Part 3: Virology

1. Introduction

Viruses are very small particles that only reproduce by infecting host cells. They are primarily known for their high pathogenicity. Depending on the species, they are capable of causing very serious diseases in humans, animals, and plants. A virus can have one or more hosts.

2. Definition of viruses and virions

Viruses are entities of organic matter; they do not possess a cellular structure. They are formed by a nucleic acid (DNA or RNA) and a protein shell called a "capsid."
Viruses can be naked or surrounded by an "envelope". Therefore, the structure of viruses is described as "acellular".

A virion is a virus that has been expelled from the host cell after its destruction. It is complete, autonomous, and ready to infect a new host. The virion lacks its own metabolism; therefore, it is inert until it infects another cell.

3. General properties of viruses

Viruses are very small and vary in size, ranging from 20 nm (e.g., Parvoviridae, parasites of vertebrates and insects) to 300 nm (e.g., Poxviridae, parasites of humans). Their main characteristic, which led to their discovery, is their ability to pass through filters that are impermeable to bacteria.

Viruses are incapable of performing any physiological function; they are classified as

"absolute obligatory parasites":

- **Parasites**: develop at the expense of a host cell by hijacking its metabolism.

- **Mandatory**: can only reproduce by using the metabolism of the host cell.

- **Absolutes**: depend on the host cell to perform all the functions necessary for survival and replication (Lwoff 1957, Tortora et al. 2010).

These acellular entities are capable of undergoing mutations and recombinations in order to evolve and adapt to changes in their environment. This allows them to resist various treatments, as is the case with HIV, and to increase their pathogenicity.

4. Structure of viruses and bacteriophages

4.1. Virus structure

Viruses are made up of a nucleic acid onto which proteins are inserted to form the capsid. This structure is called the "nucleocapsid". Depending on the type of virus, the capsid may be naked or surrounded by an envelope.

- **The viral genome**The viral genome consists of either DNA or RNA, but never both. It can be single-stranded or double-stranded: Viruses therefore possess either single-stranded DNA, double-stranded DNA, single-stranded RNA, or double-stranded RNA. This nucleic acid can be circular or linear, segmented or attached. It contains only the genes for the replication enzymes and the enzymes responsible for hijacking the host cell's metabolism.

  The total size of the viral genome varies from a few thousand to 250,000 nucleotides, hence the term "genome" rather than "chromosome" (we recall that Escherichia coli contains nearly 4 million nucleotides)
- **The capsid:**It is entirely composed of proteins. Depending on the virus, the capsid can contain one or more types of molecules. These molecules are assembled to form capsomeres (subunits of the capsid). The assembly of capsomeres is characteristic of viruses and determines the precise morphology of the capsid: helical, polyhedral, cubic, etc. (Figure 01). The capsid plays an important role in protecting the viral genome and in attaching to the host cell by determining the virus's antigenicity.
- **The envelope**It is formed by the cytoplasmic membrane or the nuclear membrane of the host cell. It therefore contains lipids, carbohydrates, and proteins.

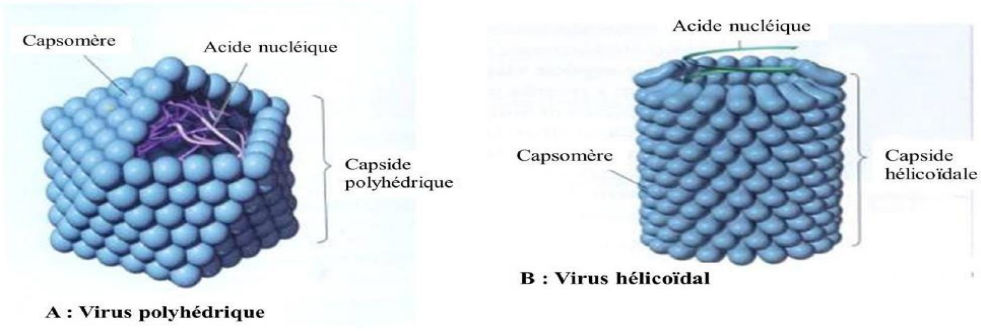

Figure 01: Examples of some capsid shapes

4.2. Structure of bacteriophages

Bacteriophages have virtually the same genome and capsid structure as other viruses; they differ primarily in the presence of a neck, a contractile sheath, tail fibers, spikes, and a terminal plaque (Figure 2). These specific structures serve for attachment to the bacterial cell wall and for the transport of nucleic acid into the bacterium. Bacteriophages can be enveloped or naked. The genome of bacteriophages is highly diverse, but the majority of species have nucleic acid composed of double-stranded DNA, single-stranded RNA, or double-stranded RNA.

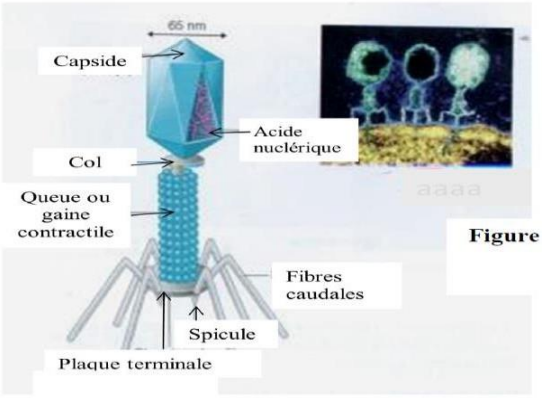

Figure 49 : Structure du bactériophage (Tortora *et al.* 2010).

5. Viral systematics

Although several parameters exist for differentiating and classifying viruses, the International Committee on Taxonomy of Viruses (ICTV) has identified only three main ones and divided viruses into families. Each family contained different genera and species. Subsequently, families with similar characteristics were grouped into orders.

The main characteristics on which the CITV based its classification of viruses were

- The type of nucleic acid: its nature (DNA or RNA), its structure (double-stranded or single-stranded, positive or negative polarity) and its shape (linear or circular, segmented or unsegmented)

- The replication mode

- The morphology of the capsid and the presence or absence of the envelope.

Order names end with the suffix "ales", e.g., Manonegavirales, Nidovirales. Family names end with "viridae", e.g., Herpesviridea, Myxoviridae, Picornaviridae. And genus names end with "virus", e.g., Myxovirus, Adenovirus.

Species are written by adding a name to the genus such as Herpesvirus simplex (HSV) or by designating them with common descriptors such as human immunodeficiency virus (HIV).

6. Viral genomes

As previously described, the virus genome can be made up of DNA or RNA. It can be single-stranded or double-stranded, circular or linear, segmented or attached (Figure 03).

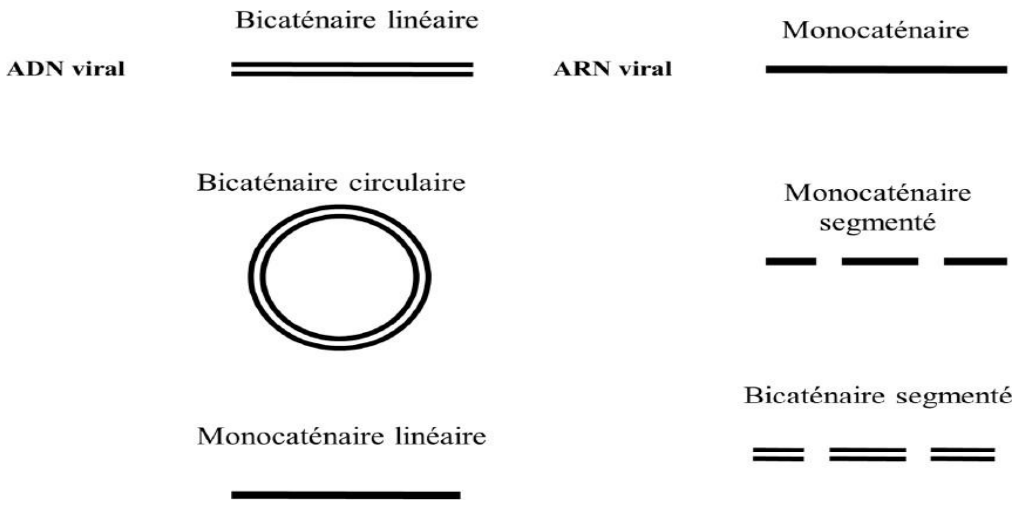

Figure 03: Possible types of viral genomes:

According to their genomes, viruses are divided as follows:

1. DNA virus

- Double-stranded DNA: linear or circular - Single-stranded DNA: linear

2. RNA virus

- Double-stranded RNA: segmented - Positive-sense single-stranded RNA (RNA+): this is an RNA that can be directly translated by ribosomes - Negative-sense single-stranded RNA (RNA-): this RNA must be transcribed into mRNA before translation. - RNA

single-stranded ambisense (RNA +/-): this is an RNA formed from a portion with positive polarity linked to another portion with negative polarity.

3. Reverse transcriptase (RT) virus: These viruses are capable of encoding an RT which allows the synthesis of DNA from RNA: - Single-stranded RNA virus (+RT) - Double-stranded DNA virus (+RT).

7. Viral replication

Virus replication occurs in the following stages:

7.1. Attachment

Viruses are unable to seek out host cells; collisions occur accidentally. However, viral particles adhere to cells via capsid proteins for non-enveloped viruses and glycoproteins for enveloped viruses. These molecules bind to specific receptors on the host cell, hence the concept of specificity between viruses and cells.

Examples: HIV It specifically infects CD4+ lymphocytes because its envelope can only attach to the CD4 molecule. Influenza viruses primarily infect respiratory cells because their envelope binds to sialic acids.

7.2. Penetration

The virus enters the cell through one of the following mechanisms:

- EndocytosisThis process is observed in enveloped and naked viruses. The virus enters the host cell in a vesicle or endosome. The endosome is then destroyed by a drop in pH, thus releasing the virus into the cytoplasm.
- Fusion: This is observed in enveloped viruses. The envelope fuses with the host's cytoplasmic membrane (fusion followed by lysis), allowing the nucleocapsid to enter through a large pore. Example: HIV penetration
- There Translocation or microphagocytosis: This is observed only in non-enveloped viruses. In this type of penetration, the virus transfers its nucleic acid and leaves the capsid outside the cell. Example: Poliovirus penetration.

7.3. Decapsulation

Once inside the cell, the virus's capsid is destroyed by cellular decapsidases; with the exception of Poxvirus, which possesses its own enzyme. The genome is then

released and can begin replication. Uncoating is observed only when penetration occurs by endocytosis or fusion.

7.4. Replication

The viral genome tends to be transcribed, translated, and then replicated. To do this, it must replace, in whole or in part, the cellular genome. The cell's metabolism is thus diverted to the benefit of the virus.

Replication can be more or less complex depending on the type of viral nucleic acid. Only DNA viruses that replicate intranuclearly can use cellular enzymes for transcription. Others must possess their own enzymes.

- **The multiplication of DNA viruses:**It is done in two phases:

- The early phase During this phase, a small portion of the genome is transcribed by a cellular DNA-dependent RNA polymerase. The "early" messenger RNAs migrate into the cell cytoplasm to be translated by the cell's ribosomes into regulatory (non-structural) proteins or enzymes involved in DNA synthesis. These enzymes, along with the cellular DNA polymerase, replicate the viral DNA to produce a large number of copies.

- The late phasewhere the newly formed DNA will serve as templates for a second transcription which results in the formation of "late" messenger RNAs. These are then translated to form the capsid proteins.

- **The multiplication of RNA viruses:**It differs depending on the type of nucleic acid:

*** Single-stranded, positive-sense RNA virus**Here, the viral RNA is used directly as mRNA and is immediately translated by cellular ribosomes. Protein synthesis is carried out by cellular protein polymerases, followed by autocleavage by a cellular protease. This mechanism yields structural proteins (of the capsid) and enzymatic proteins.

The viral RNA undergoes several replications to form identical RNAs onto which structural proteins attach.

*** Negatively polarized single-stranded RNA virus**In this case, the viral RNA must be converted into mRNA by a viral RNA replicase. The mRNA produced serves as a template for producing new viral genomic RNAs and for the synthesis of capsid proteins.

*** RNA virus and RT**Once introduced, the viral RNA is reverse-transcribed into DNA in the cytoplasm by viral RT. This results in a single-stranded viral DNA, which subsequently becomes double-stranded. The DNA migrates into the nucleus and integrates into the cellular chromosome, thus forming proviral DNA. This DNA then undergoes transcription into mRNA and translation.

7.5. Assembly and maturation

The produced genomes are surrounded by proteins to form a capsid identical to that of the original virus. This step is called "encapsidation." It can be simple, involving self-assembly of the proteins and encapsidation of the genome, or more complex, requiring the involvement of several specific viral proteins.

7.6. Release

Once replication and assembly are complete, the new viruses are released to infect new cells. For naked viruses, release occurs through the bursting of the host cell, while for enveloped viruses, it occurs through budding to form new envelopes. Some viruses, like Herpesviruses, acquire an envelope derived from the nuclear membrane of the infected cell, while others, like Retroviruses, acquire an envelope derived from the cytoplasmic membrane.

8. Plant viruses and animal viruses

8.1. Plant viruses

Because plants have a cell wall that protects their cells, viruses can only penetrate through a lesion or wound. Therefore, most plant viruses are naked. However, some viral particles are transmitted to plants through seeds, pollen, or by vectors (insects, fungi, mites, and nematodes).

8.2. Animal viruses

In animals, another particular type of virus is found. It consists of a single, inert protein. This virus is called a "prion," and its protein is called "PrP" (Prion Protein). The PrP protein is normally produced by all mammals, including humans, but in a non-pathogenic form. In contrast, the prion protein is in an abnormal and pathogenic form (PrPsc). Upon entering the cell, PrPsc alters the configuration of all the host's PrP proteins, making them abnormal. This phenomenon represents the means of prion replication. The produced PrPsc proteins then leave the host cell and infect other cells.

9. Latent infections and cytocides

9.1. Latent infections

Latent infection is an infection that remains silent for a long period (years, or even a lifetime): the host carries the virus but shows no symptoms. However, once exposed to a specific stimulus, the viral cycle is suddenly activated and viral replication becomes significant. The stimulus could be a fever, immunosuppression, etc.

Examples: - Herpes viruses can remain in cells for years without causing any harm to the host. But in cases of immunosuppression, the resulting infection is often fatal.

- The varicella-zoster virus can also lie dormant in the nerve ganglia, but changes in the immune response can activate it. The virus then causes shingles or Zosler's herpes.

9.2. Cytocidal infections

These cases represent situations where the virus remains inactive for a long period and then suddenly causes cell lysis: by multiplying rapidly and in large quantities, the virus leads to cell necrosis in just a few hours. However, a later effect can be observed when viral replication is slow. This is the case with Myxoviruses, which cause necrosis after infected cells fuse into multinucleated plasmodia.

10. The viral restriction

During a viral infection, the immune system mobilizes several cells whose objective is to destroy the virus. The most active cells are lymphocytes and plasma cells:

Dendritic cells recognize and phagocytose the virus, then insert antigenic fragments of the virus onto their membrane. This is the very principle of innate restriction.

The dendritic cells then migrate to the lymph nodes where they present the antigen to CD4 T lymphocytes. Activation of the CD4 T cell is then triggered and causes the secretion of interleukin 2. These proliferate and differentiate into effector T cells (helper T cells).

Effector T cells activate the proliferation of B lymphocytes, which transform into antibody-producing plasma cells. These plasma cells then intercept the virus. This reaction involves both innate and adaptive immunity.

On the other hand, virus-infected cells expose an antigenic fragment of the virus on their plasma membrane, which allows lymphocytes to specifically destroy them and leave healthy cells intact.

11. Viral culture and Virus detection

The viral culture is done in :

- Embryonated eggs
  - ✓ Chorioallantoic membrane
  - ✓ Amniotic and/or allantoic cavities
  - ✓ Vitellus (egg yolk)
- Cells derived from human tissue in a single cell layer (observation of characteristic cytopathogenic effects)
- Susceptible bacteria (bacteriophages) (lysis zones on bacterial plates)

Virus detection is performed in :

- Viral culture on susceptible cells
  - ✓ Cells cultured in synthetic medium
  - ✓ Culture on embryonated eggs
- Detection of specific antibodies in infected hosts
  - ✓ Serological tests [early serum, late serum (acute or latent phase)]
- Observation under electron microscopy
- Detection of viral genetic material (PCR)

12. Disease, treatment, and prevention

12.1. Diseases caused by viruses

Here are some examples of viruses and the diseases they cause :

- ❖ Pneumotropic viruses : Influenza, adenovirus, rhinovirus, respiratory syncytial virus,
- ❖ Dermotropic viruses : Smallpox, measles, rubella, chickenpox, and shingles,
- ❖ Visceralotropic viruses : Hepatitis, yellow fever, gastroenteritis, HIV, mononucleosis,
- ❖ Neurotropic viruses : Rabies, encephalitis, and poliomyelitis

12.2. Control of viral infections

❖ Antiviral drugs (treatment) :

- ✓ Amantadine: prevents influenza virus attachment
- ✓ Vidarabine: treatment of shingles and encephalitis caused by the herpes virus
- ✓ Acyclovir: treatment of chickenpox and genital herpes
- ✓ Nucleoside and non-nucleoside analogues, protease inhibitors, and fusion inhibitors: act at various stages of the HIV life cycle
- ✓ Enzyme inhibitors: polymerase

❖ Antiviral vaccines (prevention) :

- ✓ Inactivated viruses (heat or formaldehyde treatment)
- ✓ Attenuated (live) viruses
- ✓ Subunit vaccines (genetic engineering)
- ✓ DNA-based vaccines