV-1 infections result in latent infections in the trigeminal ganglia, genital HSV-2 infections lead to latently infected sacral ganglia. Viremia is more common during primary HSV-2 infections than during HSV-1 infections.

Primary HSV infections are usually mild; in fact, most are asymptomatic. Only rarely does systemic disease develop. Widespread organ involvement can result when an immunocompromised host is not able to limit viral replication and viremia ensues.

C. Latent Infection

Virus resides in latently infected ganglia in a non replicating state; only a very few viral genes are expressed. Viral persistence in latently infected ganglia lasts for the lifetime of the host. No virus can be recovered between recurrences at or near the usual site of recurrent lesions. Provocative stimuli can reactivate virus from the latent state, including axonal injury, fever, physical or emotional stress, and exposure to ultraviolet light. The virus follows axons back to the peripheral site, and replication proceeds at the skin or mucous membranes. Spontaneous reactivations occur despite HSV specific humoral and cellular immunity in the host. However, this immunity limits local viral replication, so that recurrent infections are less extensive and less severe. Many recurrences are asymptomatic, reflected only by viral shedding in secretions. When symptomatic, episodes of recurrent HSV-1 infection are usually manifested as cold sores (fever blisters) near the lip. More than 80% of the human population harbor HSV-1 in a latent form, but only a small portion experience recurrences. It is not known why some individuals have reactivations and others do not.

Clinical Findings

HSV-1 and HSV-2 may cause many clinical entities, and the infections may be primary or recurrent. Primary infections occur in persons without antibodies and in most individuals are clinically in apparent but result in antibody production and establishment of latent infections in sensory ganglia. Recurrent lesions are common.

A. Oropharyngeal Disease

Primary HSV-1 infections are usually asymptomatic. Symptomatic disease occurs most frequently in small children (1–5 years of age) and involves the buccal and gingival mucosa of the mouth. The incubation period is short (~3–5 days, with a range of 2–12 days), and clinical illness lasts 2–3 weeks. Symptoms include fever, sore throat, vesicular and ulcerative lesions, gingivostomatitis, and malaise. Gingivitis (swollen, tender gums) is the most striking and common lesion. Primary infections in adults commonly cause pharyngitis and tonsillitis. Localized lymphadenopathy may occur.

Recurrent disease is characterized by a cluster of vesicles most commonly localized at the border of the lip. Intense pain occurs at the outset but fades over 4–5 days. Lesions

progress through the pustular and crusting stages and healing without scarring is usually complete in
8–10 days. The lesions may recur, repeatedly and at various intervals, in the same location. The frequency of recurrences varies widely among individuals. Many recurrences of oral shedding are asymptomatic and of short duration (24 hours).

B. Keratoconjunctivitis

HSV-1 infections may occur in the eye, producing severe keratoconjunctivitis. Recurrent lesions of the eye are common and appear as dendritic keratitis or corneal ulcers or as vesicles on the eyelids. With recurrent keratitis, there may be progressive involvement of the corneal stroma, with permanent opacification and blindness. HSV-1 infections are second only to trauma as a cause of corneal blindness in the United States.

C. Genital Herpes

Genital disease is usually caused by HSV-2, although HSV-1 can also cause clinical episodes of genital herpes. Primary genital herpes infections can be severe, with illness lasting about 3 weeks. Genital herpes is characterized by vesiculoulcerative lesions of the penis of the male or of the cervix, vulva, vagina, and perineum of the female. The lesions are very painful and may be associated with fever, malaise, dysuria, and inguinal lymphadenopathy. Complications include extragenital lesions (~20% of cases) and aseptic meningitis (~10% of cases). Viral excretion persists for about 3 weeks. Because of the antigenic cross-reactivity between HSV-1 and HSV-2, preexisting immunity provides some protection against heterotypic infection. An initial HSV-2 infection in a person already immune to HSV-1 tends to be less severe. Recurrences of genital herpetic infections are common and tend to be mild. A limited number of vesicles appear and heal in about 10 days. Virus is shed for only a few days. Some recurrences are asymptomatic with anogenital shedding lasting less than 24 hours. Whether a recurrence is symptomatic or asymptomatic, a person shedding virus can transmit the infection to sexual partners.

D. Skin Infections

Intact skin is resistant to HSV, so cutaneous HSV infections are uncommon in healthy persons. Localized lesions caused by HSV-1 or HSV-2 may occur in abrasions that becomecontaminated with the virus (traumatic herpes). These lesions are seen on the fingers of dentists and hospital personnel (herpetic whitlow) and on the bodies of wrestlers (herpes
gladiatorum or mat herpes). Cutaneous infections are often severe and life threatening when they occur in individuals with disorders of the skin, such as eczema or burns that permit extensive local viral replication and spread. Eczema herpeticum is a primary infection, usually with HSV-1, in a person with chronic eczema. In rare instances, the illness may be fatal.

E. Encephalitis

A severe form of encephalitis may be produced by herpesvirus. HSV-1 infections are considered the most common cause of sporadic, fatal encephalitis in the United States. The disease carries a high mortality rate, and those who survive often have residual neurologic

defects. About half of patients with HSV encephalitis appear to have primary infections, and the rest appear to have recurrent infection.

F. Neonatal Herpes

HSV infection of the newborn may be acquired in utero, during birth, or after birth. The mother is the most common source of infection in all cases. Neonatal herpes is estimated to occur in about 1 in 5000 deliveries per year. The newborn infant seems to be unable to limit the replication and spread of HSV and has a propensity to develop severe disease.

The most common route of infection (~75% of cases) is for HSV to be transmitted to a newborn during birth by contact with herpetic lesions in the birth canal. To avoid infection, delivery by cesarean section has been used in pregnant women with genital herpes lesions. However, many fewer cases of neonatal HSV infection occur than cases of recurrent genital herpes, even when the virus is present at term. Neonatal herpes can be acquired postnatally by exposure to either HSV-1 or HSV-2. Sources of infection include family members and hospital personnel who are shedding virus. About 75% of neonatal herpes infections are caused by HSV-2. There do not appear to be any differences between the nature and severity of neonatal herpes in premature or fullterm infants, in infections caused by HSV-1 or HSV-2, or in disease when virus is acquired during delivery or postpartum. Neonatal herpes infections are almost always symptomatic. The overall mortality rate of untreated disease is
50%. Babies with neonatal herpes exhibit three categories of disease: (1) lesions localized to the skin, eye, and mouth; (2) encephalitis with or without localized skin involvement; and (3) disseminated disease involving multiple organs, including the central nervous system. The worst prognosis (~80% mortality rate) applies to infants with disseminated infection, many of whom develop encephalitis. The cause of death of babies with disseminated disease is usually viral pneumonitis or intravascular coagulopathy. Many survivors of severe infections are left with permanent neurologic impairment.

G. Infections in Immunocompromised Hosts

Immunocompromised patients are at increased risk of developing severe HSV infections. These include patient's immunosuppressed by disease or therapy (especially those with deficient cellular immunity) and individuals with malnutrition. Renal, cardiac, and bone marrow transplant recipients are at particular risk for severe herpes infections. Patients with hematologic malignancies and patients with AIDS have more frequent and more severe HSV infections. Herpes lesions may spread and involve the respiratory tract, esophagus, and intestinal mucosa. Malnourished children are prone to fatal disseminated HSV infections. In most cases, the disease reflects reactivation of latent HSV infection.

Immunity

Many newborns acquire passively transferred maternal antibodies. These antibodies are lost during the first 6 months of life, and the period of greatest susceptibility to primary herpes infection occurs between ages 6 months and 2 years.

During primary infections, IgM antibodies appear transiently and are followed by IgG and IgA antibodies that persist for long periods. These antibodies do not prevent reinfection or reactivation of latent virus.

Cell-mediated immunity and nonspecific host factors (natural killer cells, interferon) are important in controlling both primary and recurrent HSV infections.

Laboratory Diagnosis

A. Polymerase Chain Reaction

Polymerase chain reaction (PCR) assays can be used to detect virus and are sensitive and specific. PCR amplification of viral DNA from cerebrospinal fluid.

B. Isolation and Identification of Virus

Virus isolation remains the definitive diagnostic approach. Virus may be isolated from herpetic lesions and may also be found in throat washings, cerebrospinal fluid, and stool, both during primary infection and during asymptomatic periods.

Inoculation of tissue cultures is used for viral isolation.

HSV is easy to cultivate, and cytopathic effects usually occur n only 2–3 days. The agent is then identified by neutralization test or immunofluorescence staining with specific antiserum.

C. Cytopathology

A rapid cytologic method is to stain scrapings obtained from the base of a vesicle (eg, with Giemsa's stain); the presence of multinucleated giant cells indicates that herpesvirus .

Epidemiology

HSV are worldwide in distribution. No animal reservoirs or vectors are involved with the human viruses.

The epidemiology of HSV-1 and HSV-2 differs. HSV-1 is probably more constantly present in humans than any other virus.

Treatment, Prevention, and Control

Several antiviral drugs have proved effective against HSV infections, including acyclovir, valacyclovir, and vidarabine. All are inhibitors of viral DNA synthesis.

Experimental vaccines of various types are being developed.

Hepatitis Viruses

Viral hepatitis is a systemic disease primarily involving the liver. Most cases of acute viral hepatitis in children and adults are caused by one of the following five agents: hepatitis A virus (HAV), the etiologic agent of viral hepatitis type A (infectious hepatitis); hepatitis B virus (HBV), which is associated with viral hepatitis B (serum hepatitis); hepatitis C virus (HCV), the agent of hepatitis C (common cause of post transfusion hepatitis); hepatitis D (HDV), a defective virus dependent on coinfection with HBV; or hepatitis E virus (HEV), the agent of enterically transmitted hepatitis. Additional well-characterized viruses that can cause sporadic hepatitis, such as yellow fever virus, cytomegalovirus, Epstein-Barr virus, herpes simplex virus, rubella virus, and the enteroviruses.

Hepatitis viruses produce acute inflammation of the liver, resulting in a clinical illness characterized by fever, gastrointestinal symptoms such as nausea, vomiting, and jaundice. Regardless of the virus type, identical histopathologic lesions are observed in the liver during acute disease.

PROPERTIES OF HEPATITIS VIRUSES

The characteristics of the five known hepatitis viruses are shown in Table 1.

Virus	Hepatitis A	Hepatitis B	Hepatitis C	Hepatitis D	Hepatitis E
Family	Picornaviridae	Hepadnaviridae	Flaviviridae	Unclassified	Hepeviridae
Genus	*Hepatovirus*	*Orthohepadnavirus*	*Hepacivirus*	*Deltavirus*	*Hepevirus*
Virion	27 nm, icosahedral	42 nm, spherical	60 nm, spherical	35 nm, spherical	30–32 nm, icosahedral
Envelope	No	Yes (HBsAg)	Yes	Yes (HBsAg)	No
Genome	ssRNA	dsDNA	ssRNA	ssRNA	ssRNA
Genome size (kb)	7.5	3.2	9.4	1.7	7.2
Stability	Heat and acid stable	Acid sensitive	Ether sensitive, acid sensitive	Acid sensitive	Heat stable
Transmission	Fecal–oral	Parenteral	Parenteral	Parenteral	Fecal–oral
Prevalence	High	High	Moderate Low,	Regional	Regional
Fulminant	Rare	Rare	rare	Frequent	In pregnancy

disease					
Chronic disease	Never	Often	Often	Often	Never
Oncogenic	No	Yes	Yes	?	No

Hepatitis Type A

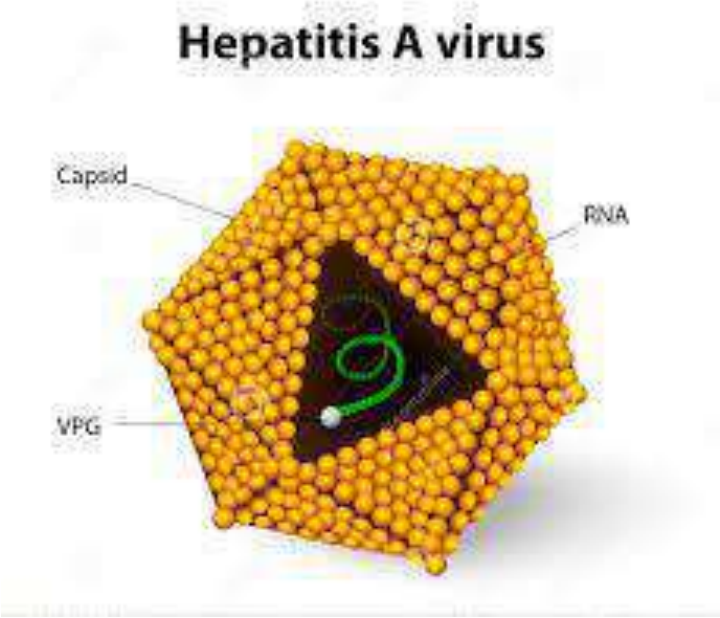
HAV is a distinct member of the picornavirus family. HAV is a 27- to 32-nm spherical particle with cubic symmetry containing a linear single-stranded RNA genome with a size of 7.5 kb. It is assigned to picornavirus genus, *Hepatovirus.* Only one serotype is known. There is no antigenic cross-reactivity with HBV or with the other hepatitis viruses. Genomic sequence analysis of a variable region involving the junction of the 1D and 2A genes divided HAV isolates into seven genotypes.

HAV is stable to treatment with 20% ether, acid (pH 1.0 for 2 hours), and heat (60°C for 1 hour), and its infectivity can be preserved for at least 1 month after being dried and stored at 25°C or for years at −20°C. The virus is destroyed by autoclaving (121°C for 20 minutes), boiling in water for 5 minutes, dry heat (180°C for 1 hour), ultraviolet irradiation (1 minute at 1.1 watts), treatment with formalin (1:4000 for 3 days at 37°C), or treatment with chlorine (10–15 ppm for 30 minutes). Heating food to above 85°C (185°F) for 1 minute and disinfecting surfaces with sodium hypochlorite (1:100 dilution of chlorine bleach) are necessary to inactivate HAV. The relative resistance of HAV to disinfection procedures emphasizes the need for extra precautions in dealing with hepatitis patients and their products.

HAV initially was identified in stool and liver preparations by using immune electron microscopy as the detection system. Sensitive serologic assays and polymerase chain reaction (PCR) methods have made it possible to detect HAV in stools and other samples and to measure specific antibody in serum. Various primate cell lines will support growth of HAV, although fresh isolates of virus are difficult to adapt and grow. Usually, no cytopathic effects are apparent. Mutations in the viral genome are selected during adaptation to tissue culture.

HAV antigens and antibodies

- Anti-HAV Antibody to HAV. Detectable at onset of symptoms; lifetime persistence.
- IgM anti-HAV IgM class antibody to HAV. Indicates recent infection with hepatitis A; positive result up to 4–6 months after infection



Hepatitis Type B

HBV is classified as a hepadnavirus. HBV establishes chronic infections, especially in those infected as infants; it is a major factor in the eventual development of liver disease and hepatocellular carcinoma in those individuals.

A. Structure and Composition

Electron microscopy of hepatitis B surface antigen (HBsAg)- positive serum reveals three morphologic forms. The most numerous are spherical particles measuring 22 nm in diameter. These small particles are made up exclusively of HBsAg—as are tubular or filamentous forms, which have the same diameter but may be more than 200 nm long—and result from overproduction of HBsAg. Larger, 42-nm spherical virions (originally referred to as Dane particles) are less frequently observed. The outer surface, or envelope, contains HBsAg and surrounds a 27-nm inner nucleocapsid core that contains hepatitis B core antigen (HBcAg). The variable length of a single-

stranded region of the circular DNA genome results in genetically heterogeneous particles with a wide range of buoyant densities.

The viral genome consists of partially double- stranded circular DNA, 3200 bp in length. Different HBV isolates share 90–98% nucleotide sequence homology. The full-length DNA minus strand (L or long strand) is complementary to all HBV mRNAs; the positive strand (S or short strand) is variable and between 50% and 80% of unit length.

There are four open reading frames that encode seven polypeptides. These include structural proteins of the virion surface and core, a small transcriptional transactivator (X), and a large polymerase (P) protein that includes DNA polymerase reverse transcriptase, and RNase H activities. The S gene has three in-frame initiation codons and encodes the major HBsAg, as well as polypeptides containing in addition pre-S2 or pre-S1 and pre-S2 sequences. The C gene has two in-frame initiation codons and encodes HBcAg plus the HBe protein, which is processed to produce soluble hepatitis B e antigen (HBeAg). The particles containing HBsAg are antigenically complex. Each contains a group-specific antigen, *a,* in addition to two pairs of mutually exclusive subdeterminants, *d/y* and *w/r*.

Thus, four phenotypes of HBsAg have been observed: *adw, ayw, adr,* and *ayr*. In the United States, *adw* is the predominant subtype. These virus-specific markers are useful in epidemiologic investigations because secondary cases have the same subtype as the index case.

The stability of HBsAg does not always coincide with that of the infectious agent. However, both are stable at −20°C for more than 20 years and stable to repeated freezing and thawing. The virus also is stable at 37°C for 60 minutes and remains viable after being dried and stored at 25°C for at least 1 week. HBV (but not HBsAg) is sensitive to higher temperatures (100°C for 1 minute) or to longer incubation periods (60°C for 10 hours). HBsAg is stable at a pH of 2.4 for up to 6 hours, but HBV infectivity is lost. Sodium hypochlorite, 0.5% (eg, 1:10 chlorine bleach), destroys antigenicity within 3 minutes at low protein concentrations, but undiluted serum specimens require higher concentrations (5%). HBsAg is not destroyed by ultraviolet

irradiation of plasma or other blood products, and viral infectivity may also resist such treatment.

HBV antigens and antibodies

HBsAg	Hepatitis B surface antigen. Surface antigen(s) of HBV detectable in large quantity in serum; several subtypes identified
HBeAg	Hepatitis B e antigen. Associated with HBV nucleocapsid; indicates viral replication; circulates as soluble antigen in serum
HBcAg	Hepatitis B core antigen
Anti-HBs	Antibody to HBsAg. Indicates past infection with and immunity to HBV, presence of passive antibody from HBIG, or immune response from HBV vaccine.
Anti-HBe	Antibody to HBeAg. Presence in serum of HBsAg carrier suggests lower titer of HBV
Anti-HBc	Antibody to HBcAg. Indicates infection with HBV at some undefined time in the past
IgM anti-HBc	IgM class antibody to HBcAg. Indicates recent infection with HBV; positive result for 4–6 months after infection

B. Replication of Hepatitis B Virus

The infectious virion attaches to cells and becomes uncoated. In the nucleus, the partially double-stranded viral genome is converted to covalently closed circular double stranded DNA (cccDNA). The cccDNA serves as template for all viral transcripts, including a 3.5-kb pregenome RNA.

The pregenome RNA becomes encapsidated with newly synthesized HBcAg. Within the cores, the viral polymerase synthesizes by reverse transcription a negative-strand DNA copy. The polymerase starts to synthesize the positive DNA strand, but the process is not completed. Cores bud from the pre-Golgi membranes, acquiring HBsAg-containing envelopes, and may exit the cell. Alternatively, cores may be reimported into the nucleus and initiate another round of replication in the same cell.

Hepatitis Type C

Clinical and epidemiologic studies and cross-challenge experiments in chimpanzees in the past had suggested that there were several non-A, non-B (NANB) hepatitis agents that, based on serologic tests, were not related to HAV or HBV. The major agent was identified

as HCV. HCV is a positive- stranded RNA virus, classified as family Flaviviridae, genus *Hepacivirus.* Various viruses can be differentiated by RNA sequence analysis into at least six major genotypes (clades) and more than 100 subtypes. Clades differ from each other by 25–35% at the nucleotide level; subtypes differ from each other by 15–25%. The genome is 9.4 kb in size and encodes a core protein, two envelope glycoproteins, and several nonstructural proteins (Figure 35-6). The expression of cDNA clones of HCV in yeast led to the development of serologic tests for antibodies to HCV. Most cases of post transfusion NANB hepatitis were caused by HCV.

Most new infections with HCV are subclinical. The majority (70–90%) of HCV patients develop chronic hepatitis, and many are at risk of progressing to chronic active hepatitis and cirrhosis (10–20%). In some countries, as in Japan, HCV infection often leads to hepatocellular carcinoma. About 25,000 individuals die annually of chronic liver disease and cirrhosis in the United States; HCV appears to be a major contributor to this burden (~40%). HCV displays genomic diversity, with different genotypes (clades) predominating in different parts of the world.

The virus undergoes sequence variation during chronic infections. This complex viral population in a host is referred to as "quasi-species." This genetic diversity is not correlated with differences in clinical disease, although differences do exist in response to antiviral therapy according to viral genotype.

HCV antibodies and antigens

Anti-HCV Antibody to HCV

Hepatitis Type D (Delta Hepatitis)

An antigen–antibody system termed the delta antigen (delta-Ag) and antibody (anti-delta) is detected in some HBV infections. The antigen is found within certain HBsAg particles. In blood, HDV (delta agent) contains delta-Ag (HDAg) surrounded by an HBsAg envelope. It has a particle size of 35–37 nm and a buoyant density of 1.24–1.25 g/mL in CsCl. The genome of HDV consists of single-stranded, circular, negative-sense RNA, 1.7 kb in size. It is the smallest of known human pathogens and resembles subviral plant pathogens (ie, viroids). No homology exists with the HBV genome. HDAg is the

only protein coded for by HDV RNA and is distinct from the antigenic determinants of HBV. HDV is a defective virus that acquires an HBsAg coat for transmission. It is often associated with the most severe forms of hepatitis in HBsAg positive patients. It is classified in the *Deltavirus* genus, which is not assigned to any virus family.

HDV antibodies and antigens

HDAg Delta antigen (delta-Ag). Detectable in early acute HDV infection

Anti-HD Antibody to delta-Ag (anti-delta). Indicates past or present infection with HDV

Hepatitis Type E

HEV is transmitted enterically and occurs in epidemic form in developing countries, where water or food supplies are sometimes fecally contaminated. It was first documented in samples collected during the New Delhi outbreak of 1955, when 29,000 cases of icteric hepatitis occurred after sewage contamination of the city's drinking water supply. An epidemic occurred in Kashmir, India, in 1978 that resulted in an estimated 1700 deaths. Pregnant women may have a high (20%) mortality rate if fulminant hepatitis develops. The viral genome has been cloned and is a positive-sense, single-stranded RNA 7.2 kb in size. The virus is classified in the virus family, Hepeviridae, in the genus *Hepevirus*. HEV resembles, but is distinct from, caliciviruses. Animal strains of HEV are common throughout the world. There is evidence of HEV or HEV-like infections in rodents, pigs, sheep, and cattle in the United States. There is the possibility of spread of virus from animals to humans.

HEV antigen and antibodies

anti-HEV IgM antibodies IgM antibodies develop after 4-5 days of infection. An early-diagnostic marker

IgG antibody to HEV diagnostic marker

Hepatitis virus infections in humans

Pathology

Hepatitis is a general term meaning inflammation of the liver. Microscopically, there is spotty parenchymal cell degeneration, with necrosis of hepatocytes, a diffuse lobular inflammatory reaction, and disruption of liver cell cords.

These parenchymal changes are accompanied by reticuloendothelial (Kupffer) cell hyperplasia, periportal infiltration by mononuclear cells, and cell degeneration. Localized areas of necrosis are frequently observed. Later in the course of the disease, there is an accumulation of macrophages near degenerating hepatocytes. Preservation of the reticulum framework allows hepatocyte regeneration so that the highly ordered architecture of the liver lobule can be ultimately regained. The damaged hepatic tissue is usually restored in 8–12 weeks. Chronic carriers of HBsAg may or may not have demonstrable evidence of liver disease. Persistent (unresolved) viral hepatitis, a mild benign disease that may follow acute hepatitis B in 8–10% of adult patients, is characterized by sporadically abnormal aminotransferase values and hepatomegaly. Histologically, the lobular architecture is preserved, with portal inflammation, swollen and pale hepatocytes (cobblestone arrangement), and slight to absent fibrosis. This lesion is frequently observed in asymptomatic carriers, usually does not progress toward cirrhosis, and has a favorable prognosis.

Chronic active hepatitis features a spectrum of histologic changes from inflammation and necrosis to collapse of the normal reticulum framework with bridging between the portal triads or terminal hepatic veins. HBV is detected in 10–50% of these patients.

Occasionally during acute viral hepatitis, more extensive damage may occur that prevents orderly liver cell regeneration. Such fulminant or massive hepatocellular necrosis is seen in 1–2% of jaundiced patients with hepatitis B. It is 10 times more common in those coinfected with HDV than in the absence of HDV.

None of the hepatitis viruses are typically cytopathogenic, and it is believed that the cellular damage seen in hepatitis is immune-mediated. Both HBV and HCV have significant roles in the development of hepatocellular carcinoma that may appear many (15–60) years after establishment of chronic infection.

Clinical Findings

In individual cases, it is not caused by the hepatitis viruses. Other viral diseases that may present as hepatitis are infectious mononucleosis, yellow fever, cytomegalovirus infection, herpes simplex, rubella, and some enterovirus infections. Hepatitis may occasionally occur as a complication of leptospirosis, syphilis, tuberculosis, toxoplasmosis, and amebiasis, all of which are susceptible to specific drug therapy. Noninfectious causes include biliary obstruction, primary biliary cirrhosis, Wilson disease, drug toxicity, and drug hypersensitivity reactions.

In viral hepatitis, onset of jaundice is often preceded by gastrointestinal symptoms such as nausea, vomiting, anorexia, and mild fever. Jaundice may appear within a few days of the prodromal period, but anicteric hepatitis is more common.

Extrahepatic manifestations of viral hepatitis (primarily type B) include a transient serum sickness-like prodrome consisting of fever, skin rash, and polyarthritis; necrotizing vasculitis (polyarteritis nodosa); and glomerulonephritis. Circulating immune complexes have been suggested as the cause of these syndromes. Diseases associated with chronic HCV infections include mixed cryoglobulinemia and glomerulonephritis. Extrahepatic manifestations are unusual with HAV infections.

Uncomplicated viral hepatitis rarely continues for more than 10 weeks without improvement. Relapses occur in 5–20% of cases and are manifested by abnormalities in liver function with or without the recurrence of clinical symptoms.

The median incubation period is different for each type of viral hepatitis. However, there is considerable overlap in timing, and the patient may not know when exposure occurred, so the incubation period is not very useful in determining the specific viral cause.

The onset of disease tends to occur abruptly with HAV (within 24 hours) in contrast to a more insidious onset with HBV and HCV. Complete recovery occurs in most hepatitis A cases; chronicity has not been observed. The disease is more severe in adults than in children, in whom it often goes unnoticed. Relapses of HAV infection can occur 1–4 months after initial symptoms have resolved.

The outcome after infection with HBV varies, ranging from complete recovery to progression to chronic hepatitis and, rarely, death from fulminant disease. In adults, 65–80% of infections are inapparent, with 90–95% of all patients recovering completely. In

contrast, 80–95% of infants and young children infected with HBV become chronic carriers, and their serum remains positive for HBsAg. The vast majority of individuals with chronic HBV remain asymptomatic for many years; there may or may not be biochemical and histologic evidence of liver disease. Chronic carriers are at high risk of developing hepatocellular carcinoma.

Fulminant hepatitis occasionally develops during acute viral hepatitis, defined as hepatic encephalopathy within the first 8 weeks of disease in patients without preexisting liver disease. It is fatal in 70–90% of cases, with survival uncommon after the age of 40 years. Fulminant HBV disease is associated with super infection by other agents, including HDV. In most patients who survive, complete restoration of the hepatic parenchyma and normal liver function is the rule. Fulminant disease rarely occurs with HAV or HCV infections.

Hepatitis C is usually clinically mild, with only minimal to moderate elevation of liver enzymes. Hospitalization is unusual, and jaundice occurs in fewer than 25% of patients. Despite the mild nature of the disease, 70–90% of cases progress to chronic liver disease. Most patients are asymptomatic, but histologic evaluation often reveals evidence of chronic active hepatitis, especially in those whose disease is acquired after transfusion.

Many patients (20–50%) develop cirrhosis and are at high risk for hepatocellular carcinoma (5–25%) decades later. About 40% of chronic liver disease is HCV related, resulting in an estimated 8000–10,000 deaths annually in the United States. End-stage liver disease associated with HCV is the most frequent indication for adult liver transplants.

Laboratory Features

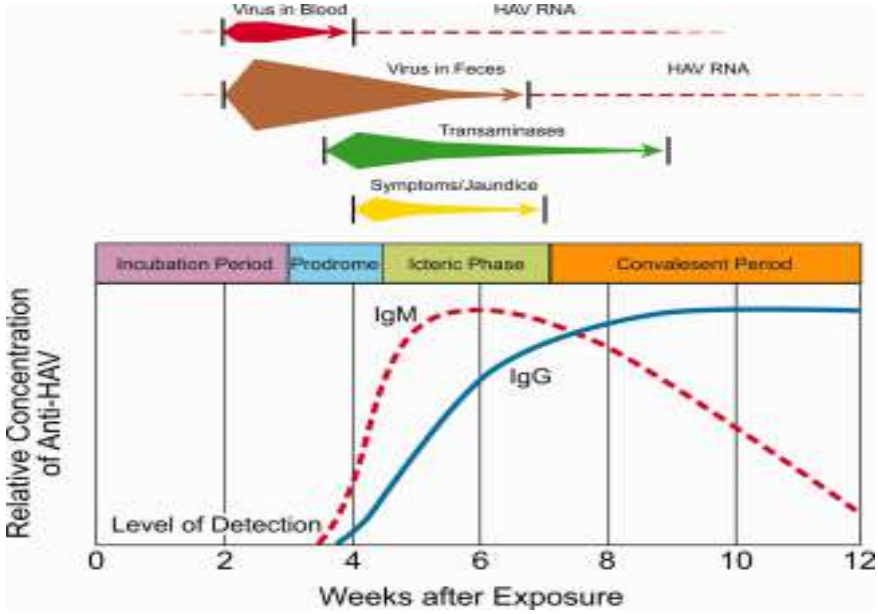
Liver biopsy permits a tissue diagnosis of hepatitis. Tests for abnormal liver function, such as serum alanine aminotransferase (ALT) and bilirubin, supplement the clinical, pathologic, and epidemiologic findings.

A. Hepatitis A

The Virus particles have been detected by immune electron microscopy in fecal extracts of hepatitis A patients (Figure 3). Virus appears early in the disease and disappears within

2 weeks after the onset of jaundice.

HAV can be detected in the liver, stool, bile, and blood of naturally infected humans and experimentally infected nonhuman primates by immunoassays, nucleic acid hybridization assays, or PCR. HAV is detected in the stool from about 2 weeks before the onset of jaundice up to 2 weeks after. Anti-HAV appears in the immunoglobulin M (IgM) fraction during the acute phase, peaking about 2 weeks after elevation of liver enzymes. Anti-HAV IgM usually declines to non detectable levels within 3–6 months. Anti- HAV IgG appears soon after the onset of disease and persists for decades. Thus, detection of IgM-specific anti-HAV in the blood of an acutely infected patient confirms the diagnosis of hepatitis A. Enzyme-linked immunosorbent assay is the method of choice for measuring HAV antibodies.



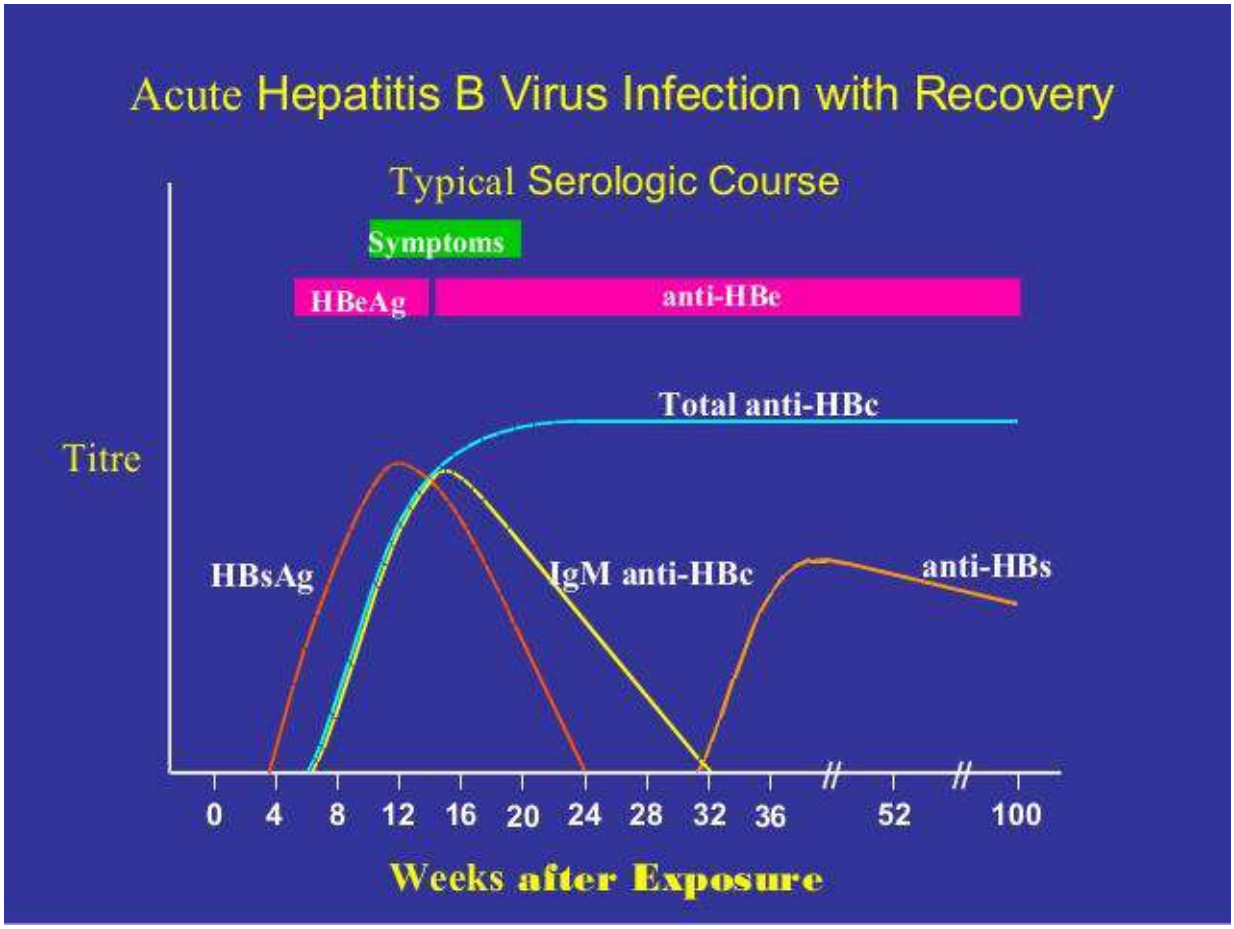
B. Hepatitis B

DNA polymerase activity, HBV DNA, and HBeAg, which are representative of the viremic stage of hepatitis B, occur early in the incubation period, concurrently or shortly after the first appearance of HBsAg. High concentrations of HBV particles may be present in the blood (up to 1010 particles/mL) during the initial phase of infection; communicability is highest at this time. HBsAg is usually detectable 2–6 weeks in advance of clinical and biochemical evidence of hepatitis and persists throughout the clinical course of the disease but typically disappears by the sixth month after exposure.

High levels of IgM-specific anti-HBc are frequently detected at the onset of clinical illness. Because this antibody is directed against the 27-nm internal core component of HBV, its appearance in the serum is indicative of viral replication. Antibody to HBsAg is first detected at a variable period after the disappearance of HBsAg. It is present in low concentrations. Before HBsAg disappears, HBeAg is replaced by anti-HBe, signaling the start of resolution of the disease. Anti-HBe levels often are no longer detectable after 6 months.

By definition, HBV chronic carriers are those in whom HBsAg persists for more than 6 months in the presence of HBeAg or anti-HBe. HBsAg may persist for years after loss of HBeAg. In contrast to the high titers of IgM-specific anti- HBc observed in acute disease, low titers of IgM anti-HBc are found in the sera of most chronic HBsAg carriers. Small amounts of HBV DNA are usually detectable in the serum as long as HBsAg is present.

The most useful detection methods are enzyme-linked immunosorbent assay for HBV antigens and antibodies and PCR for viral DNA.



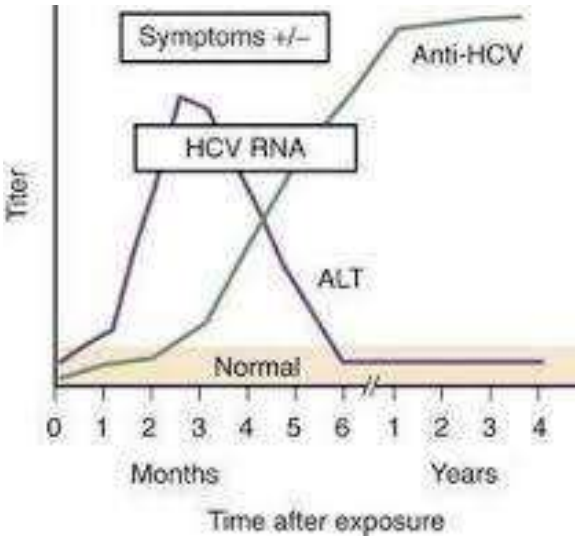
C. Hepatitis C

Most primary infections are asymptomatic or clinically mild (20–30% have jaundice; 10–20% have only nonspecific symptoms such as anorexia, malaise, and abdominal pain). Serologic assays are available for diagnosis of HCV infection. Enzyme immunoassays detect antibodies to HCV but do not distinguish among acute, chronic, or resolved infection. Anti-HCV antibodies can be detected in 50–70% of patients at the onset of symptoms, but in others, antibody appearance is delayed 3–6 weeks.

Antibodies are directed against core, envelope, and NS3 and NS4 proteins and tend to be relatively low in titer. Nucleic acid-based assays (eg, reverse transcription PCR) detect the presence of circulating HCV RNA and are useful for monitoring patients on antiviral therapy. Nucleic acid assays also are used to genotype HCV isolates.

Occult HBV infections occur frequently (~33%) in patients with chronic HCV liver disease. Occult infections are those in which patients lack detectable HBsAg but HBV DNA can be identified in liver or serum samples. These unrecognized HBV coinfections

may be clinically.



D. Hepatitis D

Because HDV depends on a coexistent HBV infection, acute type D infection occurs either as a simultaneous infection (coinfection) with HBV or as a superinfection of a person chronically infected with HBV. In the coinfection pattern, antibody to HDAg develops late in the acute phase of infection and may be of low titer. Assays for HDAg or HDV RNA in the serum or for IgM-specific anti-HD are preferable. All markers of HDV replication disappear during convalescence; even the HDV antibodies may disappear within months to years.

However, super infection by HDV usually results in persistent HDV infection (more than 70% of cases). High levels of both IgM and IgG anti-HD persist, as do levels of HDV RNA and HDAg. HDV superinfections may be associated with fulminant hepatitis.

Epidemiology

A. Hepatitis A

HAV is widespread throughout the world. Outbreaks of type A hepatitis are common in families and institutions, summer camps, day care centers, neonatal intensive care units, and among military troops. The most likely mode of transmission under these conditions is by the fecal–oral route through close personal contact.

Groups that are at increased risk of acquiring hepatitis A are travelers to developing

countries from developed countries, men who have sex with men, users of injection and noninjection drugs, persons with clotting factor disorders, and persons working with nonhuman primates. Individuals with chronic liver disease are at increased risk for fulminant hepatitis if a hepatitis A infection occurs. These groups should be vaccinated.

B. Hepatitis B

HBV is worldwide in distribution. Transmission modes and response to infection vary, depending on the age at time of infection. Most individuals infected as infants develop chronic infections. As adults, they are subject to liver disease and are at high risk of developing hepatocellular carcinoma.

The incubation period of hepatitis B is 50–180 days, with a mean between 60 and 90 days. It appears to vary with the dose of HBV administered and the route of administration, being prolonged in patients who receive a low dose of virus or who are infected by a nonpercutaneous route.

C. Hepatitis C

Infections by HCV are extensive throughout the world. The World Health Organization estimated in 1997 that about 3% of the world population has been infected, with population subgroups in Africa having prevalence rates as high as 10%. Other high-prevalence areas are found in South America and Asia.

The average incubation period for HCV is 6–7 weeks. The average time from exposure to seroconversion is 8–9 weeks, and about 90% of patients are anti-HCV positive within 5 months.

D. Hepatitis D (Delta Agent)

HDV is found throughout the world but with a non uniform distribution. Its highest prevalence has been reported in America. HDV infects all age groups. Persons who have received multiple transfusions, intravenous drug abusers, and their close contacts are at high risk.

The primary routes of transmission are believed to be similar to those of HBV. Infection depends on HBV replication because HBV provides an HBsAg envelope for HDV.

Treatment

Treatment of patients with hepatitis is supportive and directed at allowing hepatocellular damage to resolve and repair itself.

Only HBV and HCV have specific treatments, and those are only partially effective. Recombinant IFN-(and pegylated IFN) -are currently the therapy of proven benefit in the treatment of patients chronically infected with HBV or HCV. Several antiviral drugs are available for use against chronic hepatitis infections. , such as lamivudine HBV DNA levels are reduced, but the virus is rarely eliminated and viral replication resumes in the majority of patients when treatment is stopped.

Prevention and Control

Viral vaccines and protective IG preparations are available against HAV and HBV. Neither type of reagent is currently available to prevent HCV infections.

A. Standard Precautions

Simple environmental procedures can limit the risk of infection to health care workers, laboratory personnel, and others. With this approach, all blood and body fluids and materials contaminated with them are treated as if they are infectious for HIV, HBV, HCV, and other bloodborne pathogens.

B. Hepatitis A

Formalin-inactivated HAV vaccines made from cell culture adapted virus were licensed in the United States in 1995. The vaccines are safe, effective, and recommended for use in persons more than 1 year of age.

Immune globulin (IG) is prepared from large pools of normal adult plasma and confers passive protection in about 90% of those exposed when given within 1–2 weeks after exposure to hepatitis A.

C. Hepatitis B

A vaccine for hepatitis B has been available since 1982. Although plasma-derived

vaccines (purifying HBsAg from healthy HBsAg-positive carriers and treating the particles with virus-inactivating agents) are still in use in certain countries, they have been replaced in the United States by recombinant DNA-derived vaccines.

Hepatitis B vaccination is the most effective measure to prevent HBV and its consequences.

Studies on passive immunization using specific hepatitis B immune globulin (HBIG) have shown a protective effect if it is given soon after exposure.

D. Hepatitis C

There is no vaccine for hepatitis C although several candidate vaccines are undergoing tests. Control measures focus on prevention activities that reduce risks for contracting HCV.

E. Hepatitis D

Delta hepatitis can be prevented by vaccinating HBV susceptible persons with hepatitis B vaccine. However, vaccination does not protect hepatitis B carriers from superinfection by HDV.

Orthomyxoviruses (Influenza Viruses)

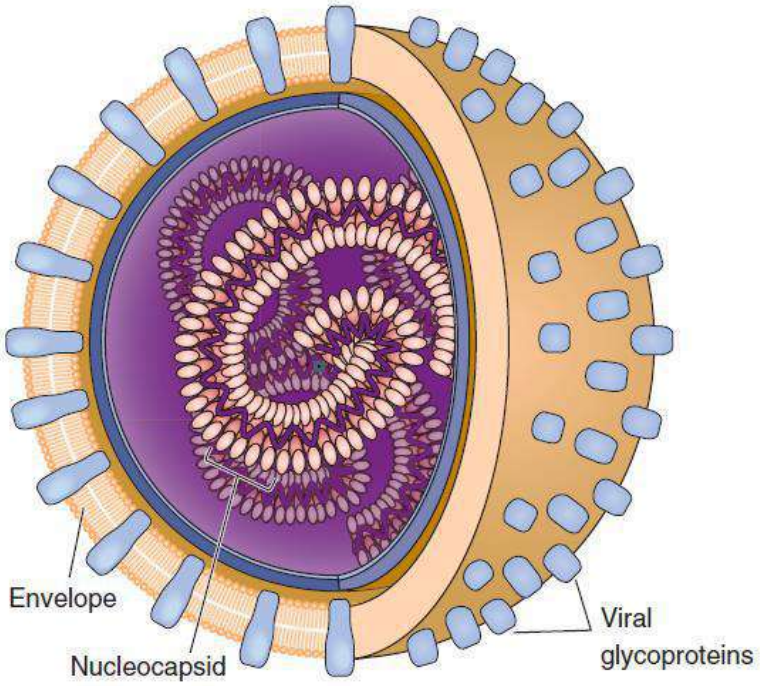
The Orthomyxoviridae (influenza viruses) are a major determinant of morbidity and mortality caused by respiratory disease, and outbreaks of infection sometimes occur in worldwide epidemics. Mutability and high frequency of genetic reassortment and resultant antigenic changes in the viral surface glycoproteins make influenza viruses formidable challenges for control efforts. Influenza type A is antigenically highly variable and is responsible for most cases of epidemic influenza. Influenza type B may exhibit antigenic changes and sometimes causes epidemics. Influenza type C is antigenically stable and causes only mild illness in immunocompetent individuals.

PROPERTIES OF ORTHOMYXOVIRUSES

Three immunologic types of influenza viruses are known, designated A, B, and C. Whereas antigenic changes continually occur within the type A group of influenza viruses and to a lesser degree in the type B group, type C appears to be antigenically stable. Influenza A strains are also known for aquatic birds, chickens, ducks, pigs, horses, and seals. Some of these strains isolated from animals are antigenically similar to strains circulating in the human population.

Virion: Spherical, pleomorphic, 80–120 nm in diameter (helical nucleocapsid, 9 nm)
Composition: RNA (1%), protein (73%), lipid (20%), carbohydrate (6%)
Genome: Single-stranded RNA, segmented (eight molecules), negative-sense, 13.6 kb overall size
Proteins: Nine structural proteins, one nonstructural
Envelope: Contains viral hemagglutinin and neuraminidase proteins
Replication: Nuclear transcription; capped 5′ termini of cellular RNA scavenged as primers; particles mature by budding from plasma membrane
Outstanding characteristics: Genetic reassortment common among members of the same genus Influenza viruses cause worldwide epidemics

[a]Description of influenza A virus, genus *Influenzavirus A*.



Structure and Composition

Influenza virus particles are usually spherical and about 100 nm in diameter (80–120 nm), although virions may display great variation in size. 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. A lipid envelope derived from the cell surrounds the virus particle. Two virus-encoded glycoproteins, hemagglutinin (HA) and neuraminidase (NA), are inserted into the envelope and are exposed as spikes about 10 nm long on the surface of the particle. Because of the segmented nature of the genome, when a cell is coinfected by two different viruses of a given type, mixtures of parental gene segments may be assembled into progeny virions. This phenomenon, called **genetic reassortment**, may result in sudden changes in viral surface antigens.

Classification and Nomenclature

Genus *Influenzavirus A* contains human and animal strains of influenza type A,

Influenzavirus B contains human strains of type B, and *Influenzavirus C* contains influenza type C viruses of humans and swine. Antigenic differences exhibited by two of the internal structural proteins, the nucleocapsid (NP) and matrix (M) proteins, are used to divide influenza viruses into types A, B, and C. The standard nomenclature system for influenza virus isolates includes the following information: type, host of origin, geographic origin, strain number, and year of isolation.

Structure and Function of Hemagglutinin

The HA protein of influenza virus binds virus particles to susceptible cells and is the major antigen against which neutralizing (protective) antibodies are directed. Variability in HA is primarily responsible for the continual evolution of new strains and subsequent influenza epidemics. HA derives its name from its ability to agglutinate erythrocytes under certain conditions.

Structure and Function of Neuraminidase

The antigenicity of NA, the other glycoprotein on the surface of influenza virus particles, is also important in determining the subtype of influenza virus isolates. The NA functions at the end of the viral replication cycle. It facilitates release of virus particles from infected cell surfaces during the budding process and helps prevent self-aggregation of virions by removing sialic acid residues from viral glycoproteins. It is possible that NA helps the virus negotiate through the mucin layer in the respiratory tract to reach the target epithelial cells.

Antigenic Drift and Antigenic Shift

Influenza viruses are remarkable because of the frequent antigenic changes that occur in HA and NA. Antigenic variants of influenza virus have a selective advantage over the parental virus in the presence of antibody directed against the original strain. This phenomenon is responsible for the unique epidemiologic features of influenza. Other respiratory tract agents do not display significant antigenic variation. The two surface antigens of influenza undergo antigenic variation independent of each other. Minor antigenic changes are termed **antigenic drift**; major antigenic changes in HA or NA, called **antigenic shift**, result in the appearance of a new subtype. Antigenic shift is most likely to result in an epidemic. Antigenic drift is caused by the accumulation of point

mutations in the gene, resulting in amino acid changes in the protein. Sequence changes can alter antigenic sites on the molecule such that a virion can escape recognition by the host's immune system. The immune system does not cause the antigenic variation but rather functions as a selection force that allows new antigenic variants to expand. A variant must sustain two or more mutations before a new, epidemiologically significant strain emerges. Antigenic shift reflects drastic changes in the sequence of a viral surface protein, caused by genetic reassortment between human, swine, and avian influenza viruses. Influenza B and C viruses do not exhibit antigenic shift because few related viruses exist in animals.

INFLUENZA VIRUS INFECTIONS IN HUMANS

A comparison of influenza A virus with other viruses that infect the human respiratory tract is shown in Table 39-3. Influenza virus is considered here.

TABLE 39-3 Comparison of Viruses That Infect the Human Respiratory Tract

Virus	Disease	Number of Serotypes	Lifelong Immunity to Disease	Vaccine Available	Viral Latency
RNA viruses					
Influenza A virus	Influenza	Many	No	+	–
Metapneumovirus	Croup, bronchiolitis	Several	No	–	–
Parainfluenza virus	Croup	Many	No	–	–
Respiratory syncytial virus	Bronchiolitis, pneumonia	Two	No	–	–
Rubella virus	Rubella	One	Yes	+	–
Measles virus	Measles	One	Yes	+	–
Mumps virus	Parotitis, meningitis	One	Yes	+	–
Rhinovirus	Common cold	Many	No	–	–
Coronavirus	Common cold	Many	No	–	–
Coxsackievirus	Herpangina, pleurodynia	Many	No	–	–
DNA viruses					
Herpes simplex virus type 1	Gingivostomatitis	One	No	–	+
Epstein-Barr virus	Infectious mononucleosis	One	Yes	–	+
Varicella-zoster virus	Chickenpox, shingles	One	Yes*	+	+
Adenovirus	Pharyngitis, pneumonia	Many	No	–	+

*Lifelong immunity to reinfections with varicella (chickenpox) but not to reactivation of zoster (shingles).

Pathogenesis and Pathology

Influenza virus spreads from person to person by airborne droplets or by contact

with contaminated hands or surfaces. A few cells of respiratory epithelium are infected if deposited virus particles avoid removal by the cough reflex and escape neutralization by preexisting specific immunoglobulin A (IgA) antibodies or inactivation by nonspecific inhibitors in the mucous secretions. Progeny virions are soon produced and spread to adjacent cells, where the replicative cycle is repeated. Viral NA lowers the viscosity of the mucous film in the respiratory tract, laying bare the cellular surface receptors and promoting the spread of virus-containing fluid to lower portions of the tract. Within a short time, many cells in the respiratory tract are infected and eventually killed. The incubation period from exposure to virus and the onset of illness varies from 1 day to 4 days, depending on the size of the viral dose and the immune status of the host. Viral shedding starts the day preceding onset of symptoms, peaks within 24 hours, remains elevated for 1–2 days, and then declines over the next 5 days. Infectious virus is very rarely recovered from blood.

Clinical Findings

A. Uncomplicated Influenza

Symptoms of classic influenza usually appear abruptly and include chills, headache, and dry cough followed closely by high fever, generalized muscular aches, malaise, and anorexia. These symptoms may be induced by any strain of influenza A or B. In contrast, influenza C rarely causes the influenza syndrome, causing instead a common cold illness. children may have higher fever and a higher incidence of gastrointestinal manifestations such as vomiting. Febrile seizures can occur. Influenza A viruses are an important cause of croup, which may be severe, in children younger than 1 year of age. Finally, otitis media may develop.

B. Pneumonia

Pneumonia complicating influenza infections can be viral, secondary bacterial, or a combination of the two. Increased mucous secretion helps carry agents into the lower respiratory tract. Influenza infection enhances susceptibility of patients to bacterial superinfection. This is attributed to loss of ciliary clearance, dysfunction of phagocytic cells, and provision of a rich bacterial growth medium by the alveolar exudate. Combined

viral–bacterial pneumonia is approximately three times more common than primary influenza pneumonia.

C. Reye Syndrome

Reye syndrome is an acute encephalopathy of children and adolescents, usually between 2 and 16 years of age.

Immunity

Immunity to influenza is long lived and subtype specific. Whereas antibodies against HA and NA are important in immunity to influenza, antibodies against the other virus encoded proteins are not protective. Resistance to initiation of infection is related to antibody against the HA, but decreased severity of disease and decreased ability to transmit virus to contacts are related to antibody directed against the NA. Protection correlates with both serum antibodies and secretory IgA antibodies in nasal secretions.

Laboratory Diagnosis

Clinical characteristics of viral respiratory infections can be produced by many different viruses. Consequently, diagnosis of influenza relies on identification of viral antigens or viral nucleic acid in specimens, embryonated eggs and primary monkey kidney cells have been the isolation methods of choice for influenza viruses, or demonstration of a specific immunologic response by the patient, Antibodies to several viral proteins (HA, NA, NP, and matrix) are produced during infection with influenza virus. Nasopharyngeal swabs and nasal aspirate or lavage fluid are the best specimens for diagnostic testing and should be obtained within 3 days after the onset of symptoms.

Epidemiology

Influenza viruses occur worldwide and cause annual outbreaks of variable intensity. It is estimated that annual epidemics of seasonal influenza cause 3–5 million cases of severe illness and 250,000–500,000 deaths worldwide. The incidence of influenza peaks during the winter. A continuous person-to-person chain of transmission must exist for maintenance of the agent between epidemics. Some viral activity can be detected in large population centers throughout each year, indicating that the virus remains endemic in the population and causes a few subclinical or minor infections.

Antigenic Change

Periodic outbreaks appear because of antigenic changes in one or both surface glycoproteins of the virus. When the number of susceptible persons in a population reaches a sufficient level, the new strain of virus causes an epidemic. The change may be gradual (hence the term “antigenic drift”) because of point mutations reflected in alterations at major antigenic sites on the glycoprotein or drastic and abrupt (hence the term “antigenic shift”) owing to genetic reassortment during coinfection with an unrelated strain.

All three types of influenza virus exhibit antigenic drift. However, only influenza A undergoes antigenic shift, presumably because types B and C are restricted to humans, but related influenza A viruses circulate in animal and bird populations. These animal strains account for antigenic shift by genetic reassortment of the glycoprotein genes. Influenza A viruses have been recovered from many aquatic birds, especially ducks; from domestic poultry, such as turkeys, chickens, geese, and ducks; from pigs and horses; and even from seals and whales. Serologic surveys indicate a high prevalence of influenza virus infection in domestic cats.

Influenza outbreaks occur in waves, although there is no regular periodicity in the occurrence of epidemics. The experience in any given year will reflect the interplay between extent of antigenic drift of the predominant virus and waning immunity in the population. The period between epidemic waves of influenza A tends to be 2–3 years; the interepidemic period for type B is longer (3–6 years). Every 10–40 years, when a new subtype of influenza A appears, a pandemic results. This happened in 1918 (H1N1), 1957 (H2N2), and 1968 (H3N2). The H1N1 subtype reemerged in 1977, although no epidemic materialized. Since 1977, influenza A (H1N1) and (H3N2) viruses and influenza B viruses have been in global circulation.

A novel swine-origin H1N1 virus appeared in early 2009 and reached pandemic spread by mid-year. It was a quadruple reassortant, containing genes from both North American and Eurasian swine viruses, as well as from avian and human influenza viruses. The virus was readily transmissible among humans and spread globally, causing more than 18,000 deaths. The severity of illness was comparable to that of seasonal flu. The pandemic virus,

designated A(H1N1)pdm09, has become a seasonal influenza virus, continuing to circulate with other seasonal viruses.

Surveillance for influenza outbreaks is necessary to identify the early appearance of new strains, with the aim of preparing vaccines against them before an epidemic occurs. That surveillance may extend into animal populations, especially birds, pigs, and horses. Isolation of a virus with an altered hemagglutinin in the late spring during a mini-epidemic signals a possible epidemic the following winter. This warning sign, termed a "herald wave," has been observed to precede influenza A and B epidemics.

Avian Influenza

In 1997, in Hong Kong, the first documented infection of humans by avian influenza A virus (H5N1) occurred. The source was domestic poultry. By 2006, the geographic presence of this highly pathogenic H5N1 avian influenza virus in both wild and domestic birds had expanded to include many countries in Asia, Africa, Europe, and the Middle East. Of about 425 laboratory-confirmed human cases by May 2009, more than half were fatal. So far, isolates from human cases have contained all RNA gene segments from avian viruses, indicating that, in those infections, the avian virus had jumped directly from bird to human. All evidence to date indicates that close contact with diseased birds has been the source of human H5N1 infection. The concern is that, given enough opportunities, the highly pathogenic H5N1 avian influenza virus will acquire the ability to spread efficiently and be sustained among humans, either by reassortment or by adaptive mutation. This would result in a devastating influenza pandemic.

The pandemic strain of 2009 was a novel reassortant that contained swine origin viral genes as well as those from avian and human influenza viruses. School-age children are the predominant vectors of influenza transmission. Crowding in schools favors the aerosol transmission of virus, and children take the virus home to their families.

In 1997, in Hong Kong, the first documented infection of humans by avian influenza A virus (H5N1) occurred. The source was domestic poultry. By 2006, the geographic presence of this highly pathogenic H5N1 avian influenza virus in both wild and domestic birds had expanded to include many countries in Asia, Africa, Europe, and the Middle East. Outbreaks were the largest and most severe on record.

Prevention and Control by Vaccines

Inactivated viral vaccines are the primary means of prevention of influenza. However, certain characteristics of influenza viruses make prevention and control of the disease by immunization especially difficult. Existing vaccines are continually being rendered obsolete as the viruses undergo antigenic drift and shift.

Paramyxoviruses and Rubella Virus

The paramyxoviruses include the most important agents of respiratory infections of infants and young children (respiratory syncytial virus [RSV] and the parainfluenza viruses) as well as the causative agents of two of the most common contagious diseases of childhood (mumps and measles). All members of the **Paramyxoviridae** family initiate infection via the respiratory tract. Whereas replication of the respiratory pathogens is limited to the respiratory epithelia, measles and mumps become disseminated throughout the body and produce generalized disease. Rubella virus, although classified as a togavirus because of its chemical and physical properties can be considered with the paramyxoviruses on an epidemiologic basis.

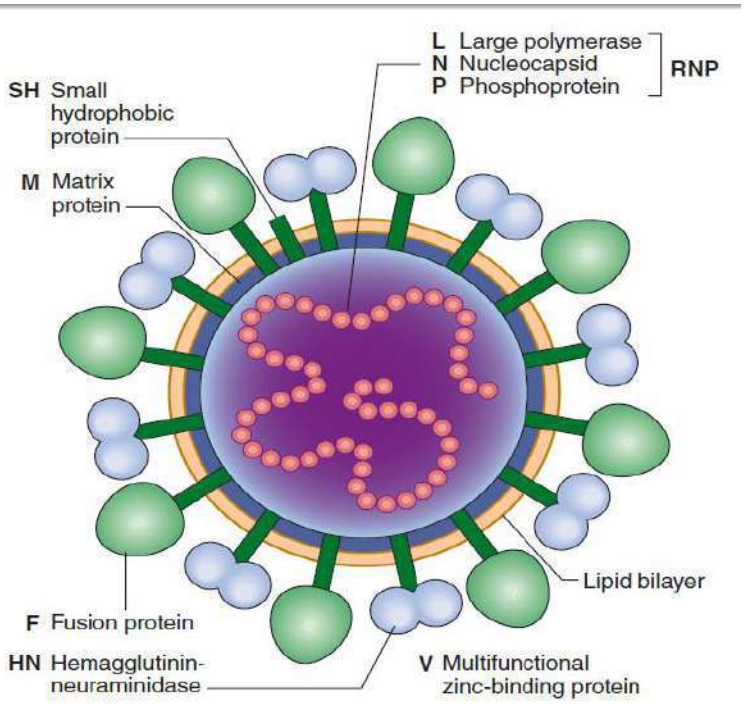
PROPERTIES OF PARAMYXOVIRUSES

Virion: Spherical, pleomorphic, 150 nm or more in diameter (helical nucleocapsid, 13 or 18 nm)
Composition: RNA (1%), protein (73%), lipid (20%), carbohydrate (6%)
Genome: Single-stranded RNA, linear, nonsegmented, negative sense, ~15 kb
Proteins: 6–8 structural proteins
Envelope: Contains viral glycoprotein (G, H, or HN) (which sometimes carries hemagglutinin or neuraminidase activity) and fusion (F) glycoprotein; very fragile
Replication: Cytoplasm; particles bud from plasma membrane
Outstanding characteristics: Antigenically stable Particles are labile yet highly infectious

Structure and Composition

The morphology of **Paramyxoviridae** is pleomorphic, the viral genome is linear, negative-sense, single-stranded, nonsegmented RNA, about 15 kb in size. Because the genome is not segmented, this negates any opportunity for frequent genetic reassortment,

resulting in antigenic stability. Most paramyxoviruses contain six structural proteins. The nucleocapsid is surrounded by a lipid envelope that is studded with 8- to 12-nm spikes of two different transmembrane glycoproteins activities of these glycoproteins help differentiate the various genera of the



family. The larger glycoprotein (HN or G) may or may not possess hemagglutination and neuraminidase activities and is responsible for attachment to the host cell. It is assembled as a tetramer in the mature virion. The other glycoprotein (F) mediates membrane fusion and hemolysin activities.

Classification

The Paramyxoviridae family is divided into two subfamilies and seven genera, six of which contain human pathogens. Most of the members are monotypic (ie, they consist of a single serotype); all are antigenically stable.

MUMPS VIRUS INFECTIONS

Mumps is an acute contagious disease characterized by non-suppurative

enlargement of one or both salivary glands.

Mumps virus mostly causes a mild childhood disease, but in adults complications including meningitis and orchitis are fairly common. More than one-third of all mumps infections are asymptomatic.

Pathogenesis and Pathology

Humans are the only natural hosts for mumps virus. Primary replication occurs in nasal or upper respiratory tract epithelial cells. Viremia then disseminates the virus to the salivary glands and other major organ systems. Involvement of the parotid gland is not an obligatory step in the infectious process. The incubation period may range from 2 to 4 weeks but is typically about 14–18 days. Virus is shed in the saliva from about 3 days before to 9 days after the onset of salivary gland swelling. About one-third of infected individuals do not exhibit obvious symptoms (in apparent infections) but are equally capable of transmitting infection.

Clinical Findings

At least one-third of all mumps infections are subclinical. The most characteristic feature of symptomatic cases is swelling of the salivary glands, which occurs in about 50% of patients. A prodromal period of malaise and anorexia is followed by rapid enlargement of parotid glands as well as other salivary glands. Central nervous system involvement is common (10–30% of cases). Mumps causes aseptic meningitis and is more common among males than females. Meningoencephalitis usually occurs 5–7 days after inflammation of the salivary glands, but up to half of patients will not have clinical evidence of parotitis. Immunity is permanent after a single infection. There is only one antigenic type of mumps virus, and it does not exhibit significant antigenic variation .

Laboratory Diagnosis

The diagnosis of typical cases usually can be made on the basis of clinical findings. However, other infectious agents, drugs, and conditions can cause similar symptoms. In cases without parotitis, the laboratory can be helpful in establishing the diagnosis. **A. Nucleic Acid Detection**

RT-PCR is a very sensitive method that can detect mumps genome sequences in clinical samples.

B. Isolation and Identification of Virus

The most appropriate clinical samples for viral isolation are saliva, cerebrospinal fluid, and urine collected within a few days after onset of illness. Monkey kidney cells are preferred for viral isolation. Samples should be inoculated shortly after collection because mumps virus is thermolabile.

C. Serology

Simple detection of mumps antibody is not adequate to diagnose an infection. Rather, an antibody rise can be demonstrated using paired sera: a fourfold or greater rise in antibody titer is evidence of mumps infection. The ELISA or HI test is commonly used.

Epidemiology

Mumps occurs endemically worldwide. Cases appear throughout the year in hot climates and peak in the winter and spring in temperate climates. Mumps is quite contagious; most susceptible individuals in a household will acquire infection from an infected member. The virus is transmitted by direct contact, airborne droplets, or fomites contaminated with saliva or urine.

Treatment, Prevention, and Control

There is no specific therapy. Immunization with attenuated live mumps virus vaccine is the best approach to reducing mumps-associated morbidity and mortality rates.

MEASLES (RUBEOLA) VIRUS INFECTIONS

Measles is an acute, highly infectious disease characterized by fever, respiratory symptoms, and a maculopapular rash. Complications are common and may be quite serious.

Pathogenesis and Pathology

Humans are the only natural hosts for measles virus, although numerous other species, including monkeys, dogs, and mice, can be experimentally infected. The virus gains access to the human body via the respiratory tract, where it multiplies locally; the infection then spreads to the regional lymphoid tissue, where further multiplication occurs. Primary viremia disseminates the virus, which then replicates in the reticuloendothelial

system. Finally, a secondary viremia seeds the epithelial surfaces of the body, including the skin, respiratory tract, and conjunctiva, where focal replication occurs. Actively replicating virus is present in the brain in this usually fatal form of disease. A rare late complication of measles is subacute sclerosing panencephalitis (SSPE). This fatal disease develops years after the initial measles infection and is caused by virus that remains in the body after acute measles infection.

Clinical Findings

Infections in nonimmune hosts are almost always symptomatic. Measles has an incubation period of 8–15 days from exposure to the onset of rash. The prodromal phase is characterized by fever, sneezing, coughing, running nose, redness of the eyes, Koplik spots, and lymphopenia. **SSPE**, the rare late complication of measles begins insidiously 5–15 years after a case of measles; it is characterized by progressive mental deterioration, involuntary movements, muscular rigidity, and coma. It is usually fatal within 1–3 years after onset.

Immunity

There is only one antigenic type of measles virus. Most so- called second attacks represent errors in diagnosis of either the initial or the second illness.

Laboratory Diagnosis

Typical measles is reliably diagnosed on clinical grounds; laboratory diagnosis may be necessary in cases of modified or atypical measles.

Detection of viral RNA by RT-PCR, Nasopharyngeal and conjunctival swabs, blood samples, respiratory secretions, and urine collected from a patient during the febrile period are appropriate sources for viral isolation. Serologically, ELISA, HI, and neutralization tests all may be used to measure measles antibodies.

Epidemiology

The virus is highly contagious, there is a single serotype, there is no animal reservoir, inapparent infections are rare, and infection confers lifelong immunity. Transmission occurs predominantly via the respiratory route (by inhalation of large droplets of infected secretions).

Treatment, Prevention, and Control

Vitamin A treatment in developing countries has decreased mortality and morbidity. Measles virus is susceptible in vitro to inhibition by ribavirin, but clinical benefits have not been proved. A highly effective and safe attenuated live measles virus vaccine has been available since 1963.

RUBELLA (GERMAN MEASLES) VIRUS INFECTIONS

Rubella (German measles; 3-day measles) is an acute febrile illness characterized by a rash and lymphadenopathy that affects children and young adults.

Classification

Rubella virus, a member of the **Togaviridae** family, is the sole member of the genus Rubivirus. Although its morphologic features and physicochemical properties place it in the togavirus group, rubella is not transmitted by arthropods. There is significant sequence diversity among rubella virus isolates. They are currently classified into two distantly related groups (clades) and nine genotypes.

POSTNATAL RUBELLA

Pathogenesis and Pathology

Neonatal, childhood, and adult infections occur through the mucosa of the upper respiratory tract. Rubella has an incubation period of about 12 days or longer. Initial viral replication probably occurs in the respiratory tract followed by multiplication in the cervical lymph nodes. Viremia develops after 7–9 days and lasts until the appearance of antibody on about day 13–15. The development of antibody coincides with the appearance of the rash, suggesting an immunologic basis for the rash. After the rash appears, the virus remains detectable only in the nasopharynx, where it may persist for several weeks (Figure 40-9). In 20–50% of cases, the primary infection is subclinical.

Clinical Findings

Rubella usually begins with malaise, low-grade fever, and a morbilli form rash appearing on the same day. The rash starts on the face, extends over the trunk and extremities, and rarely lasts more than 3 days. Unless an epidemic occurs, the disease is difficult to diagnose clinically because the rash caused by other viruses (eg, enteroviruses) is similar.

Immunity

Rubella antibodies appear in the serum of patients as the rash fades and the antibody titer rises rapidly over the next 1–3 weeks. Much of the initial antibody consists of IgM antibodies, which generally do not persist beyond 6 weeks after the illness.

Laboratory Diagnosis

Clinical diagnosis of rubella is unreliable because many viral infections produce symptoms similar to those of rubella. Certain diagnosis rests on specific laboratory studies (isolation of virus, detection of viral RNA, or evidence of seroconversion).

A. Nucleic Acid Detection

RT-PCR can be used to detect rubella virus nucleic acid directly in clinical samples or in cell cultures used for virus isolation.

B. Isolation and Identification of Virus

Nasopharyngeal or throat swabs taken 6 days before and after onset of rash are a good source of rubella virus. Various cell lines of monkey or rabbit origin may be used.

C. Serology

The HI test is a standard serologic test for rubella. However, serum must be pretreated to remove nonspecific inhibitors before testing. ELISA tests are preferred because serum pretreatment is not required and they can be adapted to detect specific IgM. Detection of IgG is evidence of immunity because there is only one serotype of rubella virus.

Epidemiology

Rubella is worldwide in distribution. Infection occurs throughout the year with a peak incidence in the spring.

Treatment, Prevention, and Control

Rubella is a mild, self-limited illness, and no specific treatment is indicated. Attenuated live rubella vaccines have been available since 1969.

CONGENITAL RUBELLA SYNDROME

Pathogenesis and Pathology

Maternal viremia associated with rubella infection during pregnancy may result in infection of the placenta and fetus. Only a limited number of fetal cells become infected. The growth rate of infected cells is reduced, resulting in fewer numbers of cells in affected organs at birth. The infection may lead to deranged and hypoplastic organ development,

resulting in structural anomalies in the newborn. Rubella infection can result in fetal death and spontaneous abortion.

Clinical Findings

Clinical features of congenital rubella syndrome may be grouped into three broad categories: (1) transient effects in infants, (2) permanent manifestations that may be apparent at birth or become recognized during the first year, and (3) developmental abnormalities that appear and progress during childhood and adolescence.

Treatment, Prevention, and Control

There is no specific treatment for congenital rubella. It can be prevented by childhood immunization with rubella vaccine to ensure that women of childbearing age are immune.

Advance virology

Picornaviruses (Enterovirus and Rhinovirus Groups)

Picornaviruses represent a very large virus family with respect to the number of members but one of the smallest in terms of virion size and genetic complexity. They include two major groups of human pathogens: enteroviruses and rhinoviruses. Enteroviruses are transient inhabitants of the human alimentary tract and may be isolated from the throat or lower intestine.

Rhinoviruses are associated with the respiratory tract and isolated chiefly from the nose and throat. Less common picornaviruses associated with human illness include hepatitis A virus, parechovirus, cardiovirus, and Aichi virus. Several genera of picornaviruses are also associated with animal, plant, and insect disease.

Many picornaviruses cause diseases in humans ranging from severe paralysis to aseptic meningitis, pleurodynia, myocarditis, vesicular and exanthematous skin lesions, mucocutaneous lesions, respiratory illnesses, undifferentiated febrile illness, conjunctivitis, and severe generalized disease of infants. However, subclinical infection is far more common than clinically manifest disease.

Etiology is difficult to establish because different viruses may produce the same syndrome, the same picornavirus may cause more than a single syndrome, and some clinical symptoms cannot be distinguished from those caused by other types of viruses. The most serious disease caused by any enterovirus is poliomyelitis. A worldwide effort is making progress toward the goal of total eradication of poliomyelitis.

TABLE 36□1 Important Properties of Picornaviruses

Virion: Icosahedral, 28–30 nm in diameter, contains 60 subunits
Composition: RNA (30%), protein (70%)
Genome: Single-stranded RNA, linear, positive sense, 7.2–8.4 kb in size, molecular weight 2.5 million, infectious, contains genome-linked protein (VPg)
Proteins: Four major polypeptides cleaved from a large precursor polyprotein. Surface capsid proteins VP1 and VP3 are major antibody-binding sites. VP4 is an internal protein.
Envelope: None
Replication: Cytoplasm
Outstanding characteristic: Family is made up of many enterovirus and rhinovirus types that infect humans and lower animals, causing various illnesses ranging from poliomyelitis to aseptic meningitis to the common cold.

Structure and Composition:

The virion of enteroviruses and rhinoviruses consists of a capsid shell of 60 subunits, each of four proteins (VP1–VP4) arranged with icosahedral symmetry around a genome made up of a single strand of positive-sense RNA. The molecular structures of poliovirus and rhinovirus have been determined. The three largest viral proteins—VP1, VP2, and VP3—have a very similar core structure in which the peptide backbone of the protein loops back on itself to form a barrel of eight strands held together by hydrogen bonds (the β barrel). The amino acid chain between the β barrel and the amino and carboxyl terminal portions of the protein contains a series of loops. These loops include the main antigenic sites that are found on the surface of the virion and are involved in the neutralization of viral infection.

The genome RNA ranges in size from 7.2 kb (human rhinovirus) to 7.4 kb (poliovirus, hepatitis A virus) to 8.4 kb (aphthovirus). The organization of the genome is similar for all (Figure 2). The genome is polyadenylated at the 3′ end and has a small viral-coded protein (VPg) covalently bound to the 5′ end. The positive-sense genomic RNA is infectious.

Whereas enteroviruses are stable at acid pH (3.0–5.0) for 1–3 hours, rhinoviruses are acid labile. Enteroviruses and some rhinoviruses are stabilized by magnesium chloride against thermal inactivation. Enteroviruses have a buoyant density in cesium chloride of

about 1.34 g/mL; human rhinoviruses, about 1.4 g/mL.

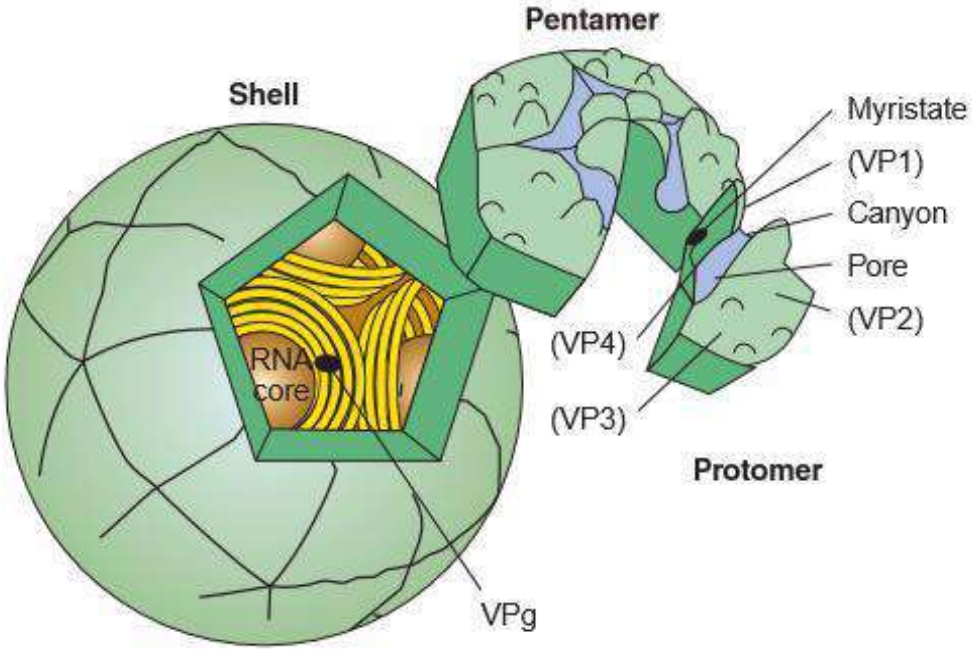


Figure 2: Structure of a typical picornavirus.

Classification:

The Picornaviridae family contains 12 genera, including Enterovirus (enteroviruses and rhinoviruses), Hepatovirus (hepatitis A virus), Kobuvirus (Aichi virus), Parechovirus (parechoviruses), Cardiovirus (cardioviruses), and Aphthovirus (foot-and-mouth disease viruses). The first five groups contain important human pathogens. Rhinoviruses historically were placed in a separate genus but are now considered to be members of the Enterovirus genus.

Enteroviruses of human origin are subdivided into seven species (human enterovirus A–D and human rhinovirus A–C) based mainly on sequence analyses.

The former taxonomy for these viruses included the following:

1- Polioviruses, types 1–3.
2- Coxsackieviruses of group A, types 1–24 (there is no type 15, 18, or 23).
3- Coxsackieviruses of group B, types 1–6.
4- Echoviruses, types 1–33 (no types 8, 10, 22, 23, 28, or 34).
5- Enteroviruses, types 68–116 (no type 72).

The host range of picornaviruses varies greatly from one type to the next and even among strains of the same type. Many enteroviruses (polioviruses, echoviruses, some coxsackieviruses) can be grown at 37°C in human and monkey cells; most rhinovirus strains can be recovered only in human cells at 33°C. Coxsackieviruses are pathogenic for newborn mice.

ENTEROVIRUS GROUP

POLIOVIRUSES:

Poliomyelitis is an acute infectious disease that in its serious form affects the central nervous system (CNS). The destruction of motor neurons in the spinal cord results in flaccid paralysis. However, most poliovirus infections are subclinical. Poliovirus has served as a model enterovirus in many laboratory studies of the molecular biology of picornavirus replication

Properties of theVirus

A. **General Properties:** Poliovirus particles are typical enteroviruses. They are inactivated when heated at 55°C for 30 minutes, but Mg2+, 1 mol/L, prevents this inactivation. Whereas purified poliovirus is inactivated by a chlorine concentration of 0.1 ppm, much higher concentrations of chlorine are required to disinfect sewage containing virus in fecal suspensions and in the presence of other organic matter. Polioviruses are not affected by ether or sodium deoxycholate.

B. **Animal Susceptibility and Growth of Virus:** Polioviruses have a very restricted host range. Most strains will infect monkeys when inoculated directly into the brain or spinal cord. Chimpanzees and cynomolgus monkeys can also be infected by the oral route; in chimpanzees, the infection is usually asymptomatic and the animals become intestinal carriers of the virus. Most strains can be grown in primary or continuous cell line cultures derived from a variety of human tissues or from monkey kidney, testis, or muscle but not from tissues of lower animals. Poliovirus

requires a primate-specific membrane receptor for infection, and the absence of this receptor on the surface of non-primate cells makes them virus resistant. This restriction can be overcome by transfection of infectious poliovirus RNA into resistant cells. Introduction of the viral receptor gene converts resistant cells to susceptible cells. Transgenic mice harboring the primate receptor gene have been developed; they are susceptible to human polioviruses.

C. **Antigenic Properties:** There are three antigenic types of polioviruses based on epitopes found in the VP1, VP2, and VP3 proteins.

Pathogenesis and Pathology:

The mouth is the portal of entry of the virus, and primary multiplication takes place in the oropharynx or intestine. The virus is regularly present in the throat and in the stools before the onset of illness. One week after infection, there is little virus in the throat, but virus continues to be excreted in the stools for several weeks even though high antibody levels are present in the blood. The virus may be found in the blood of patients with non-paralytic poliomyelitis. Antibodies to the virus appear early in the disease, usually before paralysis occurs.

It is believed that the virus first multiplies in the tonsils, the lymph nodes of the neck, Peyer's patches, and the small intestine. The CNS may then be invaded by way of the circulating blood.

Poliovirus can spread along axons of peripheral nerves to the CNS, where it continues to progress along the fibers of the lower motor neurons to increasingly involve the spinal cord or the brain. Poliovirus invades certain types of nerve cells, and in the process of its intracellular multiplication, it may damage or completely destroy these cells. Poliovirus does not multiply in muscle in vivo.

The changes that occur in peripheral nerves and voluntary muscles are secondary to the destruction of nerve cells. Some cells that lose their function may recover completely. Inflammation occurs secondary to the attack on the nerve cells. In addition to pathologic changes in the nervous system, there may be myocarditis, lymphatic hyperplasia, and ulceration of Peyer's patches.

Clinical Findings:

When an individual susceptible to infection is exposed to the virus, the response ranges from in-apparent infection without symptoms to a mild febrile illness to severe and permanent paralysis. Most infections are subclinical; only about 1% of infections result in clinical illness. The incubation period is usually 7–14 days, but it may range from 3 to 35 days.

A. Mild Diseases:

This is the most common form of disease. The patient has only a minor illness, characterized by fever, malaise, drowsiness, headache, nausea, vomiting, constipation, and sore throat in various combinations. Recovery occurs in a few days.

B. Non-paralytic Poliomyelitis (Aseptic Meningitis):

In addition to the symptoms and signs listed in the preceding paragraph, the patient with the non-paralytic form has stiffness and pain in the back and neck. The disease lasts 2–10 days, and recovery is rapid and complete. Poliovirus is only one of many viruses that produce aseptic meningitis. In a small percentage of cases, the disease advances to paralysis.

C. Paralytic Poliomyelitis:

The predominating complaint is flaccid paralysis resulting from lower motor neuron damage. However, incoordination secondary to brain stem invasion and painful spasms of non-paralyzed muscles may also occur. The amount of damage varies greatly. Maximal recovery usually occurs within 6 months, with residual paralysis lasting much longer.

D. Progressive Post poliomyelitis Muscle Atrophy:

A recrudescence of paralysis and muscle wasting has been observed in individuals decades after their experience with paralytic poliomyelitis. Although progressive post poliomyelitis muscle atrophy is rare, it is a specific syndrome. It does not appear to be a consequence of persistent infection but rather a result of physiologic and aging changes in

paralytic patients already burdened by loss of neuromuscular functions.

Laboratory Diagnosis:

The virus may be recovered from throat swabs taken soon after onset of illness and from rectal swabs or stool samples collected over long periods.
Poliovirus is uncommonly recovered from the cerebrospinal fluid, unlike some coxsackieviruses and echoviruses. Specimens should be submitted immediately to the laboratory, and frozen if testing is delayed. Cultures of human or monkey cells are inoculated, incubated, and observed.Cytopathogenic effects appear in 3–6 days.

An isolated virus is identified and typed by Neutralization with specific antiserum. Virus can also be identified more rapidly by polymerase chain reaction (PCR) assays. Paired serum specimens are required to show a rise in antibody titer during the course of the disease..

Epidemiology

Poliomyelitis has had three epidemiologic phases: endemic, epidemic, and the vaccine era. The first two reflect pre-vaccine patterns. The generally accepted explanation is that improved systems of hygiene and sanitation in cooler climates promoted the transition from endemic to epidemic paralytic disease in those societies.

Before global eradication efforts began, poliomyelitis occurred worldwide year-round in the tropics and during summer and fall in the temperate zones. Winter outbreaks were rare. The disease occurs in all age groups, but children are usually more susceptible than adults because of the acquired immunity of the adult population.

In developing areas, where living conditions favor the wide dissemination of virus, poliomyelitis is a disease of infancy and early childhood (“infantile paralysis”). In developed countries, before the advent of vaccination, the age distribution shifted so that most patients were older than age 5 years, and 25% were older than age 15 years.

The case fatality rate is variable. It is highest in the oldest patients and may reach from 5% to 10%. Humans are the only known reservoir of infection.

Prevention and Control:

Both live-virus and killed-virus vaccines are available. Formalin-inactivated vaccine (Salk) is prepared from virus grown in monkey kidney cultures. Killed-virus vaccine induces humoral antibodies but does not induce local intestinal immunity so that virus is still able to multiply in the gut.

Live attenuated vaccine (Sabin) is grown in primary monkey or human diploid cell cultures and delivered orally.

The live polio vaccine infects, multiplies, and immunizes the host against virulent strains.

AIDSandHuman immunodeficiency virus (HIV)

- Human immunodeficiency virus (HIV) types, derived from primate **lentiviruses,** are the etiologic agents of Acquired Immune Deficiency Syndrome (**AIDS**). The illness was first described in 1981, and HIV-1 was isolated by the end of 1983.Since then, AIDS has become a worldwide epidemic, expanding in scope and magnitude as HIV infections have affected different populations and geographic regions. Millions are now infected worldwide; once infected, individuals remain infected for life.

- Within a decade, if left untreated, the vast majority of HIV-infected individuals develop fatal opportunistic infections as a result of HIV-induced deficiencies in the immune system.

Lentiviruses

Important properties of lentiviruses, members of a genus in the **Retroviridae** family are:

- ✓ **Virion:** Spherical, 80–100 nm in diameter, cylindrical core.
- ✓ **Genome:** Single-stranded RNA, linear, positive-sense, diploid (The virion contains two copies of the RNA genome; hence the virion can be described as diploid. The regions of interaction between the two RNA molecules have been described as a '**kissingloop complex'**), contains up to six additional replication genes.
- ✓ **Proteins:** Envelope glycoprotein undergoes antigenic variation;reverse transcriptase enzyme contained inside virions; proteaserequired for production of infectious virus
- ✓ **Envelope:** Present
- ✓ **Replication:** Reverse transcriptase makes DNA copy from genomicRNA; provirus DNA is template for viral RNA. Genetic variability iscommon.
- ✓ **Maturation:** Particles bud from plasma membrane
- ✓ **Outstanding characteristics:1-**Members are non-oncogenic and may be cytocidal**.**

2-Infect cells of the immune system. **3-** Proviruses remain permanently associated with cells. **4-**Viral expression is restricted in some cells in vivo **5-**Cause slowly progressive, chronic diseases. **6-** Replication is usually species-specific Group includes the causative agents of AIDS**.**

Structure and Composition

- The unique morphologic characteristic of HIV is a cylindrical nucleoid in the mature virion.

- Lentiviruses contain the four genes required for a replicating retrovirus—*gag*, *pro*,*pol*, and *env*—and follow the general pattern for retrovirus replication. Up to six

additional genes regulate viral expression and are important in disease pathogenesis in vivo. Although these auxiliary genes show little sequence homology among lentiviruses, their functions are conserved.

- One early-phase replication protein, the **Tat** protein, functions in "transactivation," whereby a viral gene product is involved in transcriptional activation of other viral genesTransactivation by HIV is highly efficient and may contributeto the virulent nature of HIV infections.

- The **Rev** proteinis required for the expression of viral structural proteins (Rev facilitates the export of unspliced viral transcripts fromthe nucleus).

- The **Nef** proteinincreases viral infectivity, facilitates activation of restingT cells, and downregulates expression of CD4 and MHC class I.

- The **Vpr** protein increasestransport of the viral preintegration complex into the nucleusand also arrests cells in the G2 phase of the cell cycle.

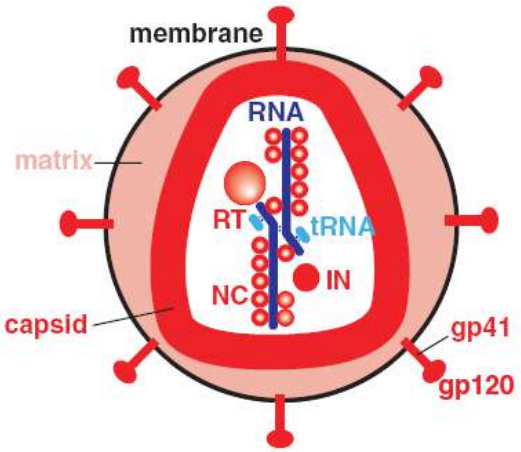
- The **Vpu** protein promotes CD4 degradation.

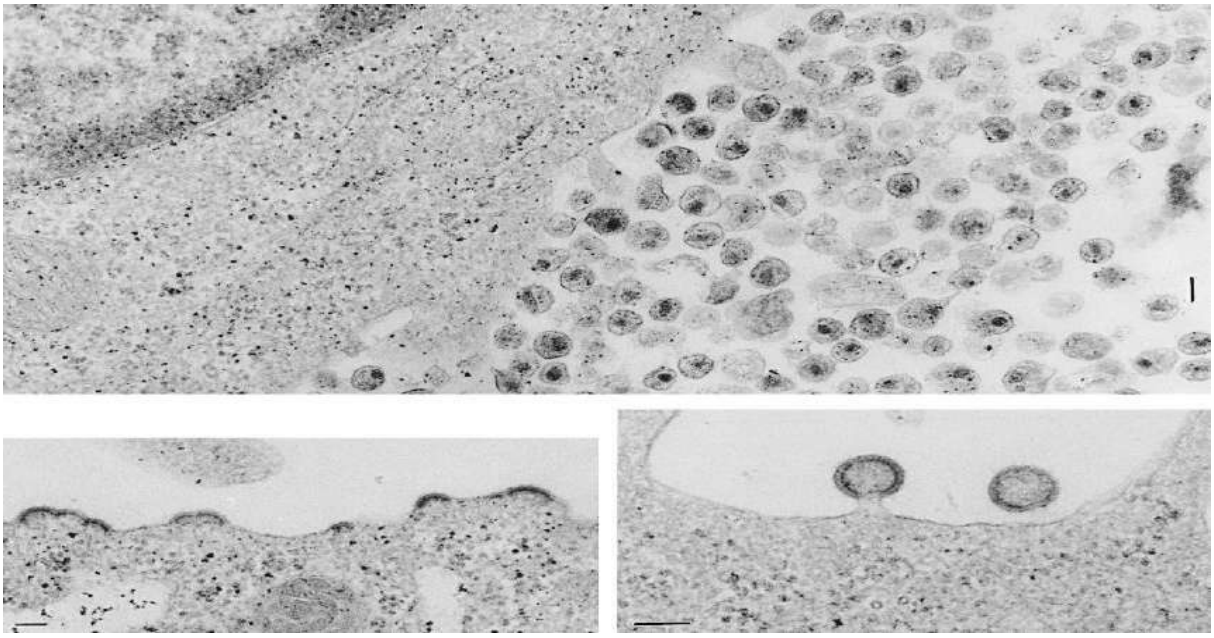
- The regionsof greatest divergence among different isolates of HIV are localizedto the ***env*** gene, which codes for the viral envelope proteins. The SU (**gp120**) product of the ***env*** gene containsbinding domains responsible for virus attachment tothe CD4 molecule and co-receptors, determines lymphocyteand macrophage tropisms, and carries the major antigenicdeterminants that elicit neutralizing antibodies.

- The divergence in the envelope of HIV complicatesefforts to develop an effective vaccine for AIDS.

Figure 17.3 *HIV-1 genome organization*

Main genes	*gag*	*g*roup-specific *a*ntigen (encodes matrix, capsid, p2, nucleocapsid, p1 and p6)
	pol	*pol*ymerase (encodes p6*, protease, reverse transcriptase, RNase H, integrase)
	env	*env*elope
Auxiliary genes	*nef*	*n*egative regulatory *f*actor
	rev	*r*egulator of *e*xpression of *v*irion proteins
	tat	*t*rans*a*ctivator of *t*ranscription
	vif	*v*irion *i*nfectivity *f*actor
	vpr	*v*iral *p*rotein *R*
	vpu	*v*iral *p*rotein *U*
Non-coding sequences	R	repeat sequence
	U3	unique sequence at 3′ end of genome
	U5	unique sequence at 5′ end of genome
Domains at the 5′ end of the genome	TAR	trans-acting response element
	Poly-A	polyadenylation signal
	PBS	primer-binding site
	DIS	dimerization initiation site (involved in formation of kissing loop complex)
	SD	splice donor site
	Psi (ψ)	main part of the packaging signal
	AUG	start codon of the *gag* gene





FIGURE; Electronmicrographs of HIV-infected lymphocytes, showing a large accumulation of freshly produced virus at the cell surface (top, 46,450×, bar = 100 nm); newly formed virus budding from cytoplasmic membrane (lower left, 49,000×, bar = 100 nm); and two virions about to be

cast off from cell surface (lower right, 75,140×, bar = 100 nm).

Types of HIV

- There are two distinct types of human AIDS viruses: **HIV-1** and **HIV-2**. The two types are distinguished on the basis of genome organization and phylogenetic (evolutionary) relationships with other primate lentiviruses. Sequence divergence between HIV-1 and HIV-2 exceeds 50%.
- Based on *env* gene sequences, HIV-1 comprises three distinct virus groups (M, N, and O); the predominant M group contains at least **11** subtypes or "clades" (A–K).
- Recombinant forms of virus are also found in circulation in humans in different geographic regions. Similarly, **eight** subtypes of HIV-2 (A–H) have been identified. Within each subtype there is extensive variability. The genetic clades do not seem to correspond to neutralization serotype groups, and there is currently no evidence that subtypes differ in biology or pathogenesis.

Common features of Lentivirus

Natural diseasepatterns vary among species, but certain common featuresare recognized.

1. Viruses are transmitted by exchange of body fluids.

2. Virus persists indefinitely in infected hosts, although itmay be present at very low levels.

3. Viruses have high mutation rates, and different mutantswill be selected under different conditions (host factors,immune responses, tissue types). Infected hosts contain"swarms" of closely related viral genomes, known asquasispecies.

4. Virus infection progresses slowly through specific stages.

- Cells in the macrophage lineage play central roles in theinfection. Lentiviruses differ from other retroviruses inthat they can infect non-dividing, terminally differentiatedcells.
- Virus is cell-associated in monocytes and macrophages,but only about one cell per million is infected. Monocytescarry the virus around the body in a form that theimmune system cannot recognize, seeding other tissues.
- Lymphocyte-tropic strains of virus tend to cause highlyproductive infections, whereas replication of macrophage tropic virus is restricted.

5. It may take years for disease to develop. Infected hostsusually make antibodies, but they do not clear theinfection, so virus persists lifelong.

- New antigenic variantsperiodically arise in infected hosts, with mostmutations occurring in envelope glycoproteins.

- Clinicalsymptoms may develop at any time, but chronic diseasetypically manifests after months to years of infection.The exceptions to long incubation periods for lentivirusdisease include **AIDS in children**.

Note: Host factors important in pathogenesis of disease includeage (the young are at greater risk), stress (may trigger disease),genetics (certain breeds of animals are more susceptible), and concurrent infections (may exacerbate disease or facilitatevirus transmission).

Virus Receptors

- All primate lentiviruses use the **CD4** molecule as a receptor, which is expressed on macrophages and T lymphocytes.
- A second **co-receptor** in addition to CD4 is necessary for HIV-1 to gain entry to cells. The second receptor is required for fusion of the virus with the cell membrane.
- These interactions cause conformational changes in the viral envelope, activating the gp41 fusion peptide and triggering membrane fusion.

- **Chemokine receptors** serve as HIV-1 second receptors. (**CCR5**) expressed in macrophage whereas (**CXCR4**) expressed in lymphocyte.
- Individuals who possess homozygous deletions in CCR5 or produce mutant forms of the protein may be protected from infection by HIV-1; mutations in the CCR5 gene promoter appear to delay disease progression.

- Another molecule, **integrin α-4 β-7**, appears to function as a receptor for HIV in the gut.

- A dendritic cell-specific **lectin** appears to bind HIV-1 but not to mediate cell entry. Rather, it may facilitate transport of HIV by dendritic cells to lymphoid organs and enhance infection of T cells.

HIV pathogenesis :

A. Course of HIV Infection

The typical course of untreated HIV infection spans abouta decade (Figure p.7). Stages include the **primary infection**,**dissemination of virus to lymphoid organs**, **clinical latency**, **elevated HIV expression**, **clinical disease**, and **death**.The duration between primary infection and progression toclinical disease averages about 10 years. In untreated cases,death usually occurs within 2 years after the onset of clinicalsymptoms.

Following primary infection, there is a 4- to 11-dayperiod between mucosal infection and **initial viremia**; the viremia is detectable for about 8–12 weeks.

Virus is widelydisseminated throughout the body during this time, and thelymphoid organs become seeded. An acute **mononucleosis like syndrome** develops in many patients (50–75%) 3–6 weeksafter primary infection.

There is a significant drop in numbersof circulating CD4 T cells at this early time. An immuneresponse to HIV occurs 1 week to 3 months after infection,plasma viremia drops, and levels of CD4 cells rebound. However,the immune response is unable to clear the infectioncompletely, and HIV-infected cells persist in the lymph nodes.

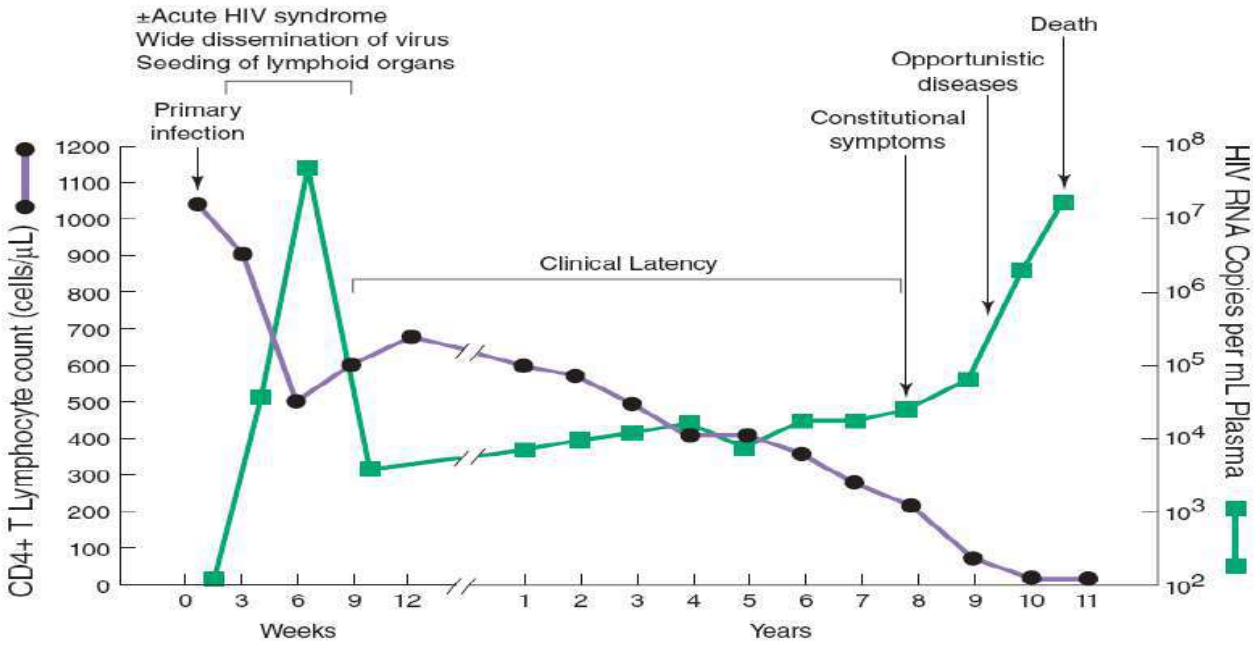
This period of **clinical latency** may last for 10 years ormore. During this time, there is a high level of ongoing viralreplication. It is estimated that 10 billion HIV particles areproduced and destroyed each day. The half-life of the virus inplasma is about 6 hours, and the virus life cycle (from the timeof infection of a cell to the production of new progeny thatinfect the next cell) averages 2.6 days. CD4 T lymphocytes,major targets responsible for virus production, appear to havesimilar high turnover rates. Once productively infected, thehalf-life of a CD4 lymphocyte is about 1.6 days.

Viral diversity studies have shown that in most cases ofsexual transmission a single HIV variant establishes a newinfection. Early in infection, viral sequences are quite homogeneous,but because of rapid viral proliferation and theinherent error rate of the HIV reverse transcriptase, **quasispecies of virus accumulate**. It is estimated that every nucleotideof the HIV genome probably mutates on a daily basis.

Eventually, the patient develops constitutional symptomsand clinically apparent disease, such as **opportunistic infections** or neoplasms.

Higher levels of virus are readilydetectable in the plasma during the advanced stages of infection.

HIV found in patients with late-stage disease is usuallymuch more virulent and cytopathic than the strains of virusfound early in infection. Often, a shift from **monocyte-tropic or macrophage-tropic** (M-tropic) strains of HIV-1 to **lymphocyte- tropic** (T-tropic) variants accompanies **progression to AIDS**.



FIGUREtypical course of untreated HIV infection. During the early period after primary infection, there is widespread disseminationof virus and a sharp decrease in the number of CD4 T cells in peripheral blood. An immune response to HIV ensues, with a decrease indetectable viremia followed by a prolonged period of clinical latency. Sensitive assays for viral RNA show that virus is present in the plasma atall times. The CD4 T-cell count continues to decrease during the following years until it reaches a critical level below which there is a substantialrisk of opportunistic diseases.

B. CD4 T Lymphocytes, Memory Cells, and Latency

The cardinal feature of HIV infection is the depletion of T helper-inducer lymphocytes—the result of HIV replication in this population of lymphocytes as well as of the death of uninfected T cells by indirect mechanisms.

C. Monocytes and Macrophages

Monocytes and macrophages play a major role in the dissemination and pathogenesis of HIV infection. Certain subsets of monocytes express the CD4 surface antigen and therefore bind to the envelope of HIV. The HIV coreceptor on monocytes and

macrophages is the CCR5 chemokine receptor. In the brain, the major cell types infected with HIV appear to be the monocytes and macrophages, and this may have important consequences for the development of neuropsychiatric manifestations associated with HIV infection.

D. Lymphoid Organs

Lymphoid organs play a central role in HIV infection. Lymphocytes in the peripheral blood represent only about 2% of the total lymphocyte pool, the remainder being located chiefly in lymphoid organs. It is in the lymphoid organs that specific immune responses are generated. The network of follicular dendritic cells in the germinal centers of lymph nodes traps antigens and stimulates an immune response.

Viral Coinfections

Activation signals are required for the establishment of a productive HIV infection. In the HIV-infected individual, a wide range of in vivo antigenic stimuli seem to serve as cellular activators. For example, active infection by *Mycobacterium tuberculosis* substantially increases plasma viremia.

Whythe infection persists in spite of immune responses against the virus?**Why HIV infection is not cleared?**

1. There are several reasons for this. One reason is that the cell types killed by HIV infection (CD4 T cells and macrophages) are those involved in the immune responses; there is also evidence that non-infected CD4 T cells are killed by apoptosis. CD4 T cells play pivotal roles as helpers for several cell types including B cells, cytotoxic T cell precursors, natural killer cells and macrophages, hence immune responses are impaired.

2. Another reason why an HIV infection is not cleared is that the virus evolves as the infection proceeds, producing new antigenic variants that may not be recognized by the antibodies (neutralizing antibodiesagainst HIV, directed against the envelope glycoprotein) and T cells present. Furthermore, in latently infected cells the virus is shielded from the immune system.

Symptoms and Clinical findings

- Symptoms of acute HIV infection are nonspecific and include **fatigue**, **rash**, **headache**, **nausea**, and **night sweats**.

- AIDS characterized by pronounced suppression of the immune systemand development of a wide variety of severe opportunisticinfections or unusual neoplasms (especially Kaposi sarcoma).
- The more serious symptoms in adults are often preceded bya prodrome ("diarrhea and dwindling") that can include**fatigue**, **malaise**, **weight loss**, **fever**, **shortness of breath**, **chronicdiarrhea**, **white patches on the tongue** (hairy leukoplakia,oral candidiasis), and **lymphadenopathy**.
- Disease symptomsin the gastrointestinal tract from the esophagus to the colonare a major cause of debility.
- With no treatment, the intervalbetween primary infection with HIV and the first appearanceof clinical disease is usually long in adults, averaging about8–10 years.
- Death occurs about 2 years later.

- **Viral load:** is a lab test that measures the number of HIV viral particles in milliliter of your blood. These particles called copies. Viral load provide the information of your health status. The plasma viral load appears tobe the best predictor of long-term clinical outcome, whereasCD4 lymphocyte counts are the best predictor of short-termrisk of developing an opportunistic disease. Plasma viral loadmeasurements are a critical element in assessing the effectivenessof antiretroviral drug therapy.

- **Pediatric AIDS**—acquiredfrom infected mothers—usually presents with clinical symptomsby 2 years of age; death follows in another 2 years. The neonate isparticularly susceptible to the devastating effects of HIV becausethe immune system has not developed at the time of primaryinfection.Clinical findings mayinclude lymphoid interstitial pneumonitis, pneumonia, severe oral candidiasis, encephalopathy,wasting, generalized lymphadenopathy, bacterial sepsis,hepatosplenomegaly, diarrhea, and growth retardation.

- A small percentageof infants (≤5%) display transient HIV infections,suggesting that some infants can clear the virus.

Opportunistic Infections

The predominant causes of morbidity and mortality among patients with late-stage HIV infection are opportunistic infections.The most common opportunistic infections in untreatedAIDS patients include the following:

1. **Protozoa:***Toxoplasma gondii*, Cryptosporidium species.
2. **Fungi:***Candida albicans*.
3. **Bacteria:***Mycobacterium avium*-intracellulare, M tuberculosis, *Listeria monocytogenes*, *Nocardiaasteroides*,Salmonella species, Streptococcus species.
4. **Viruses:** Cytomegalovirus, herpes simplex virus, varicella-zoster virus, adenovirus,,hepatitis B virus, hepatitis C virus.

Herpesvirus infections are common in AIDS patients,and multiple herpesviruses are frequently detected beingshed in saliva. Cytomegalovirus retinitis is the most commonsevere ocular complication of AIDS.

Cancers

- AIDS-associated cancers tend to be those with a viral cofactor and include non-Hodgkin lymphoma (both systemic and central nervous system types), Kaposi sarcoma, cervical cancer, and anogenital cancers.
- Epstein-Barr viral DNA is foundin the majority of B-cell malignancies classified as **Burkitt lymphoma,** Burkitt lymphoma occurs 1000 times more commonly in AIDS patients than in the general population.

- **Kaposi sarcoma** appears in skin, mucous membranes, lymph nodes, and visceral organs. Before this type of malignancy was observed in AIDS patients, it was considered to be a very rare cancer. Kaposi sarcoma is 20,000 times more common in untreated AIDS patients than in the general population.
- **Cervical cancer** is caused by high-risk papilloma viruses.
- As HIV-infected persons live longer lives due to effective antiretroviral therapy, they are developing a broad spectrum of cancers at higher frequencies than the non-infected population.

Routes of Transmission

High titers of HIV are found in two body fluids—blood and semen.

a. HIV is transmitted during sexual contact (including genital–oral sex)

- The risk increases in proportion to the number of sexual encounters with different partners.
- The presence of other sexually transmitted diseases such as syphilis, gonorrhea, or herpes simplex type 2 increases the risk of sexual HIV transmission as much as a 100-fold because the inflammation and sores facilitate the transfer of HIV across mucosal barriers. Asymptomatic virus-positive individuals can transmit the virus.

b. Transfusion of infectious blood or blood products is an effective route for viral transmission.

- Over 90%of hemophiliac recipients of contaminated clotting factor concentrates in the United States (before HIV was detected) developed antibodies to HIV.
- Injection users of illicit drugs are commonly infected through the use of contaminated needles.

c. Mother-to-infant transmission rates vary from 13% to40% in untreated women.

- Infants can become infected in utero, during the birth process, or through breastfeeding.

Diagnosis

Evidence of infection by HIV can be detected in three ways:
(1) Virus isolation; (2) serologic determination of antiviral antibodies; and (3) measurement of viral nucleic acid orantigens. (Mentioned before).

Prevention, Treatment, and Control

Antiviral Drugs

a. Therapy with combinations of antiretroviral drugs, referred to as **HAART** (highly active antiretroviral therapy). It often can suppress viral replication to below limits of detection in plasma, decrease viral loads in lymphoid tissues, allow the recovery of immune responses to opportunistic pathogens, and prolong patient survival. However, HAART has failed to cure HIV-1 infections. The virus persists in reservoirs of long-lived, latently infected cells, including memory CD4 T cells. When HAART is discontinued or there is treatment failure, virus production rebounds.

Why vaccine development is difficult for HIV?

Because HIV mutates rapidly, is not expressed in all cells that are infected, and is not completely cleared by the host immune response after primary infection.

Coronaviruses

Coronaviruses are large, enveloped RNA viruses. The human coronaviruses cause common colds, may cause lower respiratory tract infections, and have been implicated in gastroenteritis in infants. Novel coronaviruses have been identified as the cause of severe acute respiratory syndrome (SARS),
Middle East respiratory syndrome (MERS) and Covid-19. Animal coronaviruses cause diseases of economic importance in domestic animals. Coronaviruses of lower animals establish persistent infections in their natural hosts. The human viruses are difficult to culture and therefore are more poorly characterized.

PROPERTIES OF CORONAVIRUSES

- **Virion:** Spherical, 120–160 nm in diameter, helical nucleocapsid.
- **Genome:** Single-stranded RNA, linear, nonsegmented, positive sense, 27–32 kb, capped and polyadenylated, infectious.
- **Proteins:** Two glycoproteins and one phosphoprotein. Some viruses contain a third glycoprotein (hemagglutinin esterase).
- **Envelope:** Contains large, widely spaced, club- or petal-shaped spikes.
- **Replication:** Cytoplasm; particles mature by budding into endoplasmic reticulum and Golgi.
- **Outstanding characteristics:**
  Cause colds, SARS, and MERS.
  Display high frequency of recombination.
  Difficult to grow in cell culture.

Coronaviruses exhibit a high frequency of mutation during each round of replication, including the generation of a high incidence of deletion mutations. Coronaviruses undergo a high frequency of recombination during replication; this is

unusual for an RNA virus with a nonsegmented genome and may contribute to the evolution of new virus strains.

Structure and Composition

Coronaviruses are enveloped, 120- to 160-nm particles that contain an unsegmented genome of single-stranded positive sense RNA (27–32 kb), the largest genome among RNA viruses. The genomes are polyadenylated at the 3′ end. **Isolated genomic RNA is infectious**. The helical nucleocapsid is 9–11 nm in diameter. There are 20-nm-long club- or petalshaped projections that are widely spaced on the outer surface of the envelope, suggestive of a solar corona (Figure -1). The viral structural proteins include a

- phosphorylated nucleocapsid (N) protein,
- a membrane (M) glycoprotein that serves as a matrix protein embedded in the envelope lipid bilayer and interacting with the nucleocapsid,
- the spike glycoprotein that makes up the petal-shaped peplomers.
- Some viruses, including human coronavirus OC43 (HCoV-OC43), contain a third glycoprotein (HE; 65 kDa) that causes hemagglutination and has acetylesterase activity.

The gene order for the proteins encoded by all coronaviruses is Pol-S-E-M-N-3′. Several open reading frames encoding nonstructural proteins and the HE protein differ in number and gene order among coronaviruses.

The SARS virus contains a comparatively large number of interspersed genes for nonstructural proteins at the 3′ end of the genome.

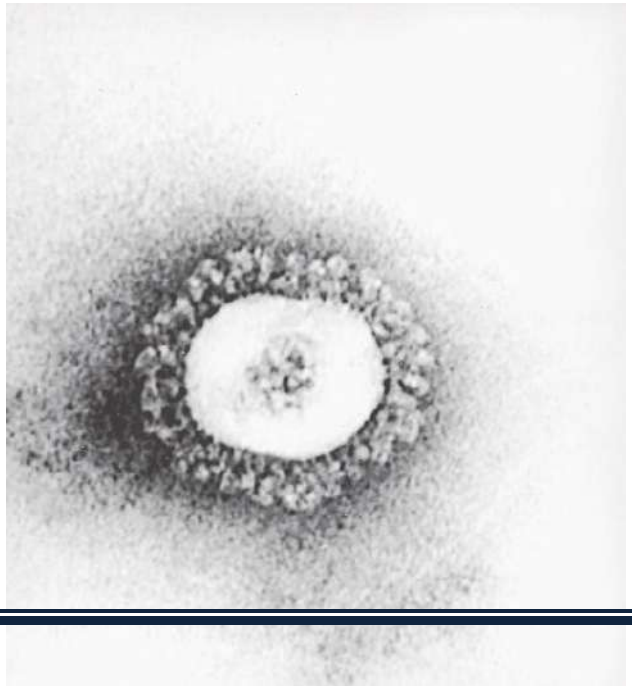


FIGURE 41-1 Human coronavirus OC43. Note the characteristic large, widely spaced spikes that form a "corona" around the virion (297,000×). (Courtesy of FA Murphy and EL Palmer.)

Members of Coronaviridae Family

There are two subfamilies:

- Coronavirinae.
- and Torovirinae.

There are six genera in the Coronaviridae family:

1. *Alphacoronavirus*.
2. *Betacoronavirus*.
3. *Gammacoronavirus*.
4. *Deltacoronavirus*.
5. *Bafinivirus*.
6. *Torovirus*.

The first two and the last genera contain viruses able to infect humans. The toroviruses are widespread in ungulates and appear to be associated with diarrheal disease.

There are seven coronaviruses that can infect humans:

- **The alpha coronaviruses 229E, and NL63.**
- **the beta coronaviruses OC43, HKU1, and SARS-CoV-1, SARS-COV-2.**
- **MERS-CoV**.

There are many coronaviruses that infect animals, with most infecting one or a few species.

Pathogenesis

Coronaviruses tend to be **highly species specific**. Most of the known animal coronaviruses display a tropism for epithelial cells of the respiratory or gastrointestinal tract. Coronavirus infections in vivo may be disseminated, such as with mouse hepatitis virus, or localized. Coronavirus infections in

humans usually, but not always, remain limited to the upper respiratory tract.

In contrast, the outbreak of SARS-CoV-1 in 2003 was characterized by serious respiratory illness, including pneumonia and progressive respiratory failure. Virus can also be detected in other organs, including kidney, liver, and small intestine, and in stool. The SARS virus probably originated in a nonhuman host, most likely bats, was amplified in palm civets, and was transmitted to humans in live animal markets.

Chinese horseshoe bats are natural reservoirs of SARS-like coronaviruses. In rural regions of southern China, where the outbreak began, people, pigs, and domestic fowl live close together, and there is widespread use of wild species for food and traditional medicine—conditions that promote the emergence of new viral strains.

The MERS-CoV outbreak beginning in 2012 was also characterized by pneumonia and respiratory failure, though most patients who died had medical comorbidities. MERS-CoV likely originated in bats and became widespread in camels as shown by seropositivity in animals in the region. It is likely that contact with either bats or camels leads to initial human infections, which can then be transmitted from person to person.

The current SARS-Cov-2 pandemic of COVID-19.

Coronaviruses are suspected of causing some gastroenteritis in humans. There are several animal models for enteric coronaviruses, including porcine **transmissible gastroenteritis virus (TGEV**). Disease occurs in young animals and is marked by epithelial cell destruction and loss of absorptive capacity. It is of interest that a novel **porcine respiratory coronavirus (PRCV)** appeared in Europe in the 1980s and caused widespread epizootics in pigs. Sequence analysis showed that PRCV was derived from TGEV by a large deletion in the S1 glycoprotein.

Clinical Findings

Coronaviruses cause respiratory and intestinal infections in animals and humans. They were not considered to be highly pathogenic to humans until the last two decades, which have seen three outbreaks of highly transmissible and pathogenic coronaviruses, including SARS-CoV (severe acute respiratory syndrome coronavirus), MERS-CoV (Middle East respiratory syndrome coronavirus), and SARS-CoV-2 (which causes the disease COVID-19). Other human coronaviruses (such as HCoV-NL63, HCoV-229E, HCoV-OC43 or HKU1) generally induce only mild upper respiratory diseases in immunocompetent hosts, although some may cause severe infections in infants, young children and elderly individuals.

The human coronaviruses produce "common colds," usually afebrile, in adults. The symptoms are similar to those produced by rhinoviruses, typified by:

- nasal discharge and malaise.
- The incubation period is from 2 to 5 days, and symptoms usually last about 1 week.
- The lower respiratory tract is seldom involved, although pneumonia may occur.
- Asthmatic children may suffer wheezing attacks, and respiratory symptoms may be exacerbated in adults with chronic pulmonary disease.

SARS-CoV causes severe respiratory disease. The incubation period averages about 6 days. Common earlysymptoms include: fever, malaise, chills, headache, dizziness, cough, and sore throat,

Followed a few days later by: shortness of breath. Many patients have abnormal chest radiographs. Some cases progress rapidly to acute respiratory distress, requiring ventilatory support.

Death from progressive respiratory failure occurs in almost 10% of cases, with the death rate highest among the elderly. SARS involves a cytokine storm, with elevated levels of multiple chemokines and cytokines in the peripheral circulation for about 2 weeks.

MERS-CoV causes mild to severe respiratory illness in children and adults. Patients

with comorbidities are more severely affected, as are the elderly. The incubation period is 2–13 days, with extended illness in some cases leading to pneumonia and death. Laboratory findings include: leukopenia, lymphopenia, thrombocytopenia, and elevated lactate dehydrogenase levels.The mortality rate is stated as up to 30%, but this is likely to be an overestimate as mild cases are not typically reported.

Clinical features of coronavirus-associated enteritis have not been clearly described. They appear to be similar to those of rotavirus infections.

Epidemiology

Coronaviruses are distributed worldwide. They are a major cause of respiratory illness in adults during some winter months when the incidence of colds is high, but the isolation of rhinoviruses or other respiratory viruses is low. They tend to be associated with well-defined outbreaks.

It is estimated that coronaviruses cause 15–30% of all colds. The incidence of coronavirus infections varies markedly from year to year, ranging in one 3-year study from 1% to 35%. Antibodies to respiratory coronaviruses appear in childhood, increase in prevalence with age, and are found in more than 90% of adults. It appears that reinfection with symptoms can occur after a period of 1 year. However, **antibodies to SARS and MERS coronaviruses are uncommon**, showing that they **have not circulated widely** in humans. Coronaviruses are commonly associated with acute respiratory disease in the elderly, along with **rhinoviruses, influenza virus, and respiratory syncytial virus**.

The frequency of coronavirus infection is estimated to be about halfthat of rhinoviruses and equivalent to those of the latter two viruses.

Coronaviruses are transmitted bycontact with: respiratory droplets, contaminated surfaces, and fomites (contaminated inanimate objects).

There is a risk of transmission in the health care setting, with documented hospital outbreaks. The outbreak of SARS erupted in southern China in late2002 and, by the time it waned in mid-2003, had resulted in over 8000 cases in 29 countries, with over 800 deaths (case fatality rate of 9.6%). In almost all cases, there was a history of close contact with a SARS patient or of recent travel to an area where SARS was reported.

International air travel allowed SARS to spread around the world with unprecedented speed. The experience with SARS illustrated that in a globalized world, an infectious disease outbreak anywhere places every country at risk.

Interestingly, a few persons with SARS were identified as “super spreaders”; each appeared to have infected more than 10 contacts. Super spreaders have been described for other diseases such as rubella, Ebola, and tuberculosis and presumably reflect a certain constellation of host, viral, and environmental factors.

The MERS coronavirus was identified in 2012 as the cause of a patient who died of respiratory failure in Saudi Arabia. Subsequently, it was determined to be the cause of multiple outbreaks of respiratory disease from several countries in the Arabian Peninsula.

The virus appears to be endemic in bats and camels in the region. Infected travelers have spread the virus in other countries, and it remains a risk for transmission from pilgrims returning from the annual Hajj in Mecca. Very little is known about the epidemiology of enteric coronavirus infections.

Laboratory Diagnosis

A. Antigen and Nucleic Acid Detection

Coronavirus antigens in cells in respiratory secretions may be detected using the ELISA test if a high-quality antiserum is available. Enteric coronaviruses can be detected by examination of stool samples by electron microscopy. Polymerase chain reaction (PCR) assays are the preferred methods todetect coronavirus nucleic acid in respiratory secretions and in stool samples. Viremia with SARS and MERS coronaviruses is detectable in the plasma by PCR.

B. Isolation and Identification of Virus

Isolation of human coronaviruses in cell culture has been difficult. However, the SARS and MERS viruses have been recovered from oropharyngeal specimens using Vero monkey kidney cells.

C. Serology

Because of the difficulty of virus isolation, serodiagnosis using acute and convalescent sera is one means of confirming coronavirus infections for epidemiologic purposes.

ELISA, indirect immunofluorescent antibody assays, and hemagglutination tests may be used. Serologic diagnosis of infections with strain 229E is possible using a passive hemagglutinationtest in which red cells coated with coronavirus antigen are agglutinated by antibody-containing sera.

Treatment, Prevention, and Control

There is no proven treatment for coronavirus infections and no vaccine. Protease inhibitors used in the treatment of human immunodeficiency virus infections (eg, lopinavir) have in vitro activity against SARS coronavirus. SARS and MERS vaccines are under development. Control measures that were effective in stopping the spread of SARS included:

- isolation of patients,
- quarantine of those who had been exposed,
- and travel restrictions,
- as well as the use of gloves, gowns, goggles, and respirators by health care workers.

There remains a high suspicion for MERS-CoV in patients returning from the Arabian Peninsula, which requires appropriate testing and infection control precautions to prevent further spread.

COVID-19 pandemic

The Causative agent of the pandemic was SARS-COV-2 virus. SARS-related coronavirus is a member of the genus Betacoronavirus and monotypic of the subgenus Sarbecovirus (subgroup B). SARSr-CoV was determined to be an early split-off from the betacoronaviruses based on a set of conserved domains that it shares with the group.

Bats serve as the main host reservoir species for the SARS-related coronaviruses like SARS-CoV-1 and SARS-CoV-2. The virus has coevolved in the bat host reservoir over a long period of time. Only recently have strains of SARS-related coronavirus evolved and made the cross-species jump from bats to humans, as in the case of the strains SARS-CoV and SARS-CoV-2. Both of these strains descended from a single ancestor but made the cross-species jump into humans separately. SARS-CoV-2 is not a direct

descendant of SARS-CoV.

The majority of COVID 19 cases (about 80%) are asymptomatic or show mild symptoms but a low percentage experience severe respiratory failure. Interestingly, the viral load in asymptomatic patients was similar to that in symptomatic patients. In a study in china it was reported that in people with normal CT scans and no clinical symptoms, who were in close contact with confirmed virus-positive patients; nasal and throat swab tests were positive on days 7, 10, and 11 after contact. Furthermore, SARS-CoV2 RNA was detectable in stool, saliva and urine samples as well as in gastrointestinal tissue in patients with COVID-19 in China. Thus, the digestive tract should also be considered as a route of infection.

The SARS-CoV-2 genome consist of 29,903nt (nucleotides) and has been assigned GenBank accession number MN908947. RNA from the virus is closely related to two bat derived SARS-like coronaviruses, with a nucleotide identity of 88.1%, but is more distant from SARS-CoV (about 79%) and MERS-CoV (about 50%).

Structure and composition:

The RNA genome of SARS-Cov-2, similar to other CoVs, contains **ten open reading frames** (ORFs). The first ORF covers two-thirds of the viral RNA, which encodes polypeptides of the viral replicase-transcriptase complex. The remaining ORFs translate four main structural proteins: spike (S), envelope (E), nucleocapsid (N), and membrane (M) proteins. The genome is packaged into a helical nucleocapsid surrounded by a host-derived lipid bilayer.

Replication cycle:

SARS-CoV-2 uses angiotensin converting enzyme 2 (ACE2) as its receptor for cellular invasion. Therefore, the pattern of ACE2 expression in different tissues can determine tropism, susceptibility, symptoms, and outcome of SARS-CoV-2 infection. ACE2 is expressed on the mucosa of oral cavity and is highly enriched in epithelial cells of the tongue which highlights the role of the oral cavity for infection with SARS-CoV-2. ACE2 is also highly expressed in vascular endothelial cells of the heart and the kidney,

and it affects cardiac function.

The viral spike glycoprotein (S protein) binds to the host cellular receptor and is therefore, considered as the main determinant of cell tropism and pathogenesis. The S protein is composed of an extracellular and a transmembrane domain (TM), as well as a short cytoplasmic tail region (CP). The extracellular domain of the S protein is composed of two subunits (S1 and S2) which are responsible for host-cell receptor recognition and membrane fusion, respectively.

The virus surface S protein mediates cell entry by binding to ACE2. Cleavage of the S glycoprotein between the S1 and S2 domains starts during viral packaging. This process is completed by the type II transmembrane serine protease TMPRSS2 which results in activation of the S2 subdomain.

The S2 subdomain then mediates the fusion of the viral genome with the host cell membrane to create a pore allowing the viral RNA and RNA-associated proteins to gain access to the cytoplasm. Another possibility is that ACE2/SARS-CoV-2 complex undergoes endocytosis. The rapid endocytosis of SARS-CoV-2 occurs through clathrin-mediated endocytosis. However; it is not completely clear how the viral genome of SARS-CoV-2 gains access to the cytoplasm.

However; after entering the cell, the viral RNA is released into the cytoplasm and translates viral proteins followed by viral genome replication. The newly formed envelope glycoproteins are inserted into the membrane of the endoplasmic reticulum or Golgi, forming nucleocapsids by assembling the genomic RNA and nucleocapsid protein. Viral particles then incorporate into the endoplasmic reticulum-Golgi intermediate compartment (ERGIC) and the vesicles containing the virus particles fuse with the plasma membrane to release the virus.

Clinical Funding:

The majority of COVID 19 cases (about 80%) are asymptomatic or show mild symptoms but a low percentage experience severe respiratory failure. Interestingly, the viral load in asymptomatic patients was similar to that in symptomatic patients. In virus-positive patients; nasal and throat swab tests were positive on days 7, 10, and 11 after contact. Furthermore, SARS-CoV2 RNA was detectable in stool, saliva and urine samples

as well as in gastrointestinal tissue in patients with COVID-19 in China. Thus, the digestive tract should also be considered as a route of infection.

COVID-19 affects different people in different ways. Most infected people will develop mild to moderate illness and recover without hospitalization.

Most common symptoms: fever, dry cough, tiredness

Less common symptoms: aches and pains, sore throat, diarrhea, conjunctivitis, headache, loss of taste or smell, a rash on skin, or discolouration of fingers or toes

Serious symptoms: difficulty breathing or shortness of breath, chest pain or pressure, loss of speech or movement

Pathogenesis:

The immune response by humans to CoV-2 virus occurs as a combination of the cell-mediated immunity and antibody production, just as with most other infections.

Although a rapid and well-coordinated immune response is necessary for a potent defense against viral infection, an excessive inflammatory response may lead to tissue damage at the systemic level. The massive production of cytokines and chemokines detected during COVID-19 infection, the so-called “cytokine storm”, is mainly responsible for the broad and uncontrolled tissue damage observed. The cytokine storm resembles the cytokine release syndrome (CRS) and results in plasma leakage, vascular permeability and disseminated vascular coagulation. These excessive proinflammatory host responses are major factors in the pathological outcomes such as acute lung injury (ALI) and acute respiratory distress syndrome (ARDS) seen in severe SARS-CoV-2 infected patients.

In addition to the dominant manifestation of respiratory symptoms, some patients have severe cardiovascular damage and individuals with underlying cardiovascular disease (CVD) have an increased risk of death. The mechanism underling the acute myocardial injury might be related to the high expression of ACE2 in the CVS.

On the other hand, the combination of cytokine storm together with respiratory dysfunction and hypoxaemia may be a mechanism by which COVID-19 results in damage to myocardial cells.

Patients also show lymphopenia and pneumonia with characteristic pulmonary

ground-glass opacity changes on chest CT. Other forms of severity including myocarditis, arrhythmia, cardiogenic shock as well as acute kidney injury have been reported in 10%–30% of ICU patients with confirmed COVID-19. In particular, neurological manifestations and pneumonia were reported in most hospitalized COVID-19 patients.

SARS-COV-2 Variants

All viruses, including SARS-CoV-2, the virus that causes COVID-19, change over time. Most changes have little to no impact on the virus' properties. However, some changes may affect the virus's properties, such as how easily it spreads, the associated disease severity, or the performance of vaccines, therapeutic medicines, diagnostic tools, or other public health and social measures.

Viruses constantly change through mutation, and new variants of a virus are expected to occur. Sometimes new variants emerge and disappear. Other times, new variants persist. Multiple variants of the virus that causes COVID-19 have been documented \ globally during this pandemic.

We are monitoring multiple variants; currently there are six notable variants in the United States:

1- B.1.1.7 (Alpha): This variant was initially detected in the United Kingdom.
2- B.1.351 (Beta): This variant was initially detected in South Africa in December 2020.
3- P.1 (Gamma): This variant was initially identified in travelers from Brazil, who were tested during routine screening at an airport in Japan, in early January.
4- B.1.427 and B.1.429 (Epsilon): These two variants were first identified in California in February 2021.
5- B.1.617.2 (Delta): This variant was initially identified in India in December 2020.

There are currently 4 main variants of concern that continue to be detected and monitored in an increasing number of countries and territories around the world.

Human Cancer Viruses

Viruses are etiologic factors in the development of several types of human tumors, including two of great significance worldwide—cervical cancer and liver cancer. At least 15–20% of all human tumors worldwide have a viral cause. The viruses that have been strongly associated with human cancers are listed in Table -1. They include human papilloma viruses (HPVs), Epstein-Barr virus (EBV), human herpesvirus 8, hepatitis B virus, hepatitis C virus, and two human retroviruses plus several candidate human cancer viruses. New cancer-associated viruses are being discovered by the use of molecular techniques. Many viruses can cause tumors in animals, either as a consequence of natural infection or after experimental inoculation.

Animal viruses are studied to learn how a limited amount of genetic information (one or a few viral genes) can profoundly alter the growth behavior of cells, ultimately converting a normal cell into a neoplastic one. Such studies reveal insights into growth regulation in normal cells. Tumor viruses are agents that can produce tumors when they infect appropriate animals. Many studies are done using cultured animal cells rather than intact animals, because it is possible to analyze events at cellular and subcellular levels. In such cultured cells, tumor viruses can cause “transformation.” However, animal studies are essential to study many steps in carcinogenesis, including complex interactions between virus and host and host responses to tumor formation. Studies with RNA tumor viruses revealed the involvement of cellular oncogenes in neoplasia; DNA tumor viruses established a role for cellular tumor suppressor genes. These discoveries revolutionized cancer biology and provided the conceptual framework for the molecular basis of carcinogenesis.

Table 1: Association of viruses with human cancers.

Virus Family	**Virus**	**Human Cancer**
papillomaviruses	Human Papillomaviridae	Genital tumors Squamous cell carcinoma

		Oropharyngeal carcinoma
Herpesviridae	Epstein-Barr virus Human herpesvirus 8	Nasopharyngeal carcinoma Burkitt lymphoma Hodgkin disease B-cell lymphoma Kaposi sarcoma
Hepadnaviridae	Hepatitis B virus	Hepatocellular carcinoma
Polyomaviridae	Merkel cell virus	Merkel cell carcinoma
Retroviridae	Human T-lymphotropic virus Human immunodefi ciency virus	Adult T-cell leukemia AIDS-related malignancies
Flaviviridae	Hepatitis C virus	Hepatocellular carcinoma

- General features of viral carcinogenesis

Tumor Viruses Are of Different Types

Like other viruses, tumor viruses are classified among different virus families according to the nucleic acid of their genome and the biophysical characteristics of their virions. Most recognized tumor viruses either have a DNA genome or generate a DNA provirus after infection of cells (hepatitis C virus is an exception). DNA tumor viruses are classified among the papilloma-, polyoma-, adeno-, herpes-, hepadna-, and poxvirus groups.

DNA tumor viruses encode viral oncoproteins that are important for viral replication but also affect cellular growth control pathways.

Most RNA tumor viruses belong to the retrovirus family. Retroviruses carry an RNA-directed polymerase (reverse transcriptase) that constructs a DNA copy of the RNA genome of the virus. The DNA copy (provirus) becomes integrated into the DNA of the infected host cell, and it is from this integrated DNA copy that all proteins of the virus are translated.

RNA tumor viruses are of two general types with respect to tumor induction. The highly oncogenic (direct transforming) viruses carry an oncogene of cellular origin. The

weakly oncogenic (slowly transforming) viruses do not contain an oncogene and induce leukemias after long incubation periods by indirect mechanisms. The two known cancer-causing retroviruses in humans act indirectly.

Hepatitis C virus, a flavivirus, does not generate a provirus and appears to induce cancer indirectly.

Multistep Carcinogenesis

Carcinogenesis is a multistep process, ie, multiple genetic changes must occur to convert a normal cell into a malignant one. Intermediate stages have been identified and designated by terms such as "immortalization," "hyperplasia," and "preneoplastic."

Tumors usually develop slowly over a long period of time. The natural history of human and animal cancers suggests a multistep process of cellular evolution, probably involving cellular genetic instability and repeated selection of rare cells with some selective growth advantage. The number of mutations underlying this process is estimated to range from five to eight.

Observations suggest that activation of multiple cellular oncogenes and inactivation of tumor suppressor genes are involved in the evolution of tumors whether or not a virus is involved. It appears that a tumor virus usually acts as a cofactor, providing only some of the steps required to generate malignant cells. Viruses are necessary—but not sufficient—for development of tumors with a viral etiology. Viruses often act as initiators of the neoplastic process and may do so by different mechanisms.

Interactions of Tumor Viruses with their Hosts

A. Persistent Infections

The pathogenesis of a viral infection and the response of the host are integral to understanding how cancer might arise from that background. The known tumor viruses establish long-term persistent infections in humans. Because of differences in individual genetic susceptibilities and host immune responses, levels of virus replication and tissue tropisms may vary among persons. Even though very few cells in the host may be infected at any given time, the chronicity of infection presents the long-term opportunity for a rare event to occur that allows survival of a cell with growth control mechanisms that are virus-

modified.

B. Host Immune Responses

Viruses that establish persistent infections must avoid detection and recognition by the immune system that would eliminate the infection. Different viral evasion strategies have been identified, including restricted expression of viral genes that makes infected cells nearly invisible to the host (EBV in B cells); infection of sites relatively inaccessible to immune responses (HPV in the epidermis); mutation of viral antigens that allows escape from antibody and T-cell recognition (human immunodeficiency virus [HIV]); modulation of host major histocompatibility complex class I molecules in infected cells (adenovirus, cytomegalovirus); inhibition of antigen processing (EBV); and infection and suppression of essential immune cells (HIV). It is believed that host immune surveillance mechanisms usually eliminate the rare neoplastic cells that may arise in normal individuals infected with cancer viruses. However, if the host is immunosuppressed, cancer cells are more likely to proliferate and escape host immune control. Immunosuppressed organ transplant recipients and HIV infected individuals are at increased risk of EBV-associated lymphomas and of HPV-related diseases. It is possible that variations in individual immune responses may contribute to susceptibility to virus-induced tumors in normal hosts.

C. Mechanisms of action by human cancer viruses

Tumor viruses mediate changes in cell behavior by means of a limited amount of genetic information. There are two general patterns by which this is accomplished: The tumor virus introduces a new “transforming gene” into the cell (directacting), or the virus alters the expression of a preexisting cellular gene or genes (indirect-acting). In either case, the cell loses control of normal regulation of growth processes. DNA repair pathways are frequently affected, leading to genetic instability and a mutagenic phenotype.

Viruses usually do not behave as complete carcinogens. In addition to changes mediated by viral functions, other alterations are necessary to disable the multiple regulatory pathways and checkpoints in normal cells to allow a cell to become completely transformed. There is no single mode of transformation underlying viral carcinogenesis. At the molecular level, oncogenic mechanisms by human tumor viruses are very diverse.

Cellular transformation may be defined as a stable, heritable change in the growth control of cells in culture. No set of characteristics invariably distinguishes transformed cells from their normal counterparts. In practice, transformation is recognized by the cells' acquisition of some growth property not exhibited by the parental cell type. Transformation to a malignant phenotype is recognized by tumor formation when transformed cells are injected into appropriate test animals. Indirect-acting tumor viruses are not able to transform cells in culture.

D. Cell susceptibility to viral infections and transformation

At the cellular level, host cells are either permissive or nonpermissive for replication of a given virus. Permissive cells support viral growth and production of progeny virus; nonpermissive cells do not. Especially with the DNA viruses, permissive cells are often killed by virus replication and are not transformed unless the viral replicative cycle that results in death of the host cell is blocked in some way; nonpermissive cells may be transformed. However, there are situations in which DNA virus replication does not lyse the host cell and such cells may be transformed. Nevertheless, transformation is a rare event. A characteristic property of RNA tumor viruses is that they are not lethal for the cells in which they replicate. Cells that are permissive for one virus may be nonpermissive for another.

Not all cells from the natural host species are susceptible to viral replication or transformation or both. Most tumor viruses exhibit marked tissue specificity, a property that probably reflects the variable presence of surface receptors for the virus, the ability of the virus to cause disseminated versus local infections, or intracellular factors necessary for viral gene expression.

Some viruses are associated with a single tumor type, whereas others are linked to multiple tumor types. These differences reflect the tissue tropisms of the viruses.

E. Retention of Tumor Virus Nucleic Acid in a Host Cell

The stable genetic change from a normal to a neoplastic cell generally requires the retention of viral genes in the cell. Often, but not always, this is accomplished by the integration of certain viral genes into the host cell genome. With DNA tumor viruses, a portion of the DNA of the viral genome may become integrated into the host cell

chromosome. Sometimes, episomal copies of the viral genome are maintained in tumor cells. With retroviruses, the proviral DNA copy of the viral RNA is integrated in the host cell DNA. Genome RNA copies of hepatitis C virus that are not integrated are maintained in tumor cells.

In some viral systems, virus-transformed cells may release growth factors that affect the phenotype of neighboring uninfected cells, thereby contributing to tumor formation.

It is also possible that as tumor cells collect genetic mutations during tumor growth, the need for the viral genes that drove tumor initiation may become unnecessary and viral markers will be lost from some cells.