1-Viral Pathogenesis
2-Viral Genetics

Viral Pathogenesis

Viral pathogenesis is the process by which a viral infection leads to disease.

Viral pathogenesis is an abnormal situation of no value to the virus.

The majority of viral infections are subclinical. It is not in the interest of the virus to severely harm or kill the host.

The consequences of viral infections depend on the interplay between **a numbers of viral** and **host factors**.

Outcome of Viral Infection

Acute Infection

Recovery with no residue effects

Recovery with residue effects e.g. acute viral encephalitis leading to neurological sequelae.

Death

Proceed to chronic infection

Chronic Infection

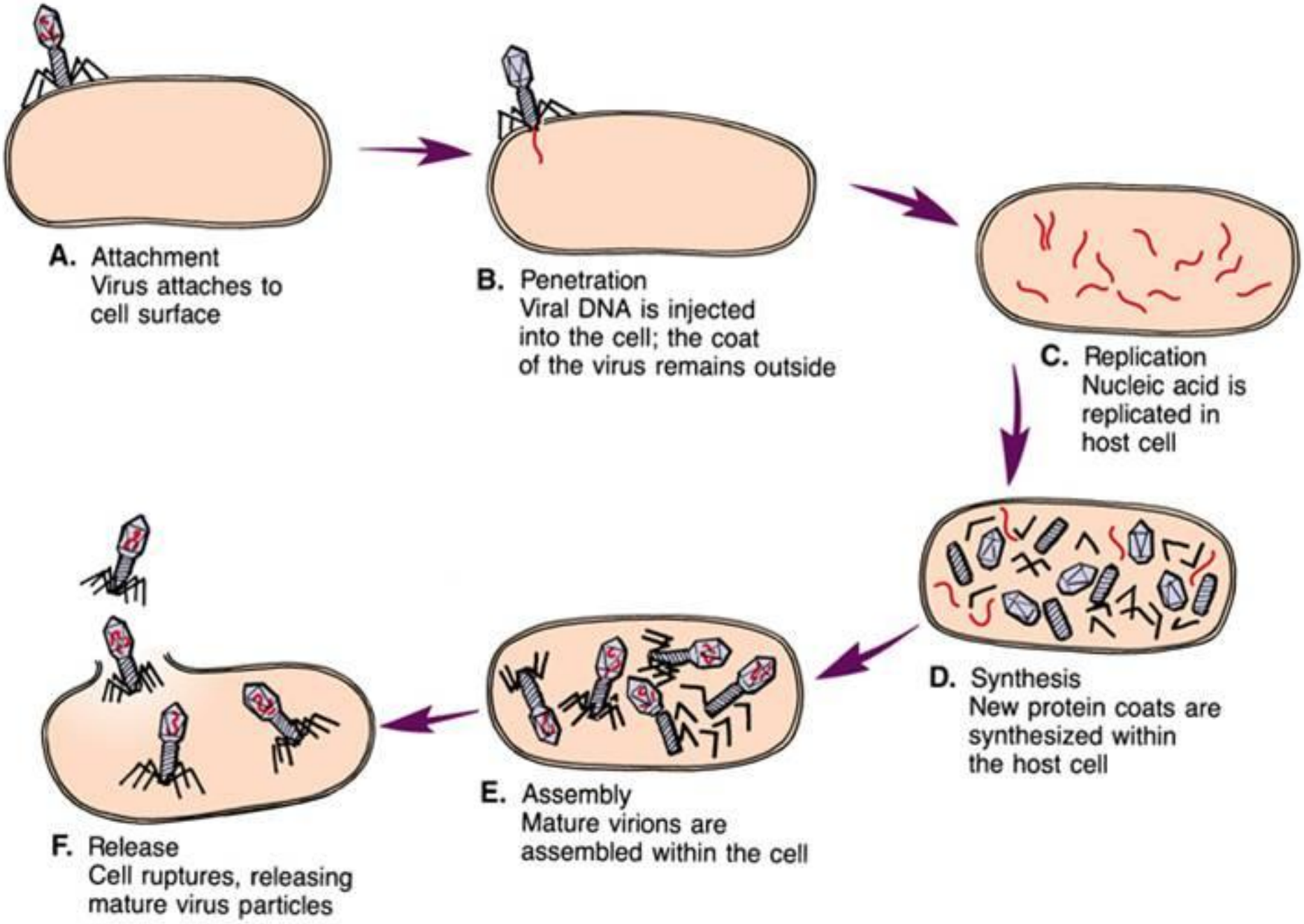
1- Silent subclinical infection for life e.g. CMV, (Epstein–Barr virus) EBV.

2- A long silent period before disease e.g. HIV, SSPE(Subacute sclerosing panencephalitis), PML(Progressive multifocal leukoencephalopathy).

3- Reactivation to cause acute disease e.g. herpes.

4- Chronic disease with relapses and exacerbations e.g. HBV, HCV.

5- Cancers e.g. (Human herpes-8)HHV-8


A. Attachment
Virus attaches to cell surface
B. Penetration
Viral DNA is injected into the cell; the coat of the virus remains outside
C. Replication
Nucleic acid is replicated in host cell
D. Synthesis
New protein coats are synthesized within the host cell
E. Assembly
Mature virions are assembled within the cell
F. Release
Cell ruptures, releasing mature virus particles

Factors included in Viral Pathogenesis

For pathogenic virus, there are a number of critical stages in replication which determines the nature of disease they produce which included;

1-Entry into the Host

2-Course of Infection (Primary Replication, Systemic Spread, Secondary Replication)

3-Cell/Tissue Tropism

4-Cell/Tissue Damage

5-Host Immune Response

6-Virus Clearance or Persistence

1- Entry into the host

The first stage in any virus infection. In the case of pathogenic infections, the site of entry can influence the disease symptoms produced. Infection can occur via:

A- Skin, dead cells therefore cannot support virus replication. Most viruses which infect via the skin require **a hole** in the physical integrity of this effective barrier, e.g. cuts or abrasions. Many viruses employ vectors, e.g. ticks, mosquitoes or vampire bats to breach the barrier.

B- Respiratory tract- In contrast to skin, the respiratory tract and all other mucosal surfaces possess sophisticated immune defense mechanisms, as well as non-specific inhibitory mechanisms(ciliated epithelium, mucus secretion, lower temperature) which virus must overcome.

C- Gastrointestinal tract- a hostile environment; gastric acid, bile salts, etc. Viruses that spread by GI tract must be adapted to this hostile environment.

D- Genitourinary tract- relatively less hostile than the others.

E-Conjunctiva and other mucous membranes - rather exposed site and relatively unprotected

2-Course of Viral Infection

Primary Replication

After entry to potential host, the virus must initiate an infection by entering a susceptible cell. This frequently determines whether the infection will remain **localized at the site** of entry **or spread to become systemic infection**.

Localized infections

Virus	primary replication
Rhinoviruses	upper respiratory tract
Rotaviruses	Intestinal epithelium
Papillomaviruses	Epidermis

Systemic Infections

Virus	primary replication	secondary replication
Enteroviruses	Intestinal epithelium	Lymphoid tissues, C.N.S
Herpesviruses	Oropharynx or G.U. tract	Lymphoid cells, C.N.S

Secondary replication

Occurs in systemic infections when a virus reaches other tissues in which it is capable of replication, e.g. poliovirus (gut epithelium- nervous in brain &spinal cord). If the virus can be prevented from reaching tissues where secondary replication can occur, **generally no disease results.**

Spread through out the host

Apart from direct cell-cell contact, there are 2 main mechanism for spread throughout the host:

Via the bloodstream

Via nervous system

Virus may get into the blood stream by direct inoculation- e.g. Arthropod vectors, blood transfusion or I.V drug abuse.

The virus may travel free in the plasma(Togaviruses, Enteroviruses) or in association with red cells (Orbiviruses) platelets (Herpes simplex virus). Primary viraemia usually proceeds and is necessary for spread to the blood stream, followed by more generalized higher titer secondary viaremia as the virus reaches other target tissues or replicates directly in blood cells.

As above, spread **to nervous system** is preceded by primary viraemia.

In some cases, spread occurs directly by contact with neurons at the primary site of infection, in other cases **via the bloodstream**. Once in peripheral nerves, the virus can spread to the CNS by axonal transport along nervous (Herpes simplex virus). Viruses can across synaptic junctions since these frequently contain virus receptors, allowing the virus to jump from one cell to another.

3- Cell/ Tissue tropism

Tropism-is the ability of a virus to replicate in particular cells or tissues- which is controlled partly by the route of infection but largely by the interaction of a virus attachment protein(V.A.P) with a specific receptor molecule on the surface of a cell and has considerable effect on pathogenesis.

Viral affinity for specific body tissues (tropism) is determined by:

- Cell receptors for virus.
- Cell transcription factors **that recognize** **viral promoters** and **enhancer sequences.**
- Ability of the cell to support virus replication.
- Physical barriers.
- Local temperature, pH, and oxygen tension enzymes and non-specific factors in body secretions.
- **Digestive enzymes and bile** in the gastrointestinal tract that may inactivate some viruses.

4-Host immune response

Has a major impact on the outcome of an infection. In the most cases the virus **is cleared completely** from the body and results in complete recovery.

In other infections, the immune response **is unable to clear** the virus completely and the virus persists.

In general, **cellular immunity plays the major role in clearing virus** infection **whereas humoral immunity** protects against reinfection.

5- Cell /Tissue damage

Virus may replicate widely throughout the body **without** any disease symptoms, if they do not cause significant cell damage or death. **Retroviruses** do not generally cause cell death, being released from the cell by budding **rather than** by cell lysis and cause persistent infections, **even being passed vertically to offspring if they infect the germ line**.

Conversely, **Picornaviruses** cause lysis and death of the cells in which they replicate, leading to fever and increased mucus secretion in the case of Rhinoviruses, paralysis or death (usually due to respiratory failure) for Poliovirus.

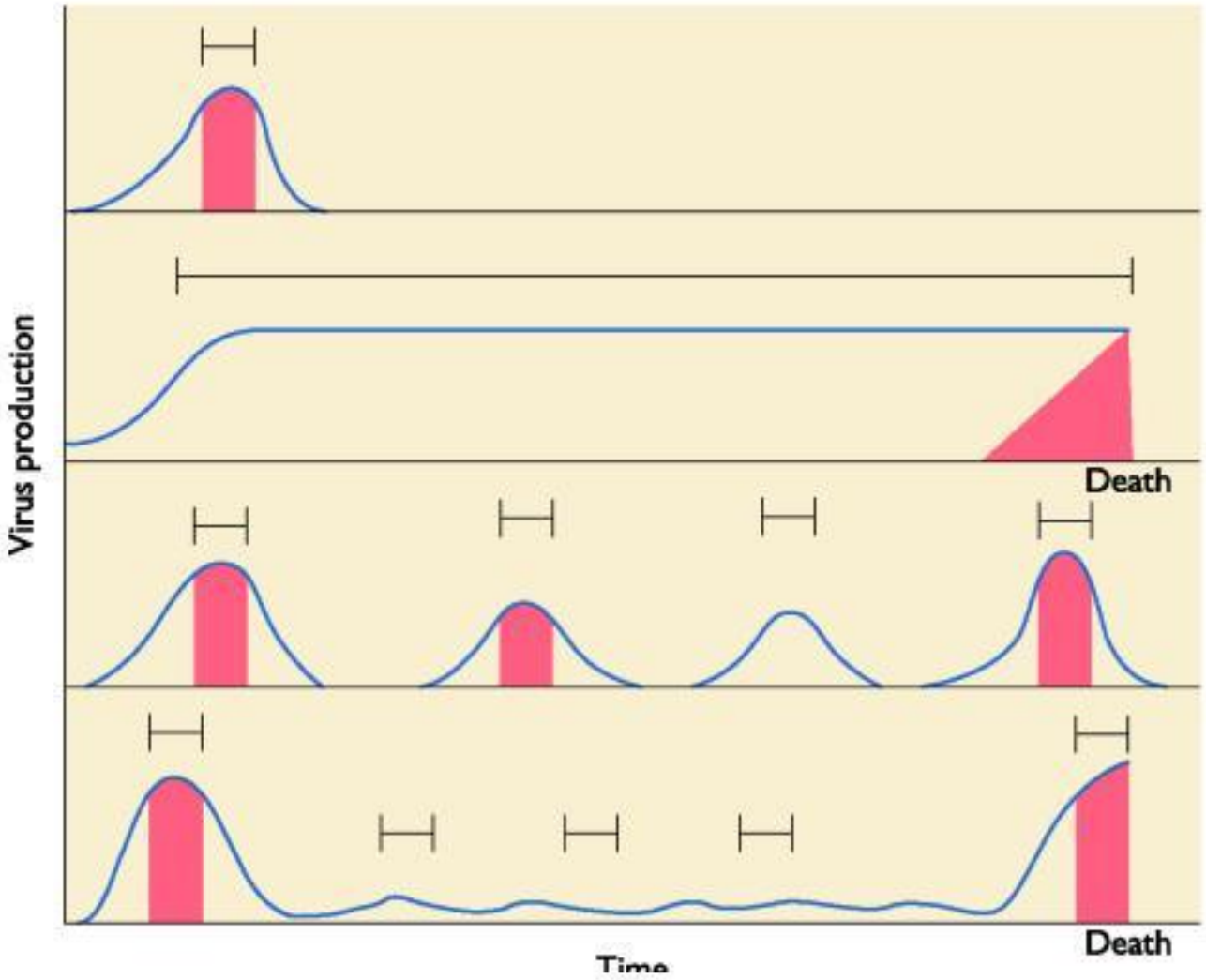
6- Viral Clearance or Persistence

The majority of viral infections are cleared but certain viruses may cause persistent infections. There are 2 types of chronic persistence infections.

1- **True Latency** -the virus remains **completely latent** following primary infection e.g. **Herpes simplex virus.**

2- Persistence - the virus replicates continuously in the body at a very low level e.g (HIV).

General patterns of infection



Acute infection
- Rhinovirus
- Rotavirus
- Influenza virus

Persistent infection
- Lymphocytic choriomeningitis virus

Latent, reactivating infection
- Herpes simplex virus

Slow virus infection
- Measles SSPE
- Human immunodeficiency virus

From Flint et al Principles of Virology

Antiviral immunity

Viruses are small, obligate intracellular parasites which cause infection by invading cells of the body and multiplying within them, within their life cycle they have **relatively short extracellular period**, prior to infecting the cells, **and longer intracellular period during** which they have undergo replication. The immune system has mechanism which can attack the virus in both these phase of its life cycle, and which involve both non-specific and specific effecter mechanism.

Non-specific mechanisms

Interferon:

Are proteins made and released by host cells in response to the presence of pathogens such as virus, bacteria etc. They allow for communication between cells to trigger the protective defenses of the immune system that eradicate pathogens or tumors.

IFNs belong to the large class of glycoproteins **known as cytokines**, they named after their ability to interfere with viral replication within host cells.

Viral infection of the cells directly stimulates the production of interferon's. **Type I interferons** lead to the induction of an antiviral state in the cells, which is characterized by inhibition of both **viral replication and cell proliferation**, and also enhancement of the **ability of natural killer cells to lyses virally infected cell.**

Mechanism of action of interferon

All interferon share several common effects; they are antiviral agent.

As an infected cell dies from a cytolytic virus, viral particles are **released that can infect nearby cells**. However, the infected cell can **warm neighboring cells** of a viral presence **by** releasing interferon to reduce protein synthesis of both viral and host gene. **Inhibited protein synthesis destroys of both the virus and infected host cells.** In addition, interferon induce production of hundreds of other proteins- known collectively as interferon-stimulated genes(ISGs)that have role in combating viruses.

Natural Killer Cells:

NK cells are subset of lymphocytes found in blood and tissues, **which lack** antigen specific surface receptors(TCR or immunoglobulin receptors). NK cells possess the ability **to recognize and lyse virally infected cell and certain tumor cell**.

Whilst not showing antigen specificity, they clearly exhibit some degree of selectivity in targeting abnormal cells for lysis. **Thus NK cells may be effective early in the course of viral infection**, and may limit the spread of infection during this early stage.

Specific Mechanism

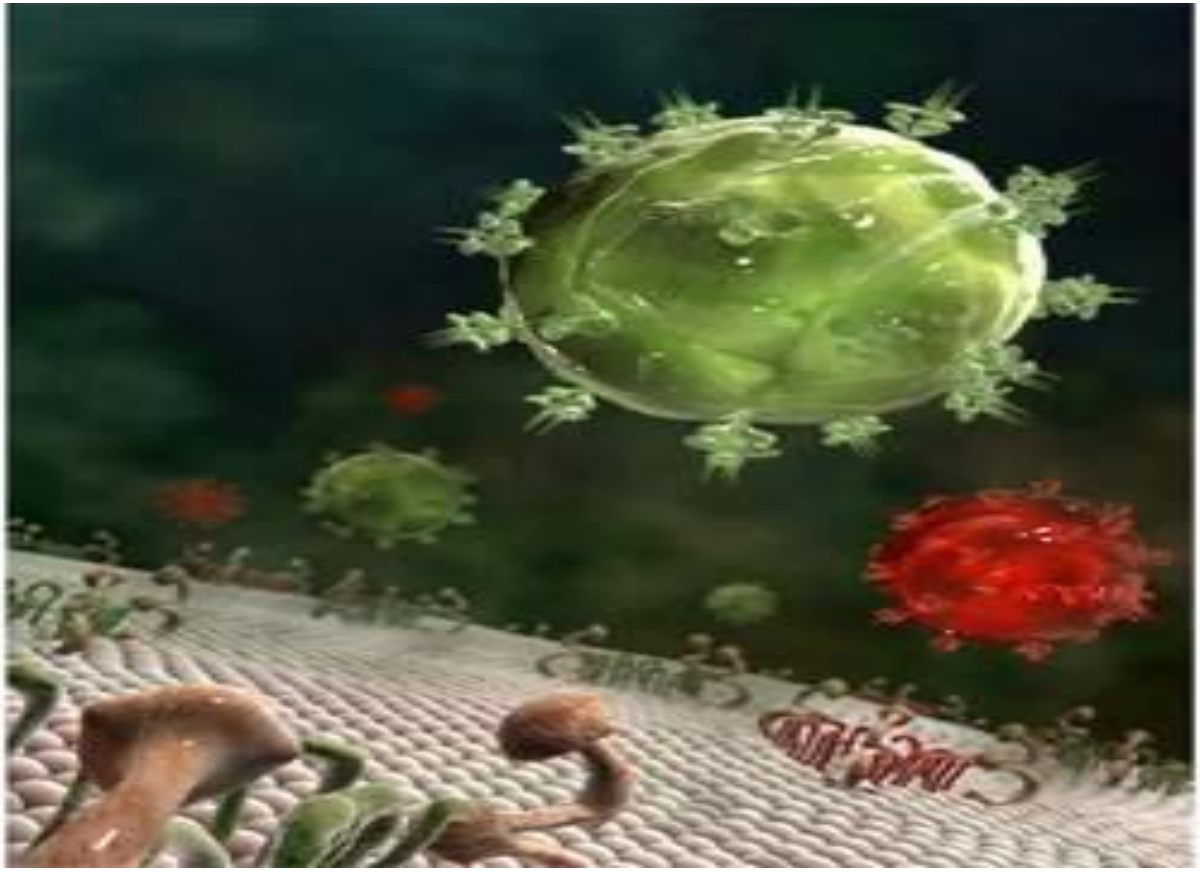
Both humoral and **cell mediated immunity** arms of the immune response play a role as specific effectors mechanism in antiviral immunity.

Antibody: Specific AB are important in protect against viral infection.
The most effective type of antiviral antibody is '**neutralizing antibody**'- this is antibody which binds to the virus, usually to the viral envelope or capsid proteins, and **which blocks the virus from binding and fast entry to the host cell**. Virus specific antibodies may also act as **opsonins in enhancing phagocytosis of virus particles-** this effect may be further enhanced by complement activation by antibody-coated virus particles.
In addition, in the case of some viral infections, viral proteins are expressed on the surface of the infected cell.

These may act as targets for virus specific antibodies, and may lead to **complement-mediated lysis of the infected cell**, or may direct subset of natural killer cells to lyse the infected cell through a process known as antibody –direct cellular cytoxicity(ADCC).

At mucosal surfaces (such as respiratory and gastrointestinal tracts), virus infection may induce the production of **specific antibodies of the IGA isotype**, which may be protective against infection at these surfaces. (This is the basis of immunization with current oral polio vaccine).

VIRAL GENETICS



GENERAL

Viruses grow rapidly, there are usually a large number of progeny virions per cell. There is, therefore, more chance of mutations occurring over a short time period. The nature of the viral genome (RNA or DNA; segmented or non-segmented) plays an important role in the genetics of the virus.

Viruses may change genetically due to **mutation or recombination**.

Viruses are simple entities, lacking an energy-generating system and having very limited biosynthetic capabilities. The smallest viruses have only a few genes; the largest viruses have as many as 200. Genetically, however, viruses have many features in common with cells. Viruses are subject to mutations, the genomes of different viruses can recombine to **form novel progeny**.

Genetic analysis is a powerful approach toward understanding the structure and function of the viral genome, its gene products and their roles infection and disease.

Variation in viral properties is of great importance for human medicine.

Viruses that have stable antigens on their surfaces (poliovirus, measles virus) **can be controlled by vaccination.**

Other viruses that exist as many antigenic types (rhinoviruses) or change frequently (influenza virus A) **are difficult to control by vaccination**; viral genetic may help develop more effective vaccines.

Some types of viral infections persist (retroviruses) in the presence of antibody and may be better controlled by antiviral drugs.

Genotype refers to the genetic structure of an organism, examples DNA.

Phenotype refers to the observable properties of an organism, which are produced by the genotype in cooperation with the environment. For examples, Hair color, eye color, weight, the ability to roll one's tongue.

The genome is the sum of the genes of an organism.

Wild-type virus; The naturally occurring, non-mutated strain of a virus or the original virus from which mutants are derived and with which the mutants are compared.

Fresh virus isolates from the natural host are referred to as field isolates or primary isolates.

A mutation is **heritable change in the genotype**, or spontaneous and random errors in the coping of viral nucleic acid. Mutation occurs when an error is incorporated in the viral genome.

The study of viral genetics falls into two general areas:

(1) Mutations and their effect on replication and pathogenesis.

(2) The interaction of two genetically distinct viruses that infect the same cell.

Mutation Rates and Outcomes

The DNA viruses have mutation rates similar to those of eukaryotic cells because, like eukaryotic DNA polymerase, their replicatory enzyme have **proofreading functions**.

The errors rate for DNA viruses have been calculated to be 10^{-8} to 10^{-11} errors per incorporated nucleotide. The proofreading function of **DNA polymerase reduces the error rate** from about one in a million basepairs to about one in a hundredthousand basepairs.

The RNA viruses, however, lack a proofreading function in their replicatory enzymes, and some have mutation rates that are many orders of magnitude higher—10^{-3} to 10^{-4} errors per incorporated nucleotide.

Defective viruses

A defective virus is one that **lacks one or more functional genes** required for viral replication.

Defective viruses require helper activity from another virus for some step in replication or maturation. e.g. **hepatitis D needs hepatitis B**. Hepatitis D (delta) virus (an RNA virus) does not code for its own envelope proteins but uses the envelope of hepatitis B virus (an DNA virus).

Phenotypic Variation by Mutations

Mutations can produce viruses with new antigenic determinants. The appearance of an antigenically **novel** virus through mutation is called **antigenic drift**. Antigenically altered viruses may be able to cause disease in previously resistant or immune hosts.

Vaccine Strains from Mutations

Mutations can produce viruses with a **reduced pathogenicity**, **altered host range**, or **altered target cell specificity but with intact antigenicity.** Such viruses can sometimes be used as **vaccine strains**.

Genetic Change in Viruses

Viruses are continuously changing as a result of genetic selection. They undergo subtle genetic changes through **mutation** and **major** genetic changes through **recombination**.

Mutation occurs when an error is incorporated in the viral genome. **Recombination** occurs when coinfecting viruses exchange genetic information, creating **a novel virus.**

When two or more virus particles infect the same host cell, they may interact in a variety of ways. They must be sufficiently related usually within the same viral family, for most types of interactions to occur.

Genetic interaction result in some progeny that are heritably (genetically) different either parent.

Progeny produced as a consequence of nongenetic interaction are similar to the parental viruses.

Origin of Mutation

Spontaneous mutations

These arise naturally during viral replication: e.g. due to errors by the **genome-replicating polymerase**

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

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

Mutations arise by one of three mechanisms:

(1) by the effects of physical mutagens (UV light, x-rays) on nucleic acids;

(2) by the natural behavior of the bases that make up nucleic acids

(3) through the weakness of the enzymes that replicate the nucleic acids.

The first two mechanisms act similarly in all viruses; hence, the effects of physical mutagens and the natural behavior of nucleotides are relatively constant.

However, viruses differ markedly in their mutation rates, which is due primarily to differences in the fidelity with which their enzymes replicate their nucleic acids. **Viruses with high-fidelity transcriptases have relatively low mutation rates and vice versa.**

Viruses Interaction: When two genetically distinct viruses infect a cell, three different phenomena can occur.

1-Recombination

Recombination involves **the exchange of genetic material** between two related viruses during coinfection of a host cell. **As a results** they production progeny virus (recombinant) that carries traits (characters) not found together in either parent.

Recombination generally occurs **between members of the same virus** type (e.g., between two influenza viruses or between two herpes simplex viruses). Two mechanisms of recombination have been observed for viruses: independent assortment and incomplete linkage. Either mechanism can produce new viral serotypes or viruses with altered virulence.

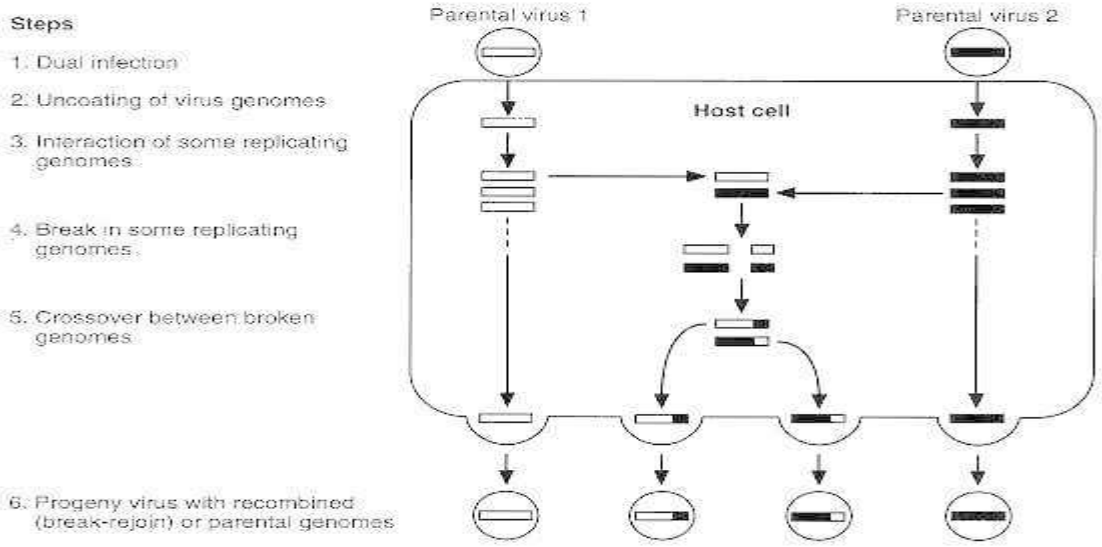
1-Recombination by Independent Assortment

Recombination by independent assortment can occur among viruses with **segmented genomes**. Genes that reside on different pieces of nucleic acid are randomly assorted. This can result in the generation of viruses with **new antigenic determinants** and **new host ranges**. Development of viruses with new antigenic determinants through independent **assortment is called antigenic shift.**

For example, for the influenza viruses (8 segments of single-stranded RNA) and for the reoviruses (10 segments of double-stranded RNA). Independent assortment between an animal and a human strain of influenza virus during a mixed infection can yield an antigenically **novel influenza** virus strain capable of infecting humans but carrying animal-strain hemagglutinin and/or neuraminidase surface molecules.

2-Recombination of Incompletely Linked Genes

Recombination also occurs between genes belong to the same piece of nucleic acid. Genes that generally separate together are called linked genes. If recombination occurs between them, the linkage is said to be incomplete. Recombination of incompletely linked genes occurs in all DNA viruses that have been studied and in several RNA viruses.



The genetic interaction of DNA viruses can result in break-rejoin recombination, in which the two DNA molecules of different viruses break and then cross over. Break-rejoin recombination results in novel progeny **viruses with some DNA sequences** of both types of parental viruses.

Phenotypic Variation from Recombination

Development of viruses with new antigenic determinants by either type of recombination may allow viruses to infect and cause disease **in previously immune hosts**.

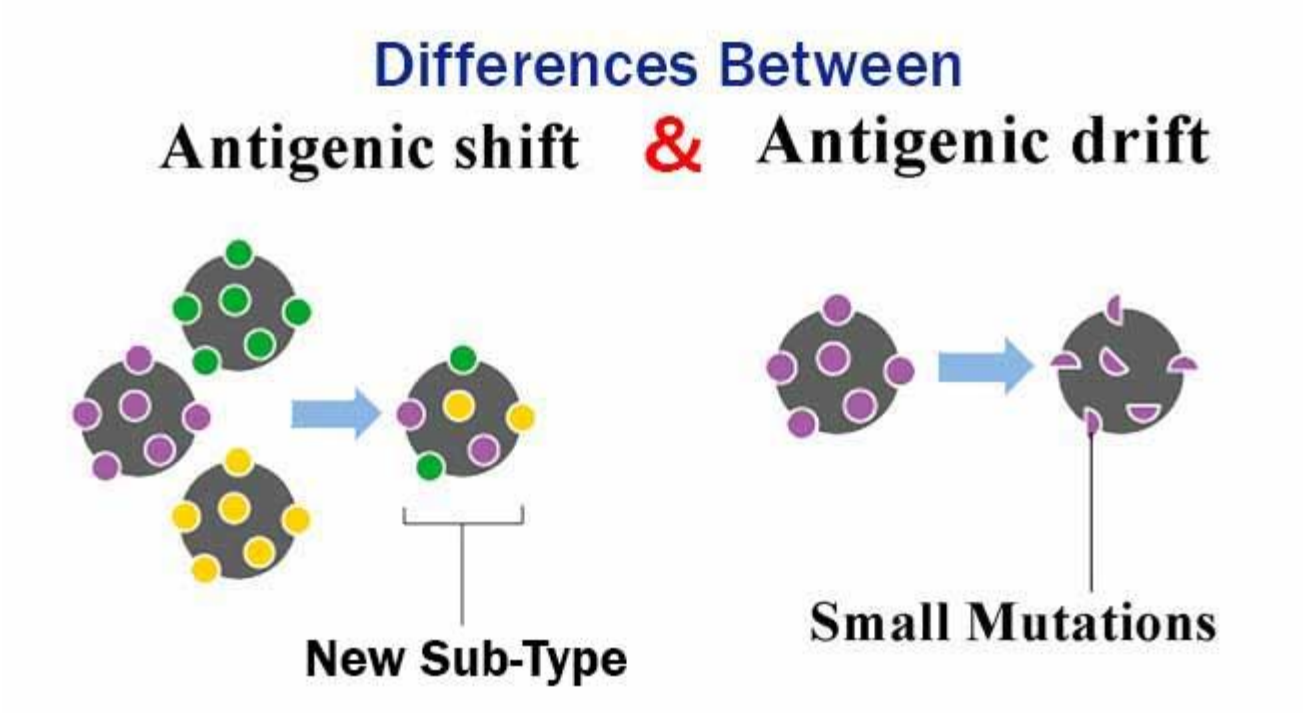
Vaccines through Recombination

Vaccine strains of viruses can be used to create recombinant viruses that carry extra genes coding for a specific immunogen.

During viral vaccination, the replicating virus will express the specific immunogen. Specific antibody production will be stimulated, and the host will be protected from the immunogen as well as from the vaccine virus.

Differences Between Antigenic Shift and Antigenic Drift

Influenza Virus are remarkable because of the frequent antigenic change that occurs in HA (hemagglutinin) or NA (neuraminidase). The two surface antigens of influenza undergo antigenic variation independent of each other. They are **Antigenic Shift** and **Antigenic Drift.**



Antigenic Shift	Antigenic drift
Major Antigenic Change, Large change in nucleotides of RNA	Minor Antigenic Change, Small mutation of RNA
Forming new sub-type (Subtype A + Subtype B –> New Subtype)	Forming new strain of virus
One or Two Viruses are Involved	Only one virus is involve
Occurs once in a time	Occurs frequently
May jump from one species to another (animal-human)	May infect animals of the same species
Occurs as a results of genome reassortment between difference subtypes.	Occurs as a result of the accumulation of point mutations in the gene.
An antigenic change which results in drastic or dramatic alternation in HA (hemagglutinin) or NA (neuraminidase) subtypes.	An antigenic change can alter antigenic sites on the molecule such that a virion can escape recognition by the host's immune system.
Large and sudden mutation and Difficult to treat (need new vaccine)and Occurs only in Influenza Virus A.	Random and Spontaneous Mutation and Easy to treat (antibody and drugs available)and Occurs in Influenza Virus A, B and C

2-Complementation

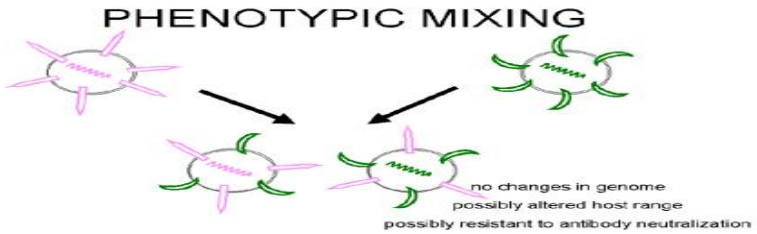
Complementation can occur when **either one or both of the two** viruses **that infect the cell** have a **mutation that** results in a nonfunctional protein. The non-mutated virus "complements" the mutated one by making a functional protein that serves for both viruses. Complementation is an important method by which a helper virus permits replication of a defective virus. One clinically important example of complementation is **Hepatitis B virus** providing its surface antigen to Hepatitis delta(D) virus, which is defective in its ability to produce its own outer protein.

3-Phenotypic mixing

The genome of virus type A can be coated with the surface proteins of virus type B. This phenotypically mixed virus can infect cells as determined by its type B protein coat. However, the progeny virus from this infection has a type A coat; it is encoded uniquely by its type A genetic material.

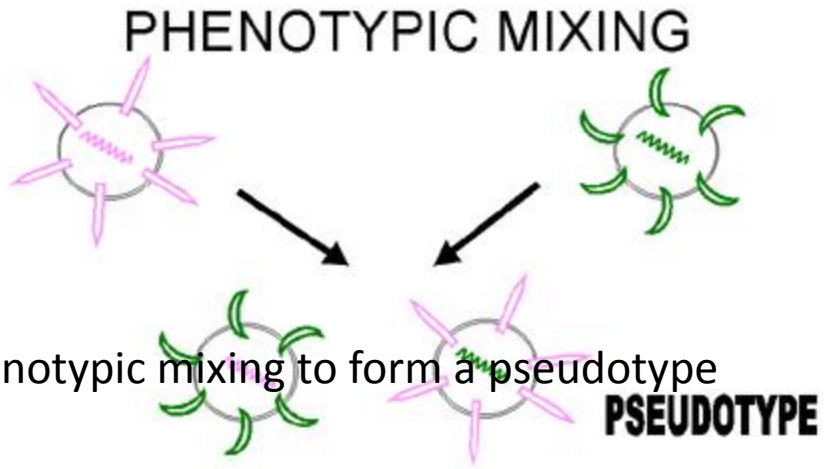
An interesting example of phenotypic mixing is that of **pseudotypes,** which consist of the **nucleocapsid** of one virus and **the envelope** of another.

Pseudotypes composed of the **nucleocapsid of vesicular stomatitis virus** (a Rhabdovirus) and the **envelope of Human immunodeficiency virus** (HIV, a Retrovirus) are currently being used to study the immune response to HIV.



Phenotypic mixing between two different viruses infecting the same cell

The **pseudotype** described above will show the **adsorption-pentration** surface antigenicity characterisistics of the rhabdovirus and will then, upon infection, behave as a retrovirus and produce progeny retroviruses. This results in **pseudotypes** having an **altered host range/tissue tropism.**



Phenotypic mixing to form a pseudotype

Interference

Infection of either **cell cultures** or **whole animals** with **two viruses** often leads to **inhibition of multiplication of one of the viruses**, an effect called interference.

Interference in animals is distinct from specific immunity.

Furthermore, interference does not occur with all viral combinations; two viruses may infect and multiply within the same cell as efficiently as in single infections.

Several mechanisms have been elucidated as causes of interference:

1- One virus may **inhibit** the ability of the second to **adsorb** to the cell, either by blocking its receptors (retroviruses, enteroviruses) or by destroying its receptors(orthomyxoviruses).Surface interference,

2- One virus may **compete** with the second for **components of the replication apparatus** (e.g. polymerase, translation initiation factor). Competition interference.

3- The first virus may cause the infected cell to **produce an inhibitor** (interferon) that prevents replication of the second virus.