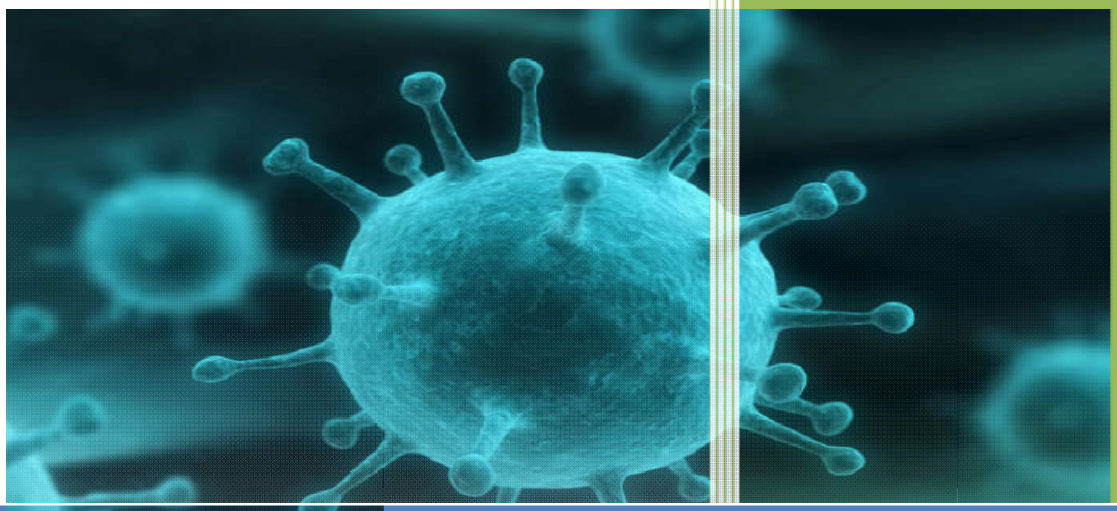

Medical Virology Lecture Note

For Medical Laboratory Sciences students (Year-III)

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Introduction to Virology

Learning Objectives

At the end of the Lesson, you will be able to:

- ✓ Describe the history of virology & explain how the present state of our knowledge of viruses was achieved
- ✓ Define what a virus is, and explain how viruses differ from other organisms
- ✓ Describe the essential properties of viruses
- ✓ Explain the structure/morphology of virus and its compositions

Introduction to Virology

Virology is a field within microbiology that encompasses the study of viruses. Medical virology deals with the study of medically important viruses and the diseases they cause or their effect on human beings. Viruses are particles consisting of protein and nucleic acid (an RNA or DNA genome). Lack both cellular structure and independent metabolic processes. They replicate solely by exploiting living cells based on the information in the viral genome.

The History of Virology

The first written record of a virus infection is from ancient Egypt (3700BC), which shows a temple priest with typical signs of paralytic poliomyelitis. Pharaoh Ramses V, who died in 1196BC and whose well-preserved mummified body is now in a Cairo museum, is believed to have died from smallpox. The comparison between the pustular lesions on the face of this mummy and those of more recent patients is startling, and traces of smallpox epidemic in the family of Ramses V of the Egyptian 20th dynasty proved by electron microscopy and immunology.

Smallpox was endemic in China by 1000BC. In response, the practice of variolation was developed. Recognizing that survivors of smallpox outbreaks were protected from subsequent infection, people inhaled the dried crusts from smallpox lesions like snuff or, in later modifications, inoculated the pus from a lesion into a scratch on the forearm. Variolation was practiced for centuries and was shown to be an effective method of disease prevention, although risky because the outcome of the inoculation was never certain.

Edward Jenner was nearly killed by variolation at the age of seven! Not surprisingly, this experience encouraged him on to find a safer alternative treatment. On May 14, 1796, he used cowpox-infected material obtained from the hand of Sarah Nemes, a milkmaid from his home village in England, to successfully vaccinate 8-year-old James Phipps. Although initially controversial, vaccination against smallpox was almost universally adopted worldwide during the 19th Century.

This early success (vaccine protection) was an achievement of scientific observation and reasoning was not based on any real understanding of the nature of infectious agents. This arose separately from another line of reasoning. Antony van Leeuwenhoek (1632-1723), a Dutch merchant, constructed the first simple microscopes and with these identified bacteria as the “animalcules” he saw in his specimens. However, it was not until Robert Koch and

Louis Pasteur in the 1880s jointly proposed the "germ theory" of disease that the significance of these organisms became apparent.

Germ theory of disease - Koch's postulates

Koch defined four famous criteria, which are now known as Koch's postulates and still generally regarded as the proof that an infectious agent is responsible for a specific disease:

- The agent must be present in every case of the disease.
- The agent must be isolated from the host and grown in vitro.
- The disease must be reproduced when a pure culture of the agent is inoculated into a healthy susceptible host.
- The same agent must be recovered once again from the experimentally infected host.

Subsequently, Pasteur worked extensively on rabies, which he identified as being caused by a virus (from the Latin for "poison"), but despite this he did not discriminate between bacteria and other agents of disease.

- In 1892, Dimitri Iwanowski, a Russian botanist, showed that extracts from diseased tobacco plants could transmit disease to other plants after being passed through ceramic filters fine enough to retain the smallest known bacteria. Unfortunately, he did not realize the full significance of these results.

- A few years later (1898), Martinus Beijerinick confirmed and extended Iwanowski's results on tobaccomosaic virus (TMV) and was the first to develop the modern idea of the virus, which he referred to as contagium vivum fluidum (soluble living germ).

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

But, despite the realization that these new found agents caused disease in animals as well as plants, people would not accept the idea that they might have anything to do with human diseases. This resistance was finally dispelled in 1909 by Karl Landsteiner and Erwin Popper, who showed that poliomyelitis was caused by a "filterable agent" - the first human disease to be recognized as being caused by a virus.

- Frederick Twort (1915) and Felix d'Herelle (1917) were the first to recognize viruses that infect bacteria, which d'Herelle called bacteriophages (eaters of bacteria)

- In the 1930s and subsequent decades, pioneering virologists such as Salvador Luria, Max Delbruck, and others used these viruses as model systems to investigate many aspects of virology, including virus structure, genetics, and replication

These relatively simple agents have since proved to be very important to our understanding of all types of viruses, including those of humans, which can be much more difficult to propagate and study. The further history of virology is the story of the development of experimental tools and systems with which viruses could be examined and that opened up whole new areas of biology, including not only the biology of the viruses themselves but inevitably also the biology of the host cells on which they are dependent.

- **Virus diversity**

There is more biological diversity within viruses than in all the rest of the bacterial, plant & animal kingdoms put together. This results from the success of viruses in parasitizing all known groups of living organisms. Understanding this diversity is the key to comprehending the interactions of viruses with their hosts. At a molecular level, protein-protein, protein-nucleic acid, & protein-lipid interactions determine the structure of virus particles, the synthesis & expression of virus genomes & the effects of viruses on the host cell.

- **Viruses are distinct from living organisms**

Viruses are submicroscopic, obligate intracellular parasites; they can only be seen with a special, very powerful microscope called an "electron microscope," However, a few groups of prokaryotic organisms that have specialized intracellular parasitic life-cycles & which puzzle the above definition. The *Rickettsiae* & *Chlamydiae* - obligate intracellular parasitic bacteria which have evolved so that they can exist outside the cells of their hosts only for a short period of time before losing viability. Therefore, it is necessary to add further clauses to the definition of what constitutes a virus.

Viruses differ from other microorganisms in a number of characteristics:

- they have no cellular structure, consisting only of proteins and nucleic acid (DNA or RNA)
- They have no metabolic systems of their own, but rather depend on the synthetic mechanism of a living host cell. Viruses exploit normal cellular metabolism by delivering their own genetic information, i.e., nucleic acid, into the host cell. One thus might call viruses “vagabond genes”
- Viruses infect other organism: bacteria (so-called bacteriophages), plants, animals, and humans.

- **Virus definition**

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

- **Viruses are energy parasites**

No known virus has the biochemical or genetic potential to generate the energy necessary to drive all biological processes (e.g. macromolecular synthesis). They are therefore absolutely dependent on the host cell for this function.

Are viruses are alive?

- One view is that inside the host cell, viruses are alive, whereas outside it they are merely complex assemblages of metabolically inert chemicals. Chemical changes may occur in extracellular virus particles, but these are in no sense the 'growth' of a living organism.
  - Viruses are infectious agents with both living and nonliving characteristics.

- **<u>Living characteristics of viruses</u>:**
  - They reproduce at a fantastic rate, but only in living host cells; they can mutate

- **<u>Nonliving characteristics of viruses</u>:**
  - They are acellular, that is, they contain no cytoplasm or cellular organelles; they carry out no metabolism on their own and must replicate using the host cell's metabolic machinery.

Viroids, Virusoids, & Prions

- Viroids are very small (200-400 nucleotides) circular RNA molecules with a rod-like secondary structure. They have no capsid or envelope & are associated with certain plant diseases.
- Virusoids are satellite, viroid-like molecules, somewhat larger than viroids (~1,000 nucleotides), which are dependent on the presence of virus replication for multiplication (hence 'satellite') - packaged into virus capsids as passengers.
- Prions are infectious agents generally believed to consist of a single type of protein molecule with no nucleic acid component.

Essential Characteristics of Viruses

- **Size**
  - 25nm (picornavirus) to 250x350nm (smallpox virus).
  - Resolving power of a light microscope: 300nm, bacteria: 500–5000nm.
- **Genome**
  - DNA or RNA: Double-stranded or single-stranded nucleic acid, depending on the species.
- **Structure**
  - Viruses are complexes comprising virus-coded protein and nucleic acid; some viral species carry cell-coded components (membranes, tRNA).

How big are viruses?

- A common mistake is that viruses are always smaller than bacteria.
- While this is true in most cases, size alone does not serve to distinguish between them.
- The largest virus particles (e.g. Granuloviruses) are 120-300nm in diameter & 300-500nm long while the smallest bacteria (e.g. *Mycoplasma*) are only 200-300nm long.
- Size alone does not differentiate viruses & bacteria!

Comparative Sizes of Viruses and Bacteria

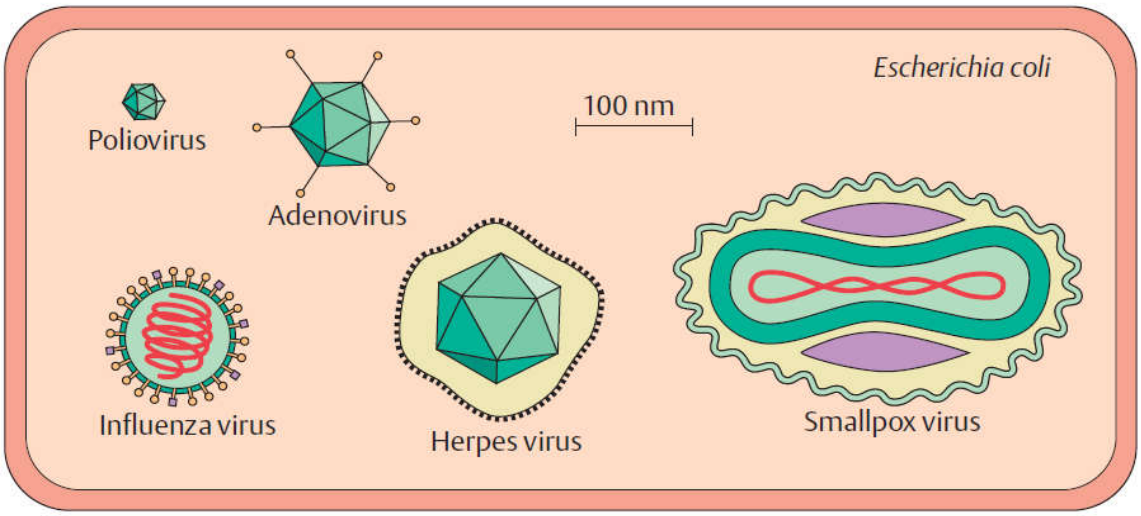


Fig. Different virus species are shown here to scale inside an *E. coli* bacterium

- **Reproduction of viruses**

Takes place only in living cells. The virus supplies the information in the form of nucleic acids and in some cases a few enzymes; the cell provides the remaining enzymes, the protein synthesizing apparatus, the chemical building blocks, the energy, and the structural framework for the synthetic steps

- **Antibiotics**

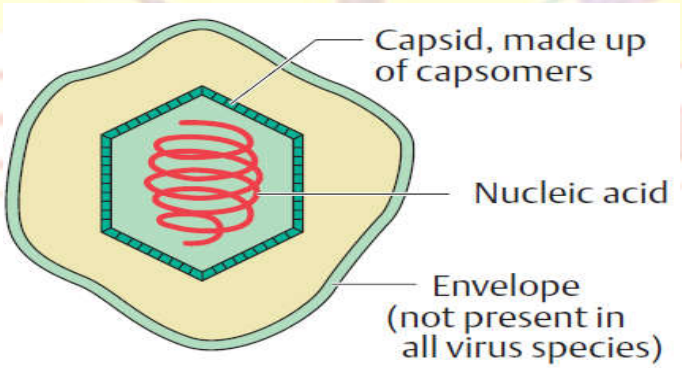
Viruses are unaffected by antibiotics, but can be inhibited by interferon and certain chemotherapeutic agents.

Morphology and Structure

A mature virus particle is also known as a virion. It consists of either two or three basic components.

- **A genome of DNA or RNA,** double-stranded or single-stranded, linear or circular, and in some cases segmented. A single-stranded nucleic acid can have plus or minus polarity.
- **The capsid:** virus-coded proteins enclosing the nucleic acid of the virus and determining its antigenicity; can have a cubic (rotational), helical or complex symmetry is made up of subunits called capsomers.
- **Envelope:** In some cases an envelope that surrounds the capsid and is always derived from cellular membranes.

Virus Particle Structure



Genome

- The viral genome is either DNA or RNA. Hence categorized as DNA or RNA viruses.
- The nucleic acid of DNA viruses is usually double-stranded (ds) and linear or circular depending on the family;
- The nucleic acid of RNA viruses is usually single-stranded (ss), with the exception of the reoviruses, and is also segmented in a number of virus families.
- Viruses with ssRNA are divided into two groups:
    - If the RNA of the genome has the same polarity as the viral mRNA and can thus function directly as messenger RNA, it is called a plus-strand (or positive-strand) or "sense" RNA strand and these viruses are sense or plus-strand viruses

- If the genome RNA has the polarity opposite to that of the mRNA, and therefore cannot be translated into proteins until it has first been transcribed into a complementary strand, it is called a minu strand (or negative-strand) or "antisense" RNA strand and the viruses are antisense or minus-strand viruses

Capsid

- The capsid is the "shell" of virus-coded protein that encloses the nucleic acid and is more or less closely associated with it.
- The combination of these two components is often termed the nucleocapsid, especially if they are closely associated as in the myxoviruses.
- The capsid is made up of subunits, the capsomers, the number of which varies but is specific and constant for each viral species.
- These are spherical or cylindrical structures composed of several polypeptides.
- The capsid protects the nucleic acid from degradation.
- In all except enveloped viruses, it is responsible for the attachment of the viruses to the host cell ("adsorption,") and determines specific viral antigenicity.

Envelope

- The envelope which surrounds the capsid in several virus families, is always dependent on cellular membranes (nuclear or cell membrane, less frequently endoplasmic reticulum).
- Both cell-coded and viral proteins are integrated in the membrane when these elements are transformed into the envelope, frequently in the form of "spikes" (or peplomers,
- Not all viruses have the envelope, and viruses can be divided into 2 kinds: enveloped virus and naked virus.
- Enveloped viruses do not adsorb to the host cell with the capsid, but rather with their envelope.
- Removing it with organic solvents or detergents reduces the infectivity of the viruses ("ether sensitivity")

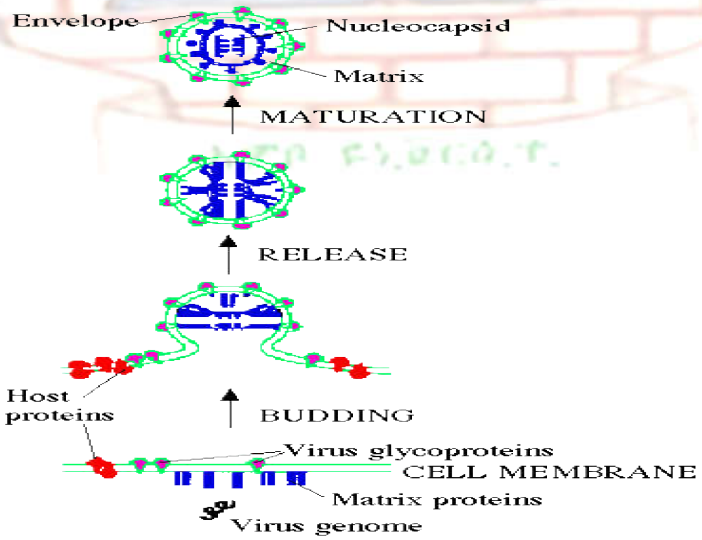
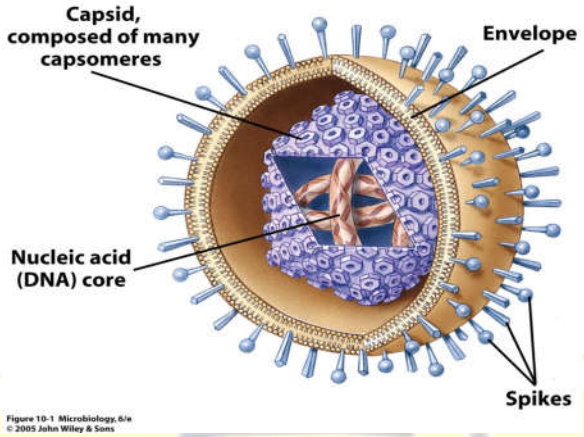


Fig. Acquisition of an envelope

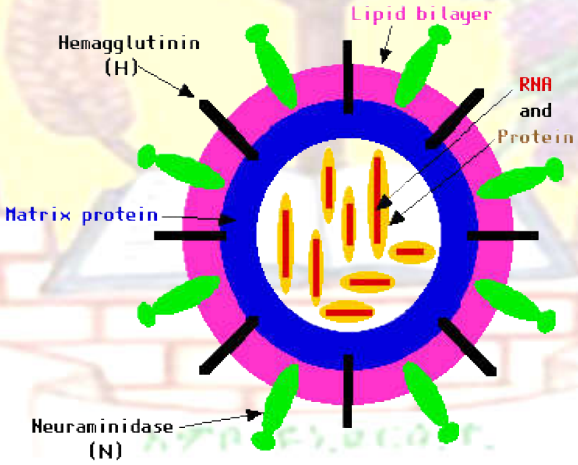
Acquisition of an envelope occurs as the nucleocapsid buds out from the cytoplasm to the extracellular surface.

Spikes

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



- Influenza Virus glycoprotein spikes serve two basic functions
  - mediate attachment to cellular receptors which is essential for infectivity (hemagglutinin)
  - they act as enzymes (neuraminidase)



Other Components of Viral Particles

- **Various enzymes**

Viruses require a number of different enzymes depending on genome type and mode of infection. In several virus species, enzymes are a component of the virus particle, for example the neuraminidase required for invasion and release of myxoviruses.

Other examples include nucleic acid polymerases such as: RNA-dependent RNA polymerases in antisense viruses, DNA polymerases in smallpox viruses and RNA-dependent DNA polymerase ("reverse transcriptase") in hepatitis B viruses and retroviruses.

- Hemagglutinin

Some viruses (above all myxoviruses and paramyxoviruses) are capable of agglutinating various different human or animal erythrocytes. These viruses bear a certain surface protein (hemagglutinin) in their envelope that enables them to do this. The hemagglutination phenomenon can be used for quantitative viral testing or—in the hemagglutination inhibition test - for virus identification and antibody identification. In biological terms, hemagglutinin plays a decisive role in adsorption and penetration of the virus into the host cell.

Viral Structural Patterns (Viral Symmetry)

- **Cubic symmetry (rotational symmetry)**

Viruses with rotational symmetry are icosahedrons (polyhedrons with 20 equilateral triangular faces and 12 corners e.g. Adenovirus). The number of capsomers per virion varies from 32 to 252 and depends on the number of capsomers (two to six) making up one side of the equilateral triangle. The capsomers in a virion need not all be the same, either in their morphology, antigen make-up or biological properties. Purified icosahedral viruses can be crystallized, so that images of them can be obtained using the methods of radiocrystallography.

- **Helical symmetry**

Helical symmetry is present when one axis of a capsid is longer than the other. The nucleic acid and capsid protein are closely associated in the ribonucleoprotein (RNP), in which the protein is tightly arrayed around the nucleic acid strand. This RNA-protein complex is known as the nucleocapsid, which takes the form of a helix inside the viral envelope. e.g. Influenza virus

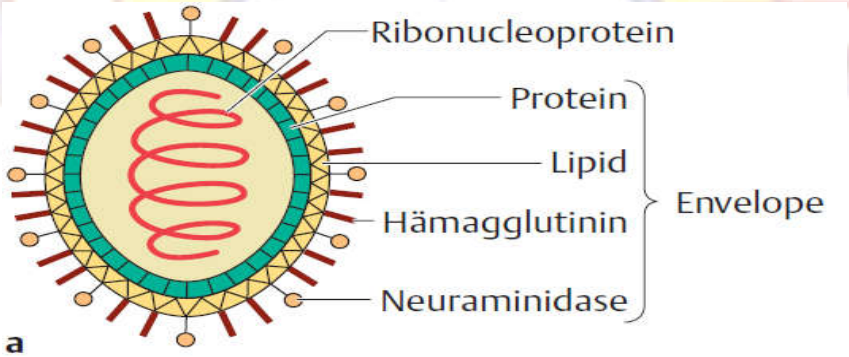
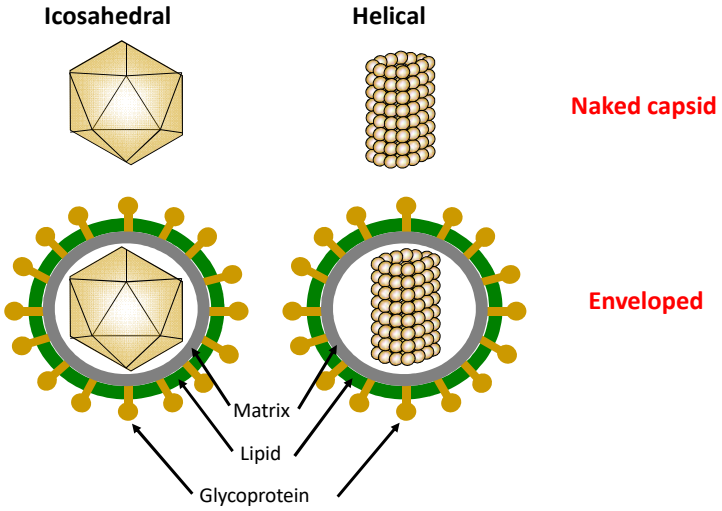


Fig. Schematic structure of a myxovirus; viruse with Helical Symmetry

- **Complex symmetry**

Complex structural patterns are found in phages and the smallpox virus**.** T-bacteriophages, for example, have an icosahedral head containing the DNA and a tubelike tail through which the DNA is injected into the host cell.

Capsid symmetry



Classification and Replication of viruses

Learning Objectives

At the end of this Lesson, you will be able to:

- Describe the classification schemes of viruses
- Mention phases/steps of viral replication
- Explain important events in the phases of viral replication
- Describe different processes in the replication of viral nucleic acids corresponding to the types and configurations of viral genome
- Describe viral protein synthesis and control mechanisms

Classification of viruses

The origins and evolution of the viruses are still largely in the dark. In contrast to the taxonomic systems used to classify the higher forms of life, we are unable to classify viruses in such evolutionary systems. An international nomenclature committee groups viruses according to various criteria and designates these groups, analogously to the higher forms, as families, genera, and species. Names used for viruses should be those approved by the International Committee on Taxonomy of Viruses (ICTV) and reported on the ICTV Virus Taxonomy website.

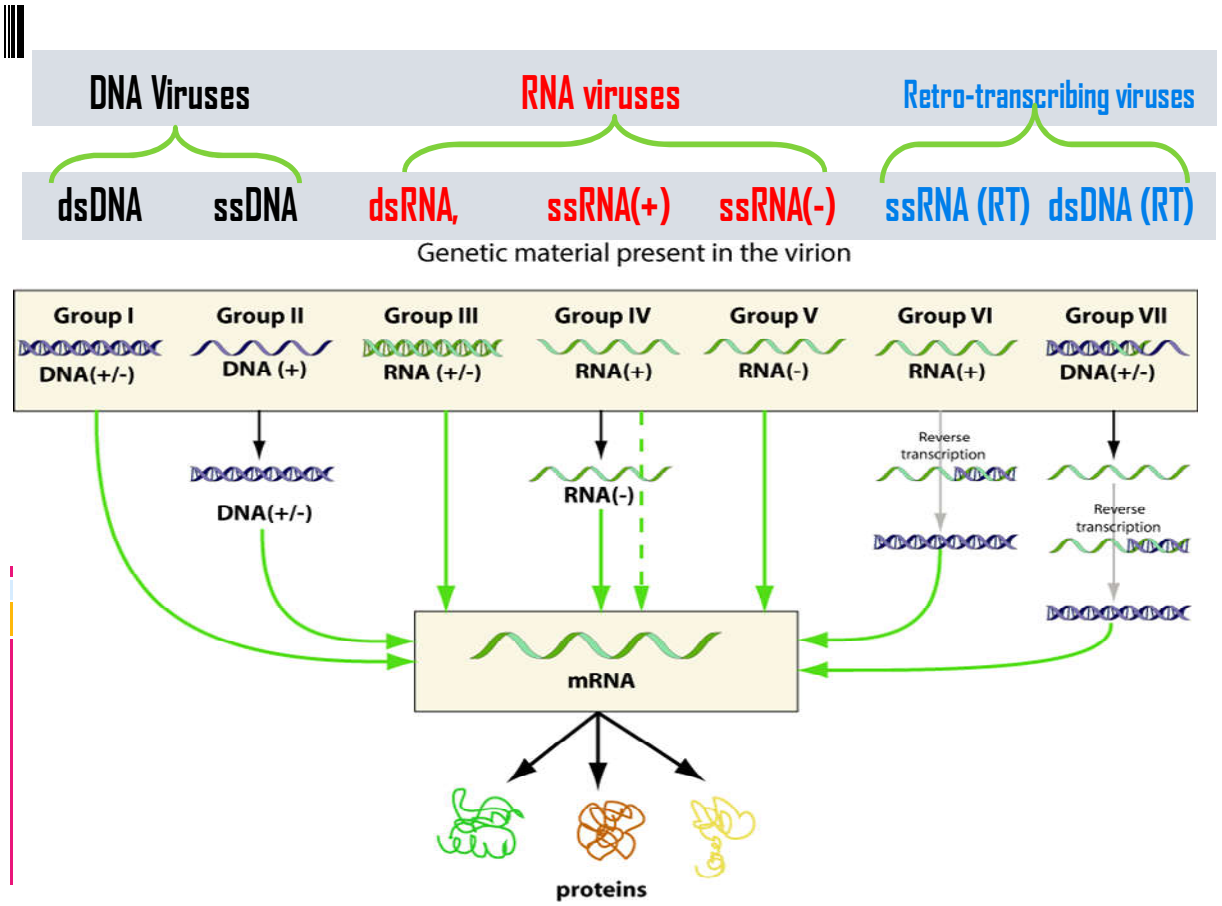
- The International Committee on Taxonomy of Viruses (ICTV) is charged with the task of developing, refining, and maintaining a universal virus taxonomy.
- This task encompasses the classification of virus species and higher-level taxa according to the genetic and biological properties of their members; naming virus taxa; maintaining a database detailing the currently approved taxonomy; and providing the database, supporting proposals, and other virus-related information from an open-access, public web site.
- The ICTV web site (http://ictv.global) provides access to the current taxonomy database in online and downloadable formats, and maintains a complete history of virus taxa back to the first release in 1971.

Baltimore classification

The Baltimore classification clusters viruses into families depending on their type of genome. Unlike LUCA (Last universal common ancestor) for cellular organism, there is no presumed common ancestor for viruses. David Baltimore (nobel Prize 1975) proposed a classification based on the genome present in virions. Under the Baltimore system, the present virus classification comprises seven trees of life.

- **DNA Viruses (Group I and II):** dsDNA and ssDNA
- **RNA viruses (Group III, IV and V):** dsRNA, ssRNA(+), ssRNA(-)
- **Retro-transcribing viruses (Group VI and VII):** ssRNA (RT), dsDNA (RT)

Baltimore system of classification



The taxonomic system used for viruses is artificial (i.e., it does not reflect virus evolution). Viruses are classified based on the following morphological and biochemical criteria:

i. Genome

DNA or RNA genome (important basic differentiation of virus types!) as well as configuration of nucleic acid structure: single-stranded (ss) or double-stranded (ds). RNA viruses are further subclassified according to plus and minus polarity.

ii. Capsid symmetry: cubic, helical, or complex symmetry

iii. Presence or absence of an envelope

iv. Diameter of the virion, or of the nucleocapsid with helical symmetry

Despite this element of "artificiality" in the system now in use, the groups appear to make biological sense and to establish order in the enormous variety of known viruses. See Tables below, Taxonomy of Viruses based on publications by the International Committee on Taxonomy of viruses.

Taxonomy of the Viruses

Nucleic acid	Nucleo-capsid symmetry	Envelope	Virus diameter (nm)	ss/ds [1] (polarity)	Family	Genus	Exemplary important species
DNA	cubic	naked	19–25	ss	*Parvoviridae*	Erythrovirus	Parvovirus B19
			55	ds	*Papillomaviridae*	Papillomavirus	Human papilloma virus (HPV)
			45	ds	*Polyomaviridae*	Polyomavirus	BK virus, JC virus
			70–90	ds	*Adenoviridae*	Mastadenovirus	Adenoviruses
		envelope	27/42[2]	ss	*Hepadnaviridae*	Ortho-hepadnavirus	Hepatitis B virus
			100/200[2]	ds	*Herpesviridae*	Simplexvirus	Herpes simplex virus
						Varicellovirus	Varicella zoster virus
						Cytomegalo-virus	Cytomegalovirus
						Roseolovirus	Human herpesvirus 6
						Lymphocrypto-virus	Epstein-Barr virus
	complex	envelope	230 × 350	ds	*Poxviridae*	Orthopox	Variola virus, vaccinia virus

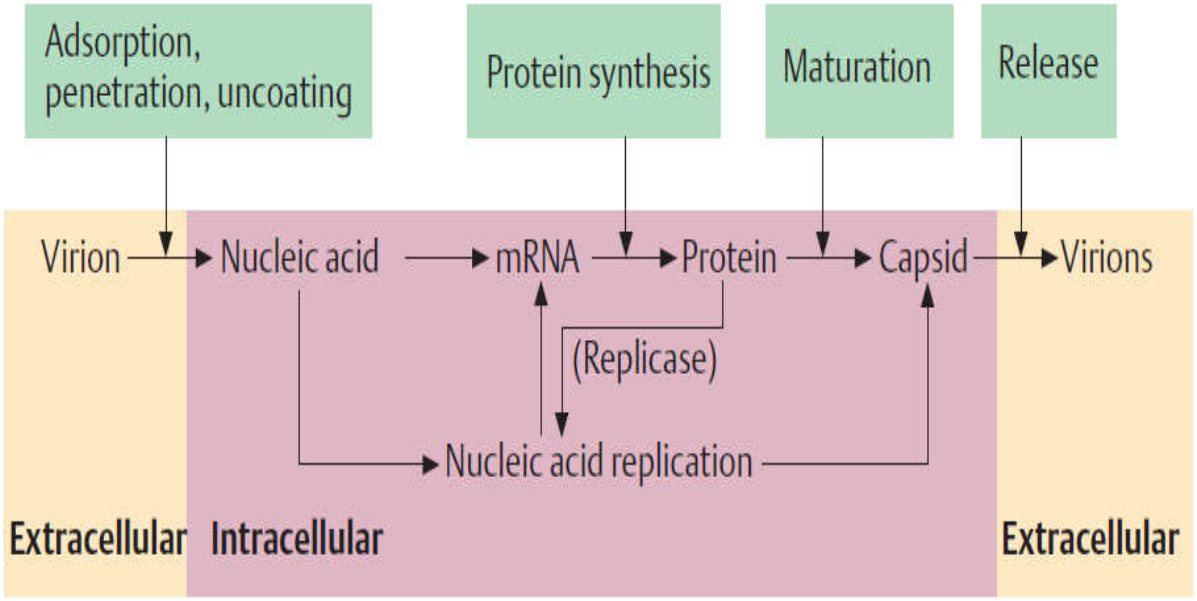
Nucleic acid	Nucleo-capsid symmetry	Envelope	Virus diameter (nm)	ss/ds [1] (polarity)	Family	Genus	Exemplary important species
RNA	cubic	naked	24–30	ss(+)	*Picornaviridae*	Enterovirus	Poliovirus, echovirus, coxsackie viruses
						Hepatovirus	Hepatitis A virus
						Rhinovirus	Rhinovirus 1–117
						Parechovirus	Parechoviruses
			30	ss(+)	*Astroviridae*	Astrovirus	Astroviruses
			33	ss(+)	*Caliciviridae*	Calicivirus	Hepatitis E virus
			60–80	ds segm.	*Reoviridae*	Coltivirus	Colorado tick fever virus
						Orthoreovirus	Reovirus 1–3
						Rotavirus	Rotaviruses
		envelope	60–70	ss(+)	*Togaviridae*	Alphavirus	Sindbis virus
						Rubivirus	Rubella virus
			40	ss(+)	*Flaviviridae*	Flavivirus	Yellow fever virus
						Hepacivirus	Hepatitis C virus
	helical	envelope	80–220	ss(+)	*Coronaviridae*	Coronavirus	SARS virus
			80–120	ss segm.(−)	*Orthomyxoviridae*	Influenzavirus	Influenza A, B, C virus
			150–300	ss(−)	*Paramyxoviridae*	Pneumovirus	Human respiratory syncytial virus
						Paramyxovirus	Human parainfluenza virus 1 and 3
						Rubulavirus	Mumps virus
						Morbillivirus	Measles virus
			60 × 180	ss(−)	*Rhabdoviridae*	Lyssavirus	Rabies virus
			80 × filament.	ss(−)	*Filoviridae*	Filovirus	Marburg virus, Ebola virus
			100	ss segm. (−)	*Bunyaviridae*	Bunyavirus	Bunyamwera virus
						Nairovirus	Crimean-Congo hemorrhagic fever virus
						Phlebovirus	Phlebotomus fever virus
						Hantavirus	Hantaan virus
	?	envelope	50–300	ss segm. (+/−)	*Arenaviridae*	Arenavirus	LCMV, Lassa virus
			100	ss segm. (+)	*Retroviridae*	HTLV-retrovirus	HTLV I and II
						Spumavirus	Spumavirus
						Lentivirus	HIV 1 and 2

[1] = Configuration of nucleic acid: ss = single-stranded, ds = double-stranded; [2] = without/with envelope

Replication of viruses

The 6 phases/steps in viral replication are as follows:

- Adsorption of the virus to specific receptors on the cell surface
- Penetration by the virus and intracellular release of nucleic acid (uncoating)
- Proliferation of the viral components: virus-coded synthesis of capsid and noncapsid proteins, replication of nucleic acid by viral and cellular enzymes
- Assembly of replicated nucleic acid and new capsid protein
- Release of virus progeny from the cell



Adsorption

It refers to the attachment of a virus to target cells**.** Virus particles can only infect cells possessing surface "receptors" specific to the particular virus species. **For example,** CD4 receptor for HIV; ICAM-1 receptor for rhinoviruses, the complement (C3) receptor that is also the receptor for the Epstein-Barr virus. It is the receptors on a cell that determine whether it can be infected by a certain virus**.** When a virus encounters such a cell, it adsorbs to it either with the capsid or, in enveloped viruses, by means of envelope proteins.

Practical consequences arise from the growing knowledge about the receptors:

- It aids in the development of antiviral therapeutics designed to inhibit the adsorption of the viruses to their target cells.
- The genetic information that codes for certain receptors can be implanted into cells or experimental animals, rendering them susceptible to viruses to which they would normally be resistant. An example of this application is the use in experimental studies of transgenic mice rendered susceptible to polioviruses instead of primates (e.g., on vaccine testing)

Penetration and uncoating

Viruses adsorbed to the cell surface receptors then penetrate into the cell by means of pinocytosis (a process also known as viropexis). In enveloped viruses, the envelope may also fuse with the cell membrane, releasing the virus into the cytoplasm. Adsorption of such an enveloped virus to two cells at the same time may result in cell fusion. The next step, known as uncoating, involves the release of the nucleic acid from the capsid and is apparently

(except in the smallpox virus) activated by cellular enzymes, possibly with a contribution from cell membranes as well.

Replication of the nucleic acid

Different processes are observed corresponding to the types and configurations of the viral genome.

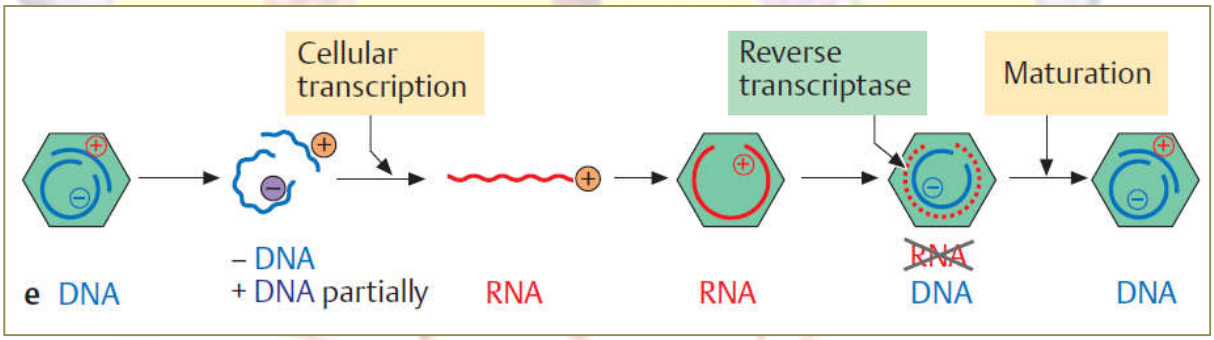
DNA viruses

- Replication of viral DNA takes place in the cell nucleus (exception: poxviruses)
- Some viruses (e.g., herpesviruses) possess replicases of their own.
- The smaller DNA viruses (e.g., polyomaviruses), which do not carry information for their own DNA polymerase, code for polypeptides that modify the cellular polymerases in such a way that mainly viral DNA sequences are replicated.

❑ **Hepadnaviruses:**

- The genome consists of an ssDNA antisense strand and a short sense strand (Fig. e).
- The infected cell transcribes an RNA sense strand ("template strand") from the antisense strand.
- This template strand is integrated in virus capsids together with an RT DNA polymerase.
- The polymerase synthesizes a complementary antisense DNA and, to "seal off" the ends of the genome, a short sense DNA from the template strand.

Replication in Partially Double Stranded DNA Viruses (hepadnaviruses)



DNA replication in hepadnaviruses: by means of cellular transcription, a sense-strand RNA is made from the viral genome (antisense DNA, partially double-stranded) and integrated in the new virion, where a virus-coded RT produces new genomic DNA (–) and destroys the RNA.

RNA viruses

- Since eukaryotic cells possess no enzymes for RNA replication, the virus must supply the RNA dependent RNA polymerase(s) **("replicase").**
- These enzymes are thus in any case virus-coded proteins, and in some cases are actually components of the virus particle.

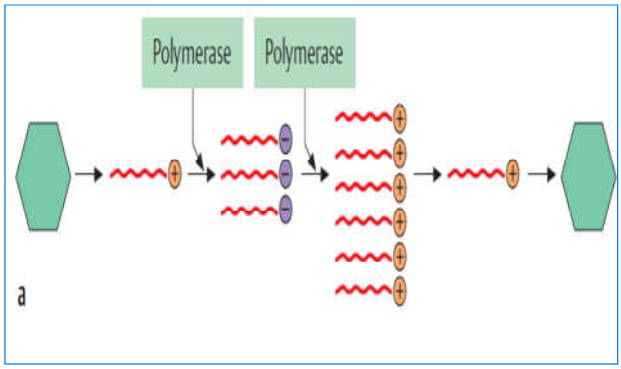
Single-stranded RNA

In sense-strand viruses, the RNA functions as mRNA "as is," meaning the information can be read off, and the replicase synthesized immediately.

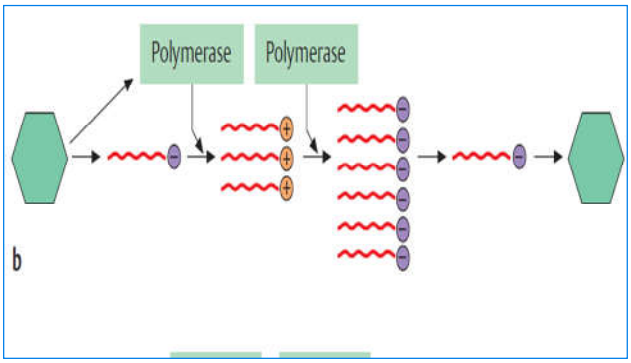
Antisense-strand viruses must first transcribe their genome into a complementary strand that can then act as mRNA. In this case, the polymerase for the first transcription is contained in the mature virion and delivered into the cell.

In ssRNA viruses, whether sense or antisense strands, complementary strands of the genome are produced first (Fig. a, b), then transcribed into daughter strands. They therefore once again show the same polarity as the viral genome and are used in assembly of the new viral progeny.

Single-stranded RNA Replicaton



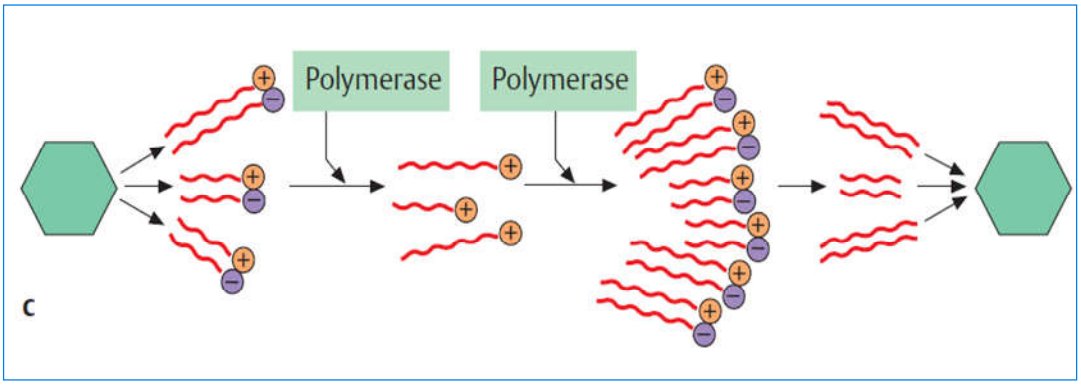
Single-stranded RNA viruses with sense-strand genome: the virus-coded RNA polymerase transcribes the viral genome (+) into complementary strands (–) and these into new genomic RNA (+). The latter is then integrated in the viral progeny.



Single-stranded RNA viruses with antisense-strand genome: the RNA polymerase in the virion transcribes the viral genome (–) into complementary strands (+), which a virus-coded polymerase then transcribes into new genomic RNA (–)

Double-stranded RNA

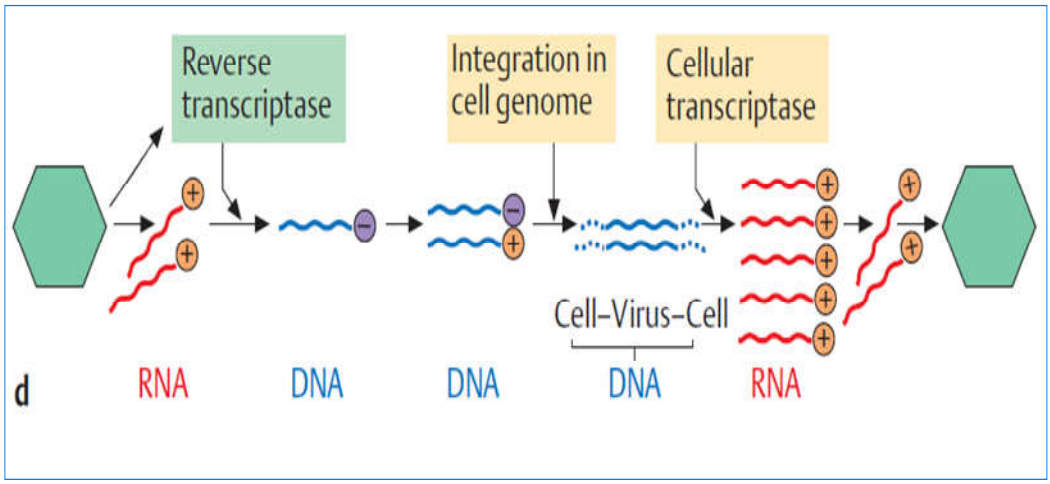
A translatable sense-strand RNA is produced from the genome, which consists of several dsRNA segments (segmented genome). This strand functions, at first, as mRNA and later as a matrix for synthesis of antisense-strand RNA (Fig. c). Here as well, an RNA-dependent RNA polymerase is part of the virus particle.



Double-stranded RNA viruses: while still in the partially decapsidated virus particle, the virus-coded polymerase transcribes complementary strands (+) from the antisense strand of the (segmented) double-stranded viral genome; these complementary strands are complemented to make the new double stranded viral genome.

RNA replication in retroviruses

Retroviruses also possess a sense-oriented RNA genome, although its replication differs from that of other RNA viruses. The genome consists of two single-stranded RNA segments with sense polarity and is transcribed by an enzyme in the virion (reverse transcriptase [RT]) into complementary DNA. The DNA is complemented to make dsDNA and integrated in the cell genome. Transcription into sense-strand RNA is the basis for both viral mRNA and the genomic RNA in the viral progeny (Fig. d).



RNA replication in retroviruses: the reverse transcriptase (RT) carried by the virion transcribes the viral genome (two sense-RNA strands) into complementary DNA (–), which is complemented to produce dsDNA and integrated in the cell genome. The viral RNA is first degraded. Cellular enzymes produce new genomic RNA (+).

Viral Protein Synthesis

- **Production of viral mRNA**

In a DNA virus infection, cellular polymerases transcribe mRNA in the nucleus of the host cell from one or both DNA strands, whereby the RNA is processed (splicing, polyadenylation, etc.) as with cellular Mrna. An exception to this procedure is the poxviruses, which use their own enzymes to replicate in the cytoplasm. In viruses with antisense-strand, ssRNA and dsRNA, the transcription of the genomic RNA into mRNA is carried out by the viral polymerases, usually without further processing of the transcript. In sense-strand ssRNA viruses, the genome can function directly as mRNA.

Certain viruses (arenaviruses) are classified as **"ambisense viruses."** Part of their genome codes in antisense (–), another part in sense (+) polarity. Proteins are translated separately from subgenomic RNA and the antisense-coded proteins are not translated until the antisense strand has been translated into a sense strand. Viral mRNA is produced for the translation process, based on both the genome of the invading virus and the nucleic acid already replicated. The actual protein synthesis procedure is implemented, coded by the viral mRNA, with the help of cellular components such as tRNA, ribosomes, initiation factors, etc. Two functionally different protein types occur in viruses:

- The "noncapsid viral proteins" (NCVP) that do not contribute to capsid assembly. These proteins frequently possess enzymatic properties (polymerases, proteases) and must therefore be produced early on in the replication cycle.

- The capsid proteins, also known as viral proteins (VP) or structural proteins, appear later in the replication process.

Protein Synthesis Control

- **Segmented genomes:** A separate nucleic acid segment is present for each protein (example: reoviruses).
- **mRNA splicing:** The correct mRNA is cut out of the primary transcript (as in the cell the exon is cut out of the hnRNA) (examples: adenoviruses, retroviruses, etc.).
- "**Early" and "late" translation:** The different mRNA molecules required for assembly of so-called early and late proteins are produced at different times in the infection cycle, possibly from different strands of viral DNA (examples, papovaviruses, herpesviruses).
- **Posttranslational control:** This process involves proteolytic cutting of the primary translation product into functional subunits. Viral proteases that recognize specific amino acid sequences are responsible for this, e.g., the two poliovirus proteases cut between glutamine and glycine or tyrosine and glycine. Such proteases, some of which have been documented in radiocrystallographic images, are potential targets for antiviral chemotherapeutics (example: HIV).

Viral maturation (morphogenesis)

In this step, the viral capsid proteins and genomes (present in multiple copies after the replication process) are assembled into new, infectious virus particles. In some viral species, these particles are also covered by an envelope.

Release

The release of viral progeny in some cases correlates closely with viral maturation, whereby envelopes or components of them are acquired when the particles "bud off" of the cytoplasmic membrane and are expelled from the cell. In nonenveloped viruses, release of viral progeny is realized either by means of lysis of the infected cell or more or less continuous exocytosis of the viral particles.

Review Questions

1. Express the following terms related with viral replication: adsorption, penetration, uncoating, biosynthesis/synthesis, Assembly, Release
2. What determines host range in viral infections?
3. Mention the mechanisms through which viruses enter into or penetrate host cells.
4. Describe the mechanisms how new viruses are released from infected cells.
5. List the types of viral symmetry.
6. Write the highlight of **Baltimore** classification of viruses

Viral Genetics

Like in higher life forms, viral genetic material is subject to change by mutation. The lack of a corrective replication "proofreading" mechanism results in a very high incidence of spontaneous mutations in RNA viruses. This results in greatly increasing the genotypic variability within each species ("**viral quasispecies**"). Furthermore, a potential for recombination of genetic material is also inherent in the replication process, not only material from different viruses but also from host cell and virus. This factor plays a major role in viral tumor induction and genetic engineering. Functional modifications arising from interactions between different viral species in mixed infections have nothing to do with genetic changes.

    - e.g. phenotype mixing, interference, and complementation
- Temporary nongenetic interactions between viruses in some cases may mimic genetic changes
- Permanent genetic changes in viruses are caused, as in the higher life forms, either by mutation or recombination of genetic material.

Mutation

- Mutations are changes in the base sequence of a nucleic acid, resulting in a more or less radical alteration of the resulting protein.
- So-called "silent mutations" (in the second or third nucleotide of a codon) do not influence the amino acid sequence of the protein
- Medically important are mutants with weakened virulence that have retained their antigenicity and replication capabilities intact.
    - These are known as "attenuated" viruses.
    - They are the raw material of live vaccines.

Recombination

- In cases where two different viral strains are replicating in the same cell, there is a chance that strand breakage and reunion will lead to new combinations of nucleic acid segments or exchanges of genome segments (influenza), so that the genetic material is redistributed among the viral strains (recombination)
- New genetic properties will therefore be conferred upon some of the resulting viral progeny, some of which will also show stable heritability.
- Genetic material can also be exchanged between virus and host cell by the same mechanism or by insertion of all, or part, of the viral genome into the cell genome.

Viruses as Vectors (viral vectors)

- The natural processes of gene transfer between viruses and their host cells can be exploited to give certain cells new characteristics by using the viruses as vectors.
- Applications of viral vectors: Basic research, Gene therapy, Vaccines
- If the vector DNA carrying the desired additional gene integrates stably in the host cell genome (e.g., retroviruses, adenoviruses, or the adeno associated virus), the host cell is permanently changed.
- This can become the basis for "gene therapy" of certain functional disorders such as cystic fibrosis or parkinsonism.
- Non integrating vectors (alphaviruses, e.g., the Sindbis virus, mengovirus, or vaccinia virus) result in temporary expression of a certain protein, which can be used, for instance, to immunize a host organism.

- By this means, wild foxes can be vaccinated against rabies using a vaccinia virus that expresses a rabies virus glycoprotein.
- Such experimental work must of course always comply with national laws on the release of genetically engineered microorganisms. It must also be mentioned here that only somatic gene therapy can be considered for use in humans.
- Human germline therapy using the methods of genetic engineering is generally rejected as unethical.

Nongenetic Interactions

- In mixed infections by two (or more) viruses, various viral components can be exchanged or they may complement (or interfere with) each other's functions (phenotype mixing, complementation or interference).
- Such processes do not result in stable heritability of new characteristics.
- In phenotypic mixing, the genome of virus A is integrated in the capsid of virus B, or a capsid made up of components from two (closely related) virus types is assembled and the genome of one of the "parents" is integrated in it.
- However, the progeny of such a "mixed" virus of course shows the genotype.

In phenotypic interference, the primary infecting virus (usually avirulent) may inhibit the replication of a second virus, or the inhibition may be mutual.

- The interference mechanism may be due to interferon production or to a metabolic change in the host cell.

In complementation, infecting viral species have genetic defects that render replication impossible.

- The "partner" virus compensates for the defect, supplying the missing substances or functions in a so-called helper effect.
- In this way, a defective and nondefective virus, or two defective viruses, can complement each other
- **Example:** murine sarcoma viruses for which leukemia virus helpers deliver capsid proteins, or the hepatitis D virus, which replicates on its own but must be supplied with capsid material by the hepatitis B virus

"Quasispecies"

When viral RNA replicates, there is no "proofreading" mechanism to check for copying errors as in DNA replication. The result is that the rate of mutations in RNA viruses is about 10^4, i.e., every copy of a viral RNA comprising 10,000 nucleotides will include on average one mutation. The consequence of this is that, given the high rate of viral replication, all of the possible viable mutants of a viral species will occur and exist together in an inhomogeneous population known as **quasispecies**. The selective pressure (e.g., host immune system efficiency) will act to select the "fittest" viruses at any given time. This explains the high level of variability seen in HIV as well as the phenomenon that a single passage of the attenuated polio vaccine virus through a human vaccine recipient produces neurovirulent revertants.

Virus-host interactions

- ❑ **Possible consequences of viral infection for the host cell:**

- **Cytocidal infection (necrosis):** viral replication results directly in cell destruction (cytopathology, so-called "cytopathic effect" in cell cultures)
- **Apoptosis:** the virus initiates a cascade of cellular events leading to cell death ("suicide"), in most cases interrupting the viral replication cycle.
- **Noncytocidal infection:** viral replication by itself does not destroy the host cell, although it may be destroyed by secondary immunological reactions

- **Latent infection:** the viral genome is inside the cell, resulting in neither viral replication nor cell destruction.
- **Tumor transformation:** the viral infection transforms the host cell into a cancer cell, whereby viral replication may or may not take place depending on the virus and/or cell type involved

Cell Destruction (Cytocidal Infection, Necrosis)

Cell death occurs eventually after initial infection with many viral species. This cytopathological cell destruction usually involves production of viral progeny. Virus production coupled with cell destruction is termed the "lytic viral life cycle." Cell destruction, whether necrotic or apoptotic is the reason (along with immunological phenomena) for the disease manifested in the macroorganism

Structural changes leading to necrosis:

- Morphological changes can often be observed in the infected cell.
- The effects seen in virally infected cell cultures are well-known and are designated by the term "cytopathic effect" (CPE).
- These effects can also be exploited for diagnostic purposes
- They include rounding off and detachment of cells from adjacent cells or the substrate, formation of multinuclear giant cells, cytoplasmic vacuoles, and inclusion bodies. Inclusion bodies are structures made up of viral and/or cellular material that form during the viral replication cycle, e.g., viral crystals in the nucleus (adenoviruses) or collections of virions and viral material in the cytoplasm (smallpox viruses).

Shutoff Phenomena

Some viruses are able to block, more or less completely, steps in cellular macromolecule synthesis not useful to them. Such shutoff phenomena apparently contribute to rapid and efficient viral replication by eliminating competing cellular synthetic processes. These shutoff phenomena of course also have a pathogenic effect since they inhibit cellular metabolism, but not in such a way as to necessarily kill the host cell. Herpesviruses, for example, which possess DNA polymerase of their own, block cellular DNA synthesis. DNA replication in adenoviruses, by contrast, is directly coupled to that of the cell.

Apoptosis

Cells possess natural mechanisms that initiate their self-destruction (apoptosis) by means of predetermined cytoplasmic and nuclear changes. Infections with some viruses may lead to apoptosis. In rapidly replicating viruses, the viral replication process must be decelerated to allow the slow, energy-dependent process of apoptosis to run its course before the cell is destroyed by virus-induced necrosis. The body rapidly eliminates apoptotic cells before an inflammatory reaction can develop, which is apparently why virus-induced apoptosis used to be overlooked so often. Apoptosis can thus be considered a defense mechanism, although certain viruses are able to inhibit it.

Virus Replication without Cell Destruction (Noncytocidal Infection)

This outcome of infection is observed with certain viruses that do not cause any extensive restructuring of the host cell and are generally released by "budding" at the cell surface. This mode of replication is seen, for example, in the oncornaviruses and myxoviruses and in the chronic form of hepatitis B virus infection. However, cell destruction can follow as a secondary result of infection. If the immune system recognizes viral antigens on the cell surface, classifies it as "foreign" and destroys it.

Latent Infection

In this infection type, the virus (or its genome) is integrated in a cell, but no viral progenies are produced. The cell is accordingly not damaged and the macroorganism does not manifest disease. This form of infection is found, for instance, with the adenovirus group and in particular the herpesviruses, which can remain latent for long periods in the human body. Latency protects these viruses from immune system activity and thus is part of their survival strategy. However, a variety of initiating events can initiate a lytic cycle leading to manifest disease and dissemination of the virus. Repeated activation of a latent virus is termed recidivation (e.g., herpes labialis).

Tumor Transformation

Infections by a number of viruses do not result in eventual host cell death, but rather cause tumor transformation of the cell. This means the cell is altered in many ways, e.g., in its growth properties, morphology, and metabolism. Following an infection with DNA tumor viruses, the type of host cell infected determines whether the cell reaction will be a tumor transformation, viral replication or lytic cycle. The transformation that takes place after infection with an RNA tumor virus either involves no viral replication (nonpermissive infection) or the cell produces new viruses but remains vital (permissive infection).

Carcinogenic Retroviruses ("Oncoviruses")

- **Genome structure and replication of the oncoviruses**

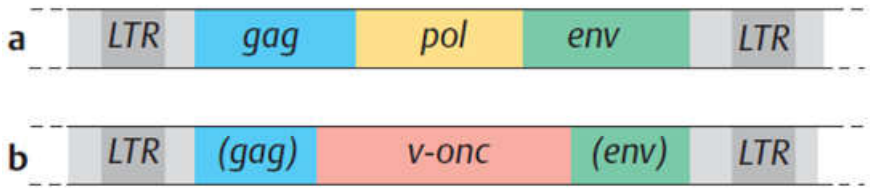
The genomes of all oncoviruses possess:

- **gag** (group-specific antigen),
- **pol** (enzymatic activities: polymerase complex with reverse transcriptase, integrase, and protease), and
- **env** (envelope glycoproteins) genes.

These coding regions are flanked by two control sequences important for regulatory functions called **LTR** (long terminal repeats). These sequences have a promoter/enhancer function and are responsible for both reverse transcription and insertion of the viral genome into the cell DNA. Certain oncoviruses possess a so-called **"onc gene"** instead of the pol region (onc gene = oncogene, refers to a cellular gene segment acquired by recombination). These viruses also often have incomplete gag and/or env regions. Such

viruses are defective and require a helper virus to replicate (complementation). An exception to this principle is the Rous sarcoma virus, which possesses both an onc gene and a complete set of viral genes and can therefore replicate itself.

Genomic Organization in Oncoviruses



a. Autonomously reproducing oncoviruses with the three replication genes: gag, pol, and env, flanked by the LTR regions.
b. Defective oncoviruses contain an onc gene instead of the entire pol region and parts of the gag and env regions.

Oncogenes

Over 100 oncogenes have been found in the course of tumor virology research to date. These genes enable tumor viruses to transform their host cells into tumor cells. The various types of oncogenes are designated by abbreviations, in most cases derived from the animal species in which the virus was first isolated. Further investigation of these viral oncogenes have now shown that these genes are not primarily of viral origin, but are rather normal, cellular genes widespread in humans and animals and acquired by the oncoviruses in their host cells, which can be transferred to new cells (transduction). Such a cellular gene, not oncogenic per se, is called a proto-oncogene.

The normal function of the proto-oncogenes concerns the regulation of cell growth in the broadest sense. Their gene products are growth factors, growth factor or hormone receptors and GTP-binding or DNA-binding proteins. Proto-oncogenes are potential contributors to tumor development that have to be "activated" before they can actually have such effects. This can occur by way of several different mechanisms:

- **Chromosomal translocation:** proto-oncogenes are moved to different chromosomes and thus placed under the influence of different cellular promoters, resulting in a chronic over expression of the corresponding protein.
- **Mutation** of the proto-oncogene.
- **Transduction** of the proto-oncogene by an oncovirus. The oncovirus promoter may induce over expression of the proto-oncogene, resulting in a tumor.

Tumor induction by oncoviruses

Both types of carcinogenic retroviruses, i.e., those with no oncogene and intact replication genes (gag, pol, env, flanked by the LTR regions) and those that have become defective by taking on an oncogene, can initiate a tumor transformation. On the whole, oncoviruses play only a subordinate role in human tumor induction.

Retroviruses without an oncogene:

LTR are highly effective promoters. Since the retrovirus genome is integrated in the cell genome at a random position, the LTR can also induce heightened expressivity in cellular protooncogenes ("promoter insertion hypothesis" or "insertion mutagenicity"), which can lead to the formation of tumors. This is a slow process (e.g., chronic leukemias) in which cocarcinogens can play an important role. The transformed cells produce new viruses.

Retroviruses with an oncogene:

A viral oncogene always represents a changed state compared with the original cellular proto-oncogene (deletion, mutation). It is integrated in the cell genome together with the residual viral genome (parts) after reverse transcription, and then expressed under the influence of the LTR, in most cases over expressed. This leads to rapid development of acute malignancies that produce no new viruses. Over production of oncogene products can be compensated by gene products from antioncogenes. The loss or mutation of such a suppressor gene can therefore result in tumor formation.

DNA Tumor Viruses

Genes have also been found in DNA tumor viruses that induce a malignant transformation of the host cell. In contrast to the oncogenes in oncoviruses, these are genuine viral genes that have presumably developed independently of one another over a much longer evolutionary period. They code for viral regulator proteins, which are among the so-called early proteins. They are produced early in the viral replication cycle and assume essential functions in viral DNA replication. Their oncogenic potential derives among other things from the fact that they bind to the products of tumor suppressor genes such as p53, Rb (antioncogenes, "antitransformation proteins") and can thus inhibit their functions. DNA viruses are more important inducers of human tumors than oncoviruses (example: HHV8, papovaviruses, hepatitis B viruses, Epstein-Barr viruses).

Viral Pathogenesis

The term "pathogenesis" covers the factors that contribute to the origins and development of a disease. In the case of viruses, the infection is by a parenteral or mucosal route. The viruses either replicate at the portal of entry only (local infection) or reach their target organ hematogenously, lympogenously or by neurogenic spread (generalized infection). In both cases, viral replication induces degenerative damage. Its extent is determined by the extent of virus-induced cell destruction and sets the level of disease manifestation. Immunological responses can contribute to elimination of the viruses by destroying the infected cells, but the same response may also exacerbate the course of the disease.

Transmission

Viruses can be transmitted horizontally (within a group of individuals or vertically (from mother to offspring). Connatal infection is the term used when offsprings are born infected.

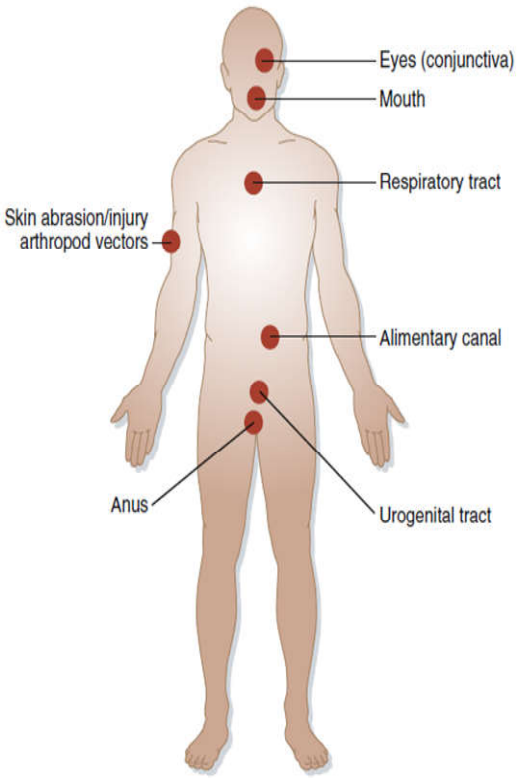
- **Table: Viral transmission patterns**

Pattern	Example
Horizontal transmission	
Human–human (aerosol)	Influenza
Human–human (fecal–oral)	Rotaviruses
Animal–human (direct)	Rabies
Animal–human (vector)	Bunyaviruses
Vertical transmission	
Placental–fetal	Rubella
Mother–child (birth)	Herpes simplex virus (HSV), human immunodeficiency virus (HIV)
Mother–child (breastfeeding)	HIV, human T-cell leukemia virus (HTLV)
Germ line	In mice, retroviruses; in humans (?)

Portal of entry

Portal of entry

- The most important portals of entry for viruses are the mucosa of the respiratory and gastrointestinal tracts.
- Intact epidermis presents a barrier to viruses, which can, however, be overcome through microtraumata (nearly always present) or mechanical inoculation (e.g., bloodsucking arthropods).



Viral dissemination in the organism

- There are two forms of infection:
  - Local infection
    - In this form of infection, the viruses spread only from cell to cell. The infection and manifest disease are thus restricted to the tissues in the immediate vicinity of the portal of entry. Example: rhinoviruses that reproduce only in the cells of the upper respiratory tract.
  - Generalized infection
    - In this type, the viruses usually replicate to some extent at the portal of entry and are then disseminated via the lymph ducts or bloodstream and reach their target organ either directly or after infecting a further organ. When the target organ is reached, viral replication and the resulting cell destruction become so widespread that clinical symptoms develop. Examples of such infection courses are seen with enteroviruses that replicate mainly in the intestinal epithelium, but cause no symptoms there**.** Clinical symptoms in these infections first arise in the target organs such as the CNS (polioviruses, echoviruses) or musculature (coxsackie viruses).
    - Another mode of viral dissemination in the macroorganism is neurogenic spread along the nerve tracts, from the portal of entry to the CNS (rabies), or in the opposite direction from the ganglions where the viruses persist in a latent state to the target organ (herpes simplex).

Examples of Localized and Systemic Virus Infections

Virus	Primary Replication	Secondary Replication
Localized infections		
Papillomaviruses	Dermis	—
Rhinoviruses	Upper respiratory tract	—
Rotaviruses	Intestinal epithelium	—
Systemic infections		
Enteroviruses	Intestinal epithelium	Lymphoid tissues, central nervous system
Herpesviruses	Oropharynx or genitourinary tract	Lymphoid cells, central nervous system

Organ Infections, Organotropism

Whether a given cell type can be infected by a given viral species at all depends on the presence of certain receptors on the cell surface**.** This mechanism explains why organotropism is observed in viruses. However, the tropism is only apparent; it is more accurate to speak of susceptible and resistant cells (and hence organs). Another observation is that cells grown in the laboratory in cell cultures can completely change their sensitivity or resistance to certain viral species compared with their organ of origin.

Course of infection

The organ damage caused by viruses is mainly of a degenerative nature; inflammatory reactions are secondary processes. The severity of the clinical symptoms depends primarily on the extent of virus-induced (or immunological) cell damage. Most of the viral progeny are produced prior to the occurrence of clinical symptoms, with consequences for epidemiology and antiviral therapies. Infections can go unnoticed if cell destruction is insignificant or lacking entirely. The terms inapparent, silent, or subclinical infection are used, in contrast to apparent viral infections with clinical symptoms. Virus replication and release do take place in inapparent infections, as opposed to latent infections in which no viral particles are produced.

Antibody-Dependent Enhancement of Viral Infection

Disease process can be worsened when viruses react with subneutralizing amounts or types of antibodies; antibodies exacerbate the infection. Fc fragment of the antibodies bound to the viruses can then react with the Fc receptors on specific cells. This makes possible for cell types to be infected that are primarily resistant to the virus in question because they possess no viral receptors (but in any case Fc receptors). ADE has been experimentally confirmed with a number of virus types to date includes herpes virus, poxvirus, reovirus, flavivirus, rhabdovirus, coronavirus, bunyavirus, and HIV species.

Virus excretion

Excretion of newly produced viruses depends on the localization of viral replication. For example, viruses that infect the respiratory tract are excreted in expired air (droplet infection). In generalized infections, not only the target organ is involved in excretion; the primary viral replication at the portal of entry also contributes to virus excretion. For example, enteroviruses replicate primarily in the intestinal wall are excreted in feces. Since the symptoms of a viral disease result from cell destruction, production, and excretion of new virus progeny precede the onset of illness. Patients are therefore contagious before they really become ill.

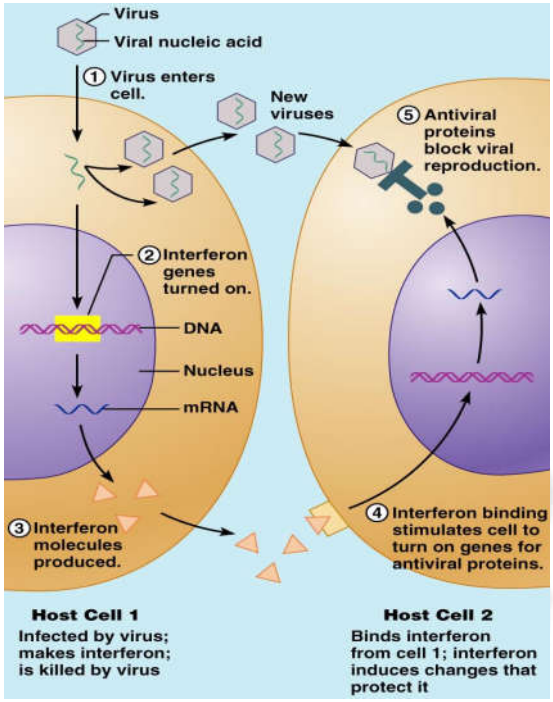
Defense Mechanisms

Defense against viral infection can be classified in two groups; non-specific and specific defense mechanisms.

In nonspecific immune defenses, interferons play a very important part. Interferons do not affect viruses directly, but rather induce cellular resistance mechanisms (synthesis of "antiviral proteins") that interfere with specific steps in viral replication.

Interferons (IFNs)

- Produced by most tissue cells when infected by a virus
- Diffuses to uninfected cells and binds to surface receptors
  - stimulates macrophages and NK-lymphocytes
  - stimulates production of **antiviral proteins** which block viral replication
  - inhibits growth of virally infected cells
  - suppresses growth of tumor cells



The specific immune defenses include the humoral immune system, consisting mainly of antibodies, and the cellular immune system, represented mainly by the T lymphocytes. Cellular immunity is more important than humoral immunity. The cellular system is capable of recognizing and destroying virus-infected cells on the surfaces of which viral antigens are expressed. The humoral system can eliminate only extracellular viruses.

Prevention

The most important prophylactic measures in the face of potential viral infections are active vaccines. Vaccines containing inactivated viruses generally provide shorter-lived and weaker protection than live vaccines. Passive immunization with human immunoglobulin is only used in a small number of cases, usually as postexposure prophylaxis.

Chemoprophylaxis, i.e., administration of chemotherapeutic agents when an infection is expected instead of after it has been diagnosed to block viral metabolism, is now justified in selected cases, e.g., in immunosuppressed patients.

There are two basic types of vaccines:

- Active immunization

The antigen (virus) is introduced into the body, either in an inactivated form, or with attenuated pathogenicity but still capable of replication, to enable the body to build up its own immunity.

- Inactivated vaccines

The immunity that develops after so-called "dead vaccines" are administered is merely humoral and generally does not last long. For this reason, booster vaccinations must be given repeatedly. The most important dead vaccines still in use today are influenza, rabies, some flavivirus, and hepatitis A and B vaccines. Some inactivated vaccines contain the most important immunogenic proteins of the virus.

Live attenuated vaccines

These vaccines confer effective and long-lasting protection after only a single dose because the viruses contained in them are capable of replication in the body, inducing not only humoral, but sometimes cellular immunity as well Such live vaccines are preferable when available. Drawbacks and risks the increased potential for contamination with other viruses, resulting in more stringent testing and the possibility that a back-mutation could produce a pathogenic strain.

Vaccines with recombinant viruses

Since only a small number of (surface) viral proteins are required to induce protective immunization, viral vectors are used in attempts to express them in vaccine recipients. Suitable vectors include the least virulent virus strains among the picornaviruses, alphaviruses, and poxviruses. There must be no generalized immunity to the vector in the population so that it can replicate in vaccine recipients and the desired protein will at the same time be expressed. E.g. a rabies vaccine containing the recombinant vaccinia virus for use in animals.

Naked DNA vaccine

Since pure DNA can be inserted into eukaryotic cells (transfection) and the information it carries can be expressed, DNA that codes for the desired (viral) proteins can be used as vaccine material. The advantages of such vaccines, now still in the trial phase, include ease of production and high stability.

Passive immunization

This type of vaccine involves the injection of antibodies using only human immunoglobulins. The protection conferred is of short duration and only effective against viruses that cause viremia.

Passive immunization is usually administered as a postexposure prophylactic measure, i.e., after an infection or in situations involving a high risk of infection, e.g., to protect against hepatitis B and rabies (locally, bite wound).

Chemotherapy

Inhibitors of certain steps in viral replication can be used as chemotherapeutic agents to treat viral infections. In practical terms, it is much more important to inhibit the synthesis of viral nucleic acid than of viral proteins. The main obstacles involved are: the low level of specificity of the agents in some cases (toxic effects, because cellular metabolism is also affected) and the necessity of commencing therapy very early in the infection cycle.

The Most Important Antiviral Chemotherapeutics

Chemotherapeutic agent	Effect/indication
Adamantanamin (amantadine)	Inhibition of uncoating in influenza viruses
Acycloguanosine (acyclovir, Zovirax)	Inhibition of DNA synthesis in HSV and VZV
Dihydropropoxymethylguanosine (DHPG, ganciclovir, Cymevene)	Inhibition of DNA synthesis in CMV
Ribavirin	Inhibition of mRNA synthesis and capping. Infections with Lassa virus and perhaps in severe paramyxovirus and myxovirus infections
Nucleoside RT inhibitors (NRTI)	Inhibition of RT in HIV (p. 454)
Phosphonoformate (foscarnet)	Inhibition of DNA synthesis in herpesviruses, HIV, HBV
Protease inhibitors	Inhibition of viral maturation in HIV
Neuraminidase inhibitors	Inhibition of release of influenza viruses
Antisense RNA	Complementary to viral mRNA, which it blocks by means of hybridization (duplexing)

Problems of chemotherapy

The close association between viruses and their host cells is a source of some essential difficulties encountered when developing virus-specific chemotherapeutics.

- Viral replication is completely integrated in cell metabolism
- The virus supplies only the genetic information for proteins to be synthesized by the cell
- Thus, any interference with viral synthesis is likely to affect physiological cellular synthetic functions.

Specific intervention is only possible with viruses that code for their own enzymes (e.g., polymerases or proteases), which enzymes also react with viral substrates.

- Another problematic aspect is the necessity of administering chemotherapeutics early, preferably before clinical symptoms manifest, since the peak of viral replication is then usually already past.

Laboratory diagnostic methods for viral infections

There are essentially three different diagnostic methods used in virology.

1. Virus isolation by growing the pathogen in a compatible host; usually done in cell cultures
2. Direct virus detection in patient material; identification of viral particles using electron microscopy, viral antigens with the methods of serology, and viral genome (components) using the methods of molecular biology
3. Antibody assay in patient serum

Virological Laboratory Diagnostics

Diagnostic approach	Methods	Detection/identification of	Advantages/ disadvantages
Isolation	Growing in cell cultures	Infectivity, pathogenicity	Slow but sensitive method
Direct detection	Electron microscopy, EIA, IF, hybridization, PCR	Viral particles, antigens, genome	Fast method, but may be less sensitive
Serology	EIA, IF, etc.	Antibodies	Retrospective method

Virus Isolation by Culturing

The virus is identified based on its infectivity and pathogenicity by inoculating in cell cultures with the specimen material. Certain changes observed in the culture indicate the presence of a virus cytopathic effect [CPE].

Cell cultures

Viruses can be grown in many types of human or animal cells available for culture. Primary cell cultures can be created with various fresh tissues and can only divide a limited number of times. Cell lines can be developed from primary cultures with unlimited in-vitro culturing capacity. Example, HeLa cells (human portio carcinoma cells) and Vero cells (monkey renal fibroblasts). Viral replication in cell cultures results in morphological changes in the cells such as rounding off, formation of giant cells, and inclusion bodies (so-called CPE). The CPE details will often suffice for an initial approximate identification of the virus involved.

Sampling and transport of diagnostic specimens

- ❑ Sampling

Selection of suitable material depends on the disease and suspected viral species. Sampling should generally be done as early as possible in the infection cycle since viral replication precedes the clinical symptoms. Sufficiently large specimens must be taken under conditions that are as sterile as possible, since virus counts in the diagnostic material are almost always quite low.

- ❑ Transportation

The half-life of viruses outside the body is often very short. Transport must be arranged quickly under cold box conditions. A number of virus transport mediums are commercially available.

Laboratory processing of the material

Before the host is inoculated with the specimen material for culturing, contaminant bacteria must be eliminated with antibiotics, centrifugation, and sometimes filtering.

Selection of a host system

The host system to be used is chosen based on the suspected (and relevant) virus infectors. Observation and incubation times, and thus how long a laboratory diagnosis will take, also depend on the viral species under investigation.

Identification

Identification of the viruses is based first on the observed cell changes, and then determined serologically using known antibodies and appropriate methods such as immunoelectron microscopy, EIA, or the neutralization test. Methods that detect the viral genome are now increasingly used.

Significance of results

It is an indicative of the etiology of the patient's disease. The most sensitive method of viral diagnostic detection, but it cannot detect all viruses in all situations. Another aspect is that the methods of virus isolation, with few exceptions, detect only mature, infectious virions and not the latent viruses integrated in the cells.

Amplification culture

The virus is grown for a brief period in a cell culture. Before the CPE is observed, the culture is tested using the antigen and genomic methods.

Direct Virus Detection

It is to find the viruses directly in the patient material without prior culturing or replication. Viruses in serous fluids such as the contents of herpes simplex or varicella- zoster blisters can be viewed under the electron microscope (EM). It must be remembered, however, that the EM is less sensitive than virus isolation in cultures by a factor of 10^5. Viral antigens can be detected in secretions using enzyme immunoassay (EIA), passive agglutination, or in smears with immunofluorescence performed with known antibodies, for instance monoclonal antibodies. The viral genome can be identified by means of filter hybridization, or in smears or tissue sections with in-situ hybridization using DNA or RNA complementary to the viral genome as a probe.

Sampling and transport of diagnostic specimens

Transport of patient material for these methods is less critical than for virus isolation**.** Cold box transport is usually not required since the virus need not remain infectious.

Significance of results

A positive result with a direct virus detection method has the same level of significance as virus isolation. A negative test result means very little, particularly with EM, due to the low level of sensitivity of this method. The antigen assay and genome hybridization procedures are more sensitive than EM, but they are selective and detect only the viruses against which the antibodies or the nucleic acid probe used, are directed.

Virus Detection Following Biochemical Amplification

Polymerase chain reaction (PCR) provides a highly sensitive test for viral genomes. First, nucleic acid is extracted from the patient material to be analyzed. Any RNA virus genome present in the material is transcribed into DNA by reverse transcriptase. This DNA, as well as the DNA of the DNA viruses, is then replicated in vitro with a DNA polymerase.

Serodiagnosis

If a viral infection induces humoral immunity, the resulting antibodies can be used in a serodiagnosis. When interpreting the serological data, one is confronted by the problem of deciding whether the observed reactions indicate a fresh, current infection or earlier contact with the virus in question. Two criteria can help with this decision. Detection of IgM (without IgG) proves the presence of a fresh primary infection. Concurrent detection of IgG and IgM in blood sampled somewhat later in the course of the disease would also indicate a fresh infection. It could, however, also indicate a reactivated latent infection or an anamnestic reaction.

Viruses as Human Pathogen

Course outline

1. DNA Viruses

1.1 Viruses with Single-Stranded DNA Genomes

- Parvoviruses

1.2 Viruses with Double-Stranded DNA Genomes

- Papillomaviruses
- Polyomaviruses
- Adenoviruses
- Herpesviruses
- Poxviruses
- Hepadnaviruses: Hepatitis B Virus and Hepatitis D Virus

2. RNA Viruse

2.1 Viruses with Single-Stranded RNA Genomes, Sense-Strand Orientation

- Picornaviruses
- Astroviruses
- Caliciviruses
- Hepatitis E Virus
- Togaviruses
- Flaviviruses
- Coronaviruses
- Retroviruses
- Human Immune Deficiency Virus (HIV)

2.2 Viruses with Double-Stranded RNA Genomes

- Reoviruses

2.3 Viruses with Single-Stranded RNA Genomes, Antisense-Strand Orientation

- Orthomyxoviruses
- Bunyaviruses
- Arenaviruses
- Paramyxoviruses
- Rhabdoviruses
- Filoviruses (Marburg and Ebola Viruses

3. Subviral Pathogens

- Viroids
- Prions

Learning Objectives

At the end of the lessons, you will be able to:

- ✓ Describe the general properties of each virus
- ✓ Discuss the pathogenesis and clinical features of each pathogenic virus
- ✓ List and elaborate the Laboratory diagnosis for each pathogenic virus
- ✓ Describe the epidemiology and prevention methods for each pathogenic virus

Viruses with Single-Stranded DNA Genomes

The groups of viruses are contained in only one family, the parvoviruses, with only a single human pathogen type. This group's only human pathogen is parvovirus B19. The Geminiviridae, Circoviridae, and many other families have circular single-stranded DNA, but infect only plants and, more rarely, animals.

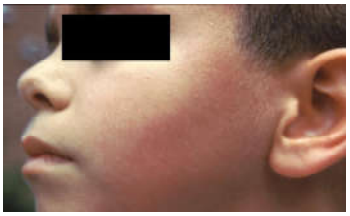
Parvoviruses

The parvoviruses are among the smallest viruses with a diameter of 19–25mm. They are icosahedral, nonenveloped, and their genome is in the form of single-stranded DNA (ssDNA). Some parvoviruses can only replicate in the presence of a helper virus (adenovirus or herpesvirus). Parvovirus B19, the only human pathogenic parvovirus identified to date, is capable of autonomic replication, i.e., it requires no helper virus. Some zoopathic strains also show this capability in rodents, dogs, and pigs.

Pathogenesis and clinical picture

Parvovirus B19 replicates in the bone marrow in erythrocyte precursor cells, which are destroyed in the process. In patients already suffering from anemia (sickle-cell anemia, chronic hemolytic anemia), such infections result in so-called aplastic crises in which the lack of erythrocyte resupply leads to a critical shortage. In healthy persons, these infections usually run an asymptomatic course. They can, however, also cause a harmless epidemic infection in children, erythema infectiosum ("slapped-cheek syndrome" or "fifth disease".

This boy's face displaying signs of erythema infectiosum, or fifth disease



The symptoms of fifth disease are usually mild and may include:

- •fever
- •runny nose
- •headache
- •rash

•This childhood disease, which used to be classified as atypical measles, is characterized by sudden onset of exanthem on the face and extremities.
•Certain forms of arthritis are considered complications of a parvovirus B19 infection.
•The virus also appears to cause spontaneous abortions in early pregnancy and fetal damage in late pregnancy (hydrops fetalis)

Epidemiology

In the transmission route of human parvovirus B19, ddroplet infection or the fecal-oral route, analogous to other parvoviruses, is suspected. Parvovirus B19 spreads through respiratory secretions, such as saliva, sputum, or nasal mucus, when an infected person coughs or sneezes. It can also spread through blood or blood products. A pregnant woman who is infected with parvovirus B19 can pass the virus to her baby.

Prevention

There is no vaccine or medicine that can prevent parvovirus B19 infection. No specific prophylactic measures are recommended. Chance of being infected or infecting others can be reduced by:

- washing hands often with soap and water
- Covering mouth and nose while coughing or sneezing
- not touching your eyes, nose, or mouth
- avoiding close contact with people who are sick
- staying home when you are sick

Once the rash occurs, the cases are probably not contagious. Once you recover from fifth disease, you develop immunity that generally protects you from parvovirus B19 infection in the future.

Treatment

Fifth disease is usually mild and will go away on its own. Children and adults who are otherwise healthy usually recover completely. Treatment usually involves relieving symptoms, such as fever, itching, and joint pain and swelling.

Viruses with Double-Stranded DNA Genomes

Viruses with double-stranded DNA genomes are classified in six families:

- papillomavirus,
- polyomavirus,
- adenovirus,
- herpesvirus,
- poxvirus, and
- hepadnavirus

Carcinogenic types have been found in all groups except the poxviruses (See DNA tumor viruses).

Papillomaviruses

The papillomaviruses have a diameter of 55nm and contain an 8kbp dsDNA genome. There are two distinct regions within the circular genome. One that codes for the regulator proteins produced early in the replication cycle and another that codes for the structural proteins synthesized later. Over 70 papillomavirus types have been described to date, all of which induce either benign or malignant tumors in natural or experimental hosts.

Pathogenesis and clinical picture

Papillomaviruses infect cells in the outer layers of the skin and mucosa and cause various types of warts by means of local cell proliferation. Specific virus types correlate with specific pathohistological wart types. Plantar and vulgar warts, flat juvenile warts, and juvenile laryngeal papillomas apparently always remain benign. By contrast, the genital warts caused by types 6 and 11 (condylomata acuminata) can show carcinomatous changes.

Of all papillomavirus-caused cervical dysplasias, 50% contain human papillomavirus (HPV) 16 and 20% HPV 18. All wart viruses induce primary proliferation of the affected cells with large numbers of viruses found in the cell nuclei. Whether a malignant degeneration will take place depends on the cell and virus type involved, but likely on the presence of cocarcinogens as well.

In carcinomas, the viral DNA is found in integrated form within the host-cell genome, whereas in premalignant changes the viral genomes are found in the episomal state.

Papillomaviruses possess oncogenes (E5, E6, and E7 genes) that bind the products of tumor suppressor genes: E6 binds the p53 gene product, E7 the Rb gene product.

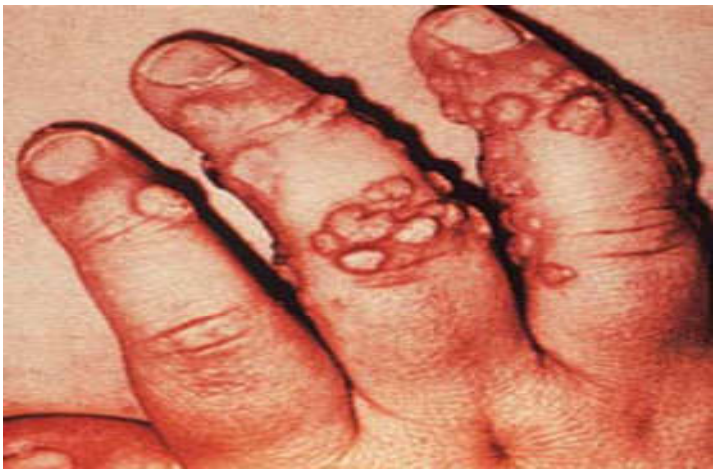
Diagnosis

Human papillomaviruses cannot be cultivated in vitro. They are detected and identified by means of histological analysis and, in malignancies in particular, by means of in-situ hybridization. Antibody assay results have a low significance level and these procedures are not standard routine.

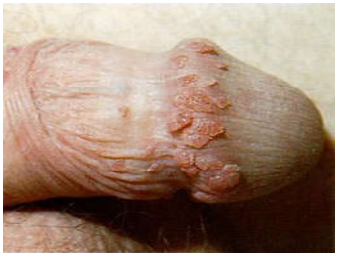
Epidemiology and prevention

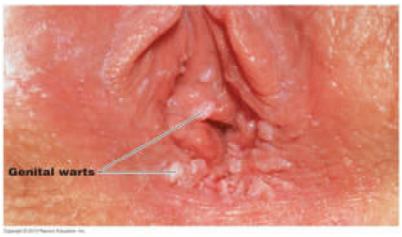
Since viruses are produced and accumulate in wart tissues, papillomaviruses are transmissible by direct contact. Warts can also spread from one part of the body to another (autoinoculation). A certain level of prophylactic protection can be achieved with hygienic measures.

Warts Caused by Papillomaviruses



Genital warts





Papillomaviruses summary

The over 70 viral types in the genus Papillomavirus are all involved in the etiology of benign tumors such as warts and papillomas, as well as malignancies, the latter mainly in the genital area (cervical carcinoma). These organisms cannot be grown in cultures. Diagnosis therefore involves direct detection of the viral genome and histological analysis. Serology is less important in this group.

Polyomaviruses

The polyomaviruses can be divided into two groups:

- in one group are the SV40 and SV40-like viruses such as human pathogen JC and BK viruses
- In the other are the true polyomaviruses such as the carcinogenic murine polyomavirus

The designations JC and BK are the initials of the first patients in whom these viral types were identified. There are also a number of other zoopathic oncogenic polyomaviruses. The name polyoma refers to the ability of this organism to produce tumors in many different organs.

Pathogenesis and clinical picture

The JC and BK viruses are widespread. Over 80% of the adult population shows antibodies to them, despite which, clinical manifestations like PML are very rare. The viruses can be reactivated by a weakening of the immune defense system. The JC virus attacks the macroglia, especially in AIDS patients, to cause PML, a demyelinating process in the brain with disseminated foci that is fatal within one year. The BK virus can cause hemorrhagic cystitis in bone marrow transplantation patients.

Diagnosis

The JC and BK viruses can be grown in cultures, albeit with great difficulty and not for diagnostic purposes. Both can be detected with PCR. BK virus can be seen under the electron microscope in urine. Antibody assays are practically useless due to the high level of generalized contamination.

Epidemiology

Despite the high level of generalized contamination, the transmission routes used by the human polyomaviruses have not been clarified. JCV and BKV have been found in sewage samples from different geographical areas in Europe, Africa, and the United States. This may indicate that ingestion of contaminated water or food could represent a possible portal of entrance of these viruses or polyomavirus DNA into the human population.

Polyomaviruses summary

A medically important polyomavirus, the JC virus, causes progressive multifocal leukoencephalopathy (PML), a demyelinating disease that has become more frequent as a sequel to HIV infections, but is otherwise rare. The same applies to the BK virus, which affects bone marrow transplantation patients. Electron microscopy or PCR are the main diagnostic tools.

Adenoviruses

Adenoviruses are nonenveloped, 70–90nm in size, and icosahedral. Their morphogenesis occurs in the cell nucleus, where they also aggregate to form large crystals. Their genome is a linear, 36–38kbp double- stranded DNA. Adenoviruses got their name from the adenoidal tissues (tonsils) in which they were first identified.

Pathogenesis and clinical picture

Adenoviruses cause a variety of diseases, which may occur singly or concurrently. The most important are infections of the upper (sometimes lower) respiratory tracts, the eyes, and the intestinal tract.

- Infections of the respiratory tract
  - Infections take the form of rhinitis or abacterial pharyngitis, depending on the virus type as well as presumably on the disposition of the patient
  - They may also develop into acute, influenza like infections or even, especially in small children, into a potentially fatal pneumonia
- The eye infections, which may occur alone but are often concurrent with pharyngitis, range from follicular conjunctivitis to a form of keratoconjunctivitis. May cause permanent partial loss of eyesight.
- Intestinal infections: the primary gastroenteritis forms are caused by the viral strains 40 and 41, which are difficult to culture
- Adenoviruses can persist for months in the regional lymph nodes or tonsils until they are reactivated.

Diagnosis

In respiratory adenovirus infections, antibody assays in patient serum are the main approaches. Serology is unreliable in the eye and intestinal infections, since hardly any antibodies are produced in response to such highly localized infections. It is possible to isolate the viruses that cause respiratory infections by inoculating cell cultures with pharyngeal material or bronchial secretion and with conjunctival smears in eye infections. Enteric adenoviruses, on the other hand, are hard to culture. The best approach to detecting them is therefore to subject stool specimens to electron microscopy, enzyme immunoassay, or passive agglutination methods.

Epidemiology and prevention

Humans are the source of infection. Generalized contamination of the population begins so early in childhood that adenovirus infections play a more significant role in children than in adults. Transmission of respiratory adenoviruses is primarily by droplet infection, but also as smear infections since the virus is also excreted in stool. Eye infections can be contracted from bathing water or, in the case of adenovirus type 8 in particular, iatrogenically from insufficiently sterilized ophthalmological instruments. The enteral infections are also transmitted by the fecal-oral route, mainly by contact rather than in water or food. Adenoviruses are the second most frequent diarrheal pathogen in children after rotaviruses.

Adenoviruses summary

- *More than 41 types of adenoviruses and they cause a wide variety of diseases*
- *Infections of the upper, less frequently the lower, respiratory tract and eye infections (follicular conjunctivitis, keratoconjunctivitis) are among the more significant clinical pictures.*
- *Intestinal infections are mainly caused by the only not culturable virus types 40 and 41*
- *Diagnosis: antibody assay in respiratory adenovirus infections.*
- *Serology is not reliable in the eye and intestinal infections.*
- *It is possible to isolate the pathogens in cell cultures from eye infections*
- *Enteral adenoviruses are detected in stool by means of electron microscopy, enzyme immunoassay, or passive agglutination*

Group Assignment and presentation

1. Emerging and reemerging viral diseases
2. Subviral Pathogens

Reading Assignment

- Disinfection, sterilization and biosafety in virology laboratory

Herpesviruses

Over 150 herpesviruses have been described in many species (vertebrate and invertebrate). Nine have been shown to cause disease in humans. No animal reservoir for viruses causing human disease. Viruses in this family are identical in morphology - icosahedral, Enveloped DNA viruses. But they show little uniformity in their biology and the clinical pictures resulting from infections. Herpesviruses have the ability to reactivate after a period of latency – property shared by all.

Herpes viruses causing disease in humans

Subfamily	Important human viruses	
Alphaherpesviruses	Herpes simplex types 1 & 2	HHV-1, HHV-2
	Varicella-zoster virus	HHV-3
Betaherpesviruses	Cytomegalovirus	HHV-5
	Human herpes virus types 6 and 7	HHV-6, HHV-7
	Simian herpes B	
Gammaherpesviruses	Epstein–Barr virus	HHV-4
	Kaposi's sarcoma-associated herpesvirus	HHV-8

Biology of the Herpesviruses

Several hundred herpesvirus species have been described in humans and animals, all with the same morphology. They have dsDNA genomes. Replication of the DNA and morphogenesis take place in the host-cell nucleus. The envelope (inner nuclear membrane) is then formed when the virus penetrates the nuclear membrane, whereby depending on the cell and viral type involved a more or less substantial number of viruses receive an envelope after reaching the cytoplasm, at the cell membrane or not at all.

The envelope is the major determinant of viral infectivity. Envelope contains mainly host-cell determinants - it provides a level of protection from host immune responses.

Common to all herpesviruses is a high level of generalized contamination (60–90% carriers) and the ability to persist in a latent state in the body over long periods. Different viral species persist in different cells, whereby the cell type is the decisive factor determining latency or replication of the virus. Herpes simplex virus and varicella- zoster virus do not produce any virus particles during latency, although they do produce one, or a few, mRNA

types and the corresponding proteins. Cytomegalovirus and Epstein-Barr virus appear to maintain continuous production of small numbers of viruses as well, so that fresh infection of a small number of new cells is an ongoing process --- these viruses would appear to produce persistent, subclinical infections concurrently with their latent status. Reactivation of viral latency is apparently initiated by a number of factors: psychological stress, solar irradiation, fever, traumata, other infections, immunosuppressive therapy.

Human herpesviruses (with the exception of the varicella-zoster virus) and many zoopathic herpes species have been implicated in the etiology of malignancies.

Human herpesviruses that infect different organs:

- Herpes simplex virus-1 (HHV-1)
- Herpes simplex virus-2 (HHV-2)
- Varicella-zoster virus (VZV), (HHV-3)
- Epstein-Barr virus (EBV), (HHV-4)
- Cytomegalo Virus (CMV), (HHV-5)
- Human herpes virus-6 (HHV-6)
- Human herpes virus-7 (HHV-7)
- Kaposi's sarcoma-associated herpesvirus (KSHV),(HHV-8)

Herpes simplex Virus (HSV)

Pathogen, pathogenesis, and clinical picture

The viral genome codes for about 90 proteins: categorized as immediate early proteins (regulatory functions), Early proteins (DNA synthesis), and late proteins (structural proteins). Herpes simplex viruses are classified in types 1 and 2 which differ both serologically and biologically (host-cell spectrum, replication temperature).

Initial infection with herpes simplex type 1 usually occurs in early childhood. The portal of entry is normally the oral mucosa ("oral type") and the infection usually manifests as a gingivostomatitis. The viruses then wander along axons into the CNS, where they persist in a latent state in the trigeminal (Gasseri) ganglion. As with all herpesviruses, the pathogen remains in the macroorganism permanently after the primary infection. Following reactivation (endogenous recidivation), the viruses follow the same route back to the periphery, where they cause the familiar vesicular exanthem ("fever blisters," herpes labialis). Despite established immunity, such recidivations can manifest repeatedly because the viruses wander within the nerve cells and do not enter the intercellular space, thus remaining beyond the reach of the immune defenses. Possible complications include keratoconjunctivitis and a highly lethal form of encephalitis.

Herpes labialis

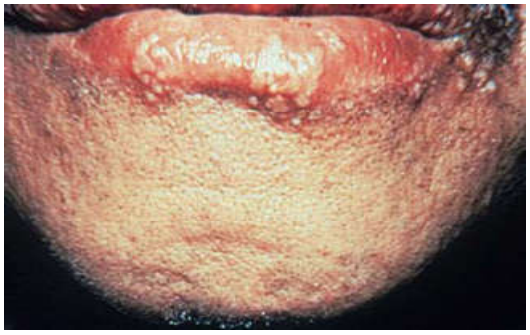
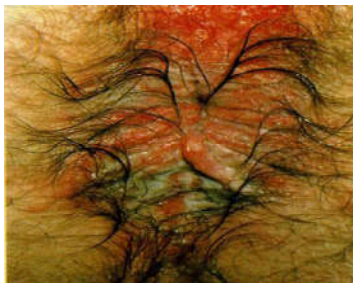
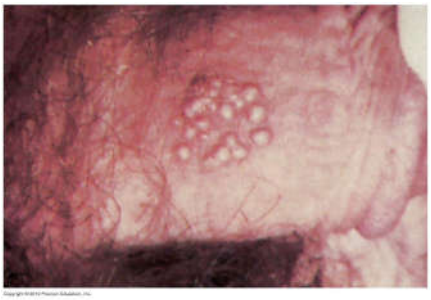


Fig. Following the initial infection, herpes simplex viruses (HSV) persist in the latent state in nerve cells of the CNS. When reactivated, they travel down the axons of these cells to the periphery, where they cause the typical vesicular exanthem.

The initial infection with HSV type 2 normally affects the urogenital area ("genital type") and can be contracted despite an existing HSV type 1 infection. HSV type 2 persists in the latent state in the lumbosacral ganglia or peripheral tissues, from where it causes episodes of manifest herpes genitalis. Neurological complications are very rare and more benign than in HSV type 1. On the other hand, infections of newborn children (herpes neonatorum), e.g., in cases of maternal genital herpes, are feared for their high lethality rate.

Genital herpes: recurrent, very painful vesicles





Diagnosis

Cultivating the pathogen from pustule contents is the method of choice in labial and genital herpes.

In HSV encephalitis, the cerebrospinal fluid will contain few viruses or none at all. In such cases, they can only be cultivated from tissues (biopsy or autopsy material). Virus detection by means of cerebrospinal fluid PCR is worth a try. Direct detection of the viruses under an

electron microscope is only practicable if the specimen contains large numbers of viruses, which in practice will normally only be the case in blister contents.

The virus can also be detected directly in patient specimens using immunofluorescence or in-situ hybridization, but the material must contain virus-infected cells, i.e., blister contents are not as suitable here as in electron microscopy and virus isolation.

Serological investigation results in HSV lack significance due to the high level of general contamination in the population.

Epidemiology, prevention, and therapy

HSV type 1 is transmitted by contact, and possibly by smear infection as well. Contamination with HSV therefore begins in early childhood. Transmission of HSV type 2 usually occurs during sexual intercourse, so that infections are generally not observed until after puberty**.** No immune prophylaxis (vaccination) is currently available for HSV**.** Acycloguanosine is used prophylactically in immunosuppressed patients**.** Specific therapy is possible with acycloguanosine. Used in time, this chemotherapy can save lives in HSV encephalitis.

Varicella-zoster Virus (VZV)

Pathogen, pathogenesis, clinical picture

VZ virus differs substantially from HSV, both serologically and in many biological traits. For instance, it can only be grown in primate cell cultures, in which it grows much more slowly and more cell-associated than is the case with HSV. No subtypes have been described. The initial infection with VZV manifests in the great majority of persons as chickenpox, an episodic papulous exanthema.

The portals of entry are the nasopharyngeal space and the conjunctiva. From there, the virus undergoes a viremic phase in which it is transported by the blood to the skin, where the typical exanthem is produced. The disease confers an effective immunity. In immunodeficient patients, a VZV infection (or reactivation) can affect other organs (lungs, brain) and manifest a severe, frequently lethal, course. After the symptoms of chickenpox have abated, the VZV persists in the spinal ganglia and perhaps in other tissues. Following reactivation, zoster (shingles) develops, whereby the virus once again spreads neurogenically and causes neuralgia as well as the typical zoster efflorescence in the skin segment supplied by the sensitive nerves. Reactivation is induced by internal or external influences and becomes possible when cellular VZV immunity drops off, i.e., after about the age of 45 assuming normal immune defenses.

Herpes zoster

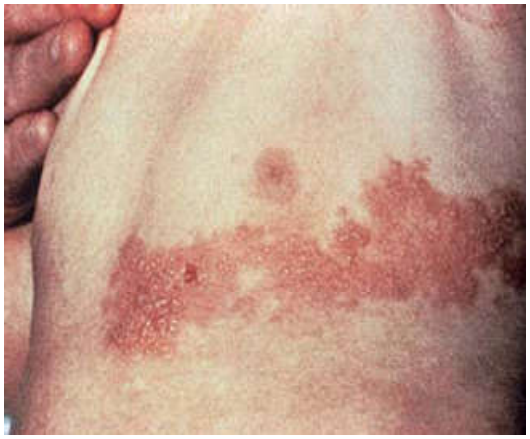


Fig. varicella zoster viruses (VZV) persist in the latent state in spinal ganglia cells. When reactivated, they cause dermal efflorescences in the corresponding dermatome

Diagnosis

VZV can be detected with a wide spectrum of methods

- Electron microscopy,
- PCR,
- Isolation,
- Detection of viral antigens using immunofluorescence in tissue specimens or cell smears
- Serological methods based on antibody titer increases or IgM detection.

Epidemiology, prevention, and therapy

VZV is highly contagious and is transmitted aerogenically. The primary infection, which manifests as chickenpox, is still almost exclusively a childhood disease today. A vaccine containing attenuated viruses is available for prevention of chickenpox and possibly zoster, but its use is currently a matter of controversy. In immunosuppressed patients, hyperimmunoglobulin can be used for passive immunization or postexposure immunity. Acycloguanosine is used both prophylactically and in treatment of VZV infections.

Cytomegalovirus (CMV)

CMV is characterized by a narrow spectrum of hosts**,** slow replication**,** frequently involving formation of giant cells and late and slow development of cytopathology.

An initial infection with cytomegaly is inapparent in most persons, even in very early—perinatal or postnatal—infections. The virus apparently persists in the latent state in mononuclear cells.

Reactivation can also run an asymptomatic course. Symptoms may also develop that are generally relatively mild, mononucleosis like clinical pictures, mild forms of hepatitis or other febrile illnesses.

Droplet infection is the most frequent route of transmission, but smear infections are also possible. Generalized contamination of the adult population with the pathogen is over 90%. The patient is not primarily ill due to a CMV infection. The situation is different in AIDS, transplantation or malignancy patients, in whom a fresh CMV infection or reactivation similarly to HSV and VZV— can result in severe generalized infections with lethal outcome. The liver and lungs are the main organs involved. Retinitis is also frequent in AIDS patients.

Another feared CMV-caused condition is an intrauterine fetal infection results from a primary infection in the mother. In 10% of cases, the infection results in severe deformities CMV is a member of TORCH infections.

Diagnosis

Amplification cultures from saliva, urine, buffy coat, tissue, or BAL (bronchoalveolar lavage) are a suitable method of confirming a florid CMV infection. PCR results must be interpreted with a clear idea of how sensitive the method used can be, since the numbers of viruses found may be clinically insignificant. Serological results are hardly useful in clarifying a florid infection due to the high level of generalized contamination.

Epidemiology, prevention, and therapy

CMV is transmitted by contact or smear infection, usually in childhood or adolescence. Immunosuppressed patients can be treated with hyperimmunoglobulin to provide passive immunity against infection or recidivation. Ganciclovir and foscarnet are therapeutically useful in transplantation, and particularly in AIDS patients, to combat CMV-induced pneumonia, encephalitis, and retinitis.

Epstein - Barr virus (EBV)

Pathogen, pathogenesis, clinical picture

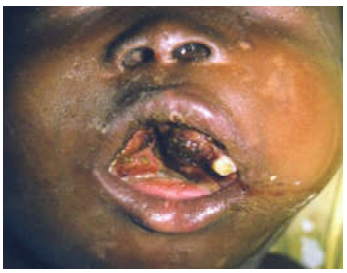
EBV infects only a narrow spectrum of hosts and replicates very slowly. It persists in a latent state in B lymphocytes and can lead to their immortalization and tumor transformation. EBV enters the body through the mucosa. It replicates in epithelial cells of the oropharynx or cervix and enters B lymphocytes, where it continues to replicate. This results in the clinical picture of infectious mononucleosis (kissing disease or Pfeiffer disease) which is characterized by fever and a generalized but mainly cervical swelling of the lymph nodes, typically accompanied by tonsillitis, pharyngitis, and some cases of mild hepatic involvement.

This virus also persists in latency, probably for the life of the patient, in (immortalized) B cells. EBV and EBV-specific sequences and antigens are isolated in cases of Burkitt lymphoma and nasopharyngeal carcinoma.

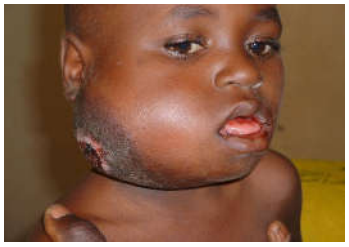
The higher incidence of Burkitt lymphoma in parts of Africa is attributed to a cofactor arising from the hyperendemic presence of malaria there. EBV exacerbates the B-cell proliferation resulting from a malaria infection. EBV has also been implicated in Hodgkin disease and T-cell lymphomas. These tumor forms also result from the interaction of EBV with other mechanisms of cell damage. In immunocompetent persons, the following lymphoproliferative diseases are sequelae of EBV infections:

- a benign polyclonal B-cell hyperplasia,
- its malignant transformation into a polyclonal B-cell lymphoma, and
- a malignant, oligoclonal or monoclonal B-cell lymphoma.

Picture of a mouth of a patient with Burkitt's lymphoma showing disruption of teeth and partial obstruction of airway



Seven-year-old boy with a several month history of jaw swelling which had been treated with antibiotics. The tumor was ulcerated and draining



Diagnosis

Heterogenetic antibodies that agglutinate the erythrocytes of several animal species and antibodies to a variety of viral antigens are found in acute infectious mononucleosis:

- VCA (viral capsid antigen): antibodies to VCA appear early and persist for life
- EA (early antigen): antibodies to EA are only detectable during the active disease.
- EBNA (Epstein-Barr nuclear antigen): antibodies to EBNA are not produced until two to four weeks after disease manifestation, and then persist for life.
- Chronic mononucleosis is characterized by antibodies to VCA and EA

The diagnostic procedures in lymphoproliferative diseases involve histology and cellular immunotyping.

Epidemiology, prevention, and therapy

EBV is excreted in saliva and pharyngeal secretions and is transmitted by close contact ("kissing disease"). As with all herpesviruses the level of generalized contamination is high, with the process beginning in childhood and continuing throughout adolescence. Neither

immunoprophylactic nor chemoprophylactic measures have been developed as yet. Lymphoproliferative diseases involving viral replication can be treated with acyclovir and ganciclovir.

Human Herpesvirus (HHV) 6

Pathogen, pathogenesis, clinical picture

HHV-6 was isolated in 1986 in patients suffering from lymphoproliferative diseases and AIDS. The virus shows T-cell tropism and is biologically related to the cytomegalovirus. HHV-6 exists in two variants, HHV-6A and HHV-6B. The pathogenic implications of their reactivation have not yet been described. HHV-6B is the causal pathogen in exanthema subitum (roseola infantum); a disease that is nearly always harmless, characterized by sudden onset with high fever and manifests as a typical exanthem in small children. Reports of HHV-6-caused illness in adults are rare and the clinical pictures described resemble mononucleosis (EBV-negative mononucleosis). Apparently, however, this virus can also cause severe infections in bone marrow transplant patients (pulmonary and encephalitic infections). HHV-6A has not yet been convincingly implicated in any clinical disease.

Diagnosis and epidemiology

HHV-6 can be cultured in stimulated umbilical lymphocytes. Potentially useful diagnostic tools include antibody assay and PCR. Generalized contamination with HHV 6 begins in early childhood, eventually reaching levels exceeding 90% in the adult population. The virus persists in latent form in the salivary gland, so that mother-to-child transmission is most likely to be in saliva.

Human Herpesvirus (HHV) 8

HHV 8 has recently been identified as a decisive cofactor in induction of Kaposi sarcoma. The classic, sporadic form of this malignancy was described in 1872 in the Mediterranean area. It occurs following organ transplantations and is a significant cause of death in AIDS patients. The contribution of HHV 8 to the pathogenesis of Kaposi sarcoma appears to lie in dysregulation of cytokine and hormone production. In transplantation- association Kaposi sarcoma the virus can also be transmitted by the transplant.

Diagnosis: Antibody assay (EIA, IF, Western blot).

Poxviruses

The viruses of the pox group are the largest viruses of all, 230x350nm, within the resolution range of LM. They have a complex structure. The only DNA viruses that replicates in a defined area within the host-cell cytoplasm, the so-called “virus factory”. The diseases smallpox (variola major) and the milder form alastrim (variola minor) now no longer occur in the human population because a worldwide vaccination program during the 1970s. The last person infected by smallpox was registered in Somalia in 1977. Eradication of the disease was formally proclaimed in 1980.

The Family Poxviridae

This virus family comprises several genera:

- Orthopoxviruses
  - Variola and alastrim viruses
  - Vaccinia virus: used in smallpox vaccines
  - Cowpox viruses, monkeypox, mousepox, and rabbitpox viruses
- Parapoxviruses
  - orf virus (poxvirus of sheep and goat): transmitted to humans by sheep and goats)
  - Milker's nodule virus (not to be confused with cowpox)
    transmitted to humans by cows
- Molluscipoxviruses
  - Molluscum contagiosum virus

Unique properties of Poxviruses

- the largest, most complex viruses, have complex, oval to brick-shaped morphology with internal structure
- have a linear, double-stranded DNA genome with fused ends
- are **DNA viruses that replicate in the cytoplasm**
- Virus encodes and carries all proteins necessary for mRNA synthesis.
- Virus also encodes proteins for functions such as DNA synthesis, nucleotide scavenging, and immune escape mechanisms
- Virus is assembled in **inclusion bodies** (Guarnieri bodies), where it acquires its outer membranes

Disease mechanisms of Poxvirus

Smallpox is initiated by respiratory tract infection and is spread mainly by the lymphatic system and cell-associated viremia. Molluscum contagiosum and zoonoses are transmitted by contact. Virus may cause initial stimulation of cell growth and then cell lysis. Virus encodes immune escape mechanisms. Cell-mediated immunity and humoral immunity are important for resolution. Most poxviruses share antigenic determinants allowing preparation of "safe" live vaccines from animal poxviruses.

Vaccinia virus

A distinct viral type of unknown origins and it is not an attenuated variola virus. Formerly used as a vaccine virus to protect against smallpox. The vaccination caused a pustular exanthem around the vaccination site, usually accompanied by fever. Encephalitis was a feared complication; It is assumed that an autoimmune reaction was the decisive factor. Other complications include generalized vaccinia infection and vaccinial keratitis. Vaccinia infections and their complications disappeared for the most part when smallpox was eradicated. The vaccinia virus does not occur in nature and any human infections are now accidental (laboratory infections). Vaccinia viruses are still frequently used as vectors in molecular biology laboratories.

Cowpox, orf, and milker's nodule viruses

Infections with the viruses are rare & usually harmless. The lesions remain localized on the skin (contact site), accompanied by a local lymphadenitis. These are typical occupational infections (farmers, veterinarians).

Molluscum contagiosum virus

Infections with this virus do not confer immunity. The infection causes epidermal, benign tumors, molluscum contagiosum warts.

Summary of diseases associated with poxviruses

Virus	Disease	Source	Location
Variola	Smallpox (now extinct)	Humans	Extinct
Vaccinia	Used for smallpox vaccination	Laboratory product	-
Orf	Localized lesion	Zoonosis-sheep, goats	Worldwide
Cowpox	Localized lesion	Zoonosis-rodents, cats, cows	Europe
Pseudocowpox	Milker's nodule	Zoonosis-dairy cows	Worldwide
Monkeypox	Generalized disease	Zoonosis-monkeys, squirrels	Africa
Bovine papular stomatitis virus	Localized lesion	Zoonosis-calves, beef cattle	Worldwide
Tanapox	Localized lesion	Rare zoonosis-monkeys	Africa
Yabapox	Localized lesion	Rare zoonosis-monkeys, baboons	Africa
Molluscum contagiosum	Many skin lesions	Humans	Worldwide

Diagnosis

Poxviruses are relatively easy to recognize under an electron microscope in pustule contents. This is provided the pustules have not yet dried out or been superinfected with bacteria. Orthopoxviruses and parapoxviruses can be differentiated morphologically. But the viruses within each genus share the same morphology. Molluscum contagiosum is diagnosed histologically.

Properties of Natural Smallpox That Led to Its Eradication

- **Viral Characteristics**
  - Exclusive human host range (no animal reservoirs or vectors)
  - Single serotype (immunization protected against all infections)
- **Disease Characteristics**
  - Consistent disease presentation with visible pustules (identification of sources of contagion allowed quarantine and vaccination of contacts)
- **Vaccine**
  - Immunization with animal poxviruses protects against smallpox
  - Stable, inexpensive, and easy-to-administer vaccine
  - Presence of scar indicating successful vaccination
- **Public Health Service**
  - Successful worldwide WHO program combining vaccination and quarantine

Hepadnaviruses: HBV and HDV

Hepatitis B virus

It is the main representative of the family of hepadnaviruses, Hepadnaviridae. The name of the family is an acronym of the disease caused by the virus and its genomic type. Hepadnavirus = hepa/dna/virus; hepatitis, DNA virus.

Its genome consists of partially double-stranded DNA. The replication cycle of the HBV includes a transient RNA phase. HBV possess an envelope made up of a cellular double lipid layer in which are integrated the HBs antigen, a 25 kDa polypeptide, and its precursor stages PreS1 (40 kDa) and PreS2 (33 kDa).

The envelope encloses the actual capsid, which consists of the hepatitis B core (HBc) antigen and the genome together with the DNA polymerase (a reverse transcriptase). The complete, infectious virion, also known as a Dane particle after its discoverer, has a diameter of 42 nm, the inner structure 27 nm. The virus replicates in liver cells. The Dane particles and the HBs antigen, but not the HBc antigen, are released into the bloodstream. A further viral protein is the HBe antigen, which represents a posttranslational, truncated form of the HBc antigen

and is no longer capable of spontaneous capsid formation. It is also released from the hepatic cells into the blood

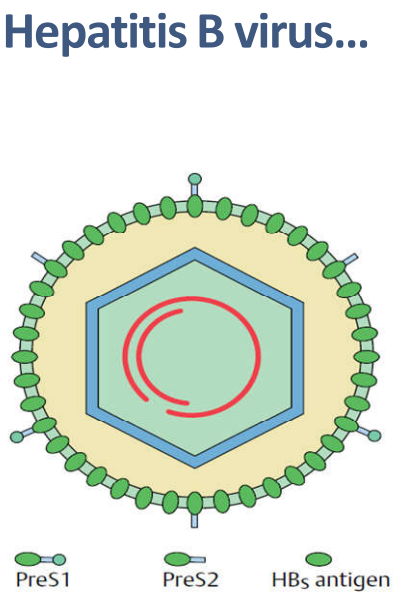


Fig.

•The capsid, which consists of **Hbc** and **Hbe antigens,** encloses the entire DNA antisense strand, the incomplete sense strand, and the reverse transcriptase (not shown here).

•The envelope contains the three forms of the **Hbs antigen**:

- **•PreS1** = complete protein,
- **•PreS2** = shortened form of PreS1,
- **•HBs antigen** = HB surface antigen, shortened form of PreS2.

Hepatitis B Mutants

It is detected using molecular biological methods refined in recent years. More and more, HBV mutants have been found with one or more amino acid exchanges in certain proteins. HBs or PreS mutants are so-called "escape" mutants that can cause a new infection or recidivation despite immune protection by antibodies to HBs. Similarly, pre-HBVc or HBVc mutants can lead to a reactivation of HBV replication and thus to a chronic hepatitis, since they block formation of the HBe antigen and thus the point of attack for the cellular immune defenses. HBc mutants are frequently observed under interferon therapy.

Hepatitis D virus

A certain percentage of HBV-infected persons are also infected by a second hepatitis virus It was discovered at the end of the seventies in Italy, the delta agent or HDV. It was originally thought to be a new HBV antigen. In fact, it is an unclassified RNA virus that codes for the delta antigen. Its capsid consists of HBs antigen, i.e., HBV-coded material. For this reason, the virus can only replicate in persons infected with HBV (helper virus).

Pathogenesis and clinical picture

IP of hepatitis B is four to 12weeks, followed by the acute infection phase, icteric, or anicteric course, once again with a variable duration of two to 12 weeks. The hepatic cell damage resulting from an HBV infection is not primarily due to cytopathic activity of the virus. But rather to a humoral and cellular immune response directed against the virus-induced membrane antigens (HBs, HBc) on the surface of the infected hepatocytes. 0.5–1%

of those infected experience a fulminant, often lethal, hepatitis. In 80–90% of cases, the infection runs a benign course with complete recovery and elimination of the HBV from the body.

A chronic infection develops in 5–10%. Three forms are differentiated, but mixed forms are possible:

- healthy HBV carriers,
- chronic persistent hepatitis (CPH) without viral replication, and finally
- chronic aggressive hepatitis (CAH) with viral replication and a progressive course

A chronic infection can result in development of carcinoma (hepatocellular carcinoma, HCC) or cirrhosis of the liver. HDV appears to have an unfavorable influence on the clinical course, usually making the disease more aggressive, increasing the number of complications and worsening the prognosis.

Chronic hepatitis B

Development of a chronic hepatitis B infection is revealed by a changed antigen-antibody profile: the two antigens HBs and HBc (and raised transaminases) persist for over six months, whereby antibodies to HBe and HBs are not produced. A subsequent "late seroconversion" of HBe antigen to anti-HBe antibodies supports a better prognosis. Thorough clarification of chronic cases must include either immunohistological testing for HBV antigens in liver biopsies or PCR testing for the presence of viral DNA, and thus Dane particles, in patient serum.

Diagnosis

Hepatitis B is diagnosed by identifying the various HBV antigens or the antibodies directed against them. Both antigens and antibodies can be detected in patient blood using a solid phase test (EIA). HDV is diagnosed by detection of delta antigen or possibly antibodies to delta (IgM) in the blood.

Interpretation of Hepatitis B Serologic Test Results

Hepatitis B serologic testing involves measurement of several hepatitis B virus (HBV)-specific antigens and antibodies. Different serologic "markers" or combinations of markers are used to identify different phases of HBV infection and to determine whether a patient has acute or chronic HBV infection, is immune to HBV as a result of prior infection or vaccination, or is susceptible to infection.

HBsAg **anti-HBc** **anti-HBs**	negative negative negative	Susceptible ==➔Consider vaccine
HBsAg **anti-HBc** **anti-HBs**	negative positive positive	Immune due to natural infection ==➔ Resolved HBV infection
HBsAg **anti-HBc** **anti-HBs**	negative negative positive	Immune due to hepatitis B vaccination
HBsAg **anti-HBc** **IgM anti-HBc** **anti-HBs**	positive positive positive negative	Acutely infected
HBsAg **anti-HBc** **IgM anti-HBc** **anti-HBs**	positive positive negative negative	Chronically infected
HBsAg **anti-HBc** **anti-HBs**	negative positive negative	Interpretation unclear; four possibilities: 1. Resolved infection (most common) 2. False-positive anti-HBc, thus susceptible 3. "Low level" chronic infection 4. Resolving acute infection

Adapted from: A Comprehensive Immunization Strategy to Eliminate Transmission of Hepatitis B Virus Infection in the United States: Recommendations of the Advisory Committee on Immunization Practices. Part I: Immunization of Infants, Children, and Adolescents. MMWR 2005;54(No. RR-16).

- **Hepatitis B surface antigen (HBsAg):** A protein on the surface of hepatitis B virus; it can be detected in high levels in serum during acute or chronic hepatitis B virus infection. The presence of HBsAg indicates that the person is infectious. The body normally produces antibodies to HBsAg as part of the normal immune response to infection. HBsAg is the antigen used to make hepatitis B vaccine.
- **Hepatitis B surface antibody (anti-HBs):** The presence of anti-HBs is generally interpreted as indicating recovery and immunity from hepatitis B virus infection. Anti-HBs also develops in a person who has been successfully vaccinated against hepatitis B.
- **Total hepatitis B core antibody (anti-HBc):** Appears at the onset of symptoms in acute hepatitis B and persists for life. The presence of anti-HBc indicates previous or ongoing infection with hepatitis B virus in an undefined time frame.
- **IgM antibody to hepatitis B core antigen (IgM anti-HBc):** Positivity indicates recent infection with hepatitis B virus (≤6 mos). Its presence indicates acute infection.

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Hepatitis B e antigen (HBeAg) is a marker of viral replication. When viral replication slows, HBeAg disappears, and antibody to HBeAg (anti-HBe) is detected. Anti-HBe may persist for years. The first antibody to appear is antibody to HBcAg (anti-HBc). Initially, it is of the immunoglobulin M (IgM) class.

Epidemiology and prevention

Humans are the sole reservoir of HBV**.** Transmission is parenteral, either with blood or body fluids containing HBV (sexual intercourse) that come into contact with mucosa, lesions, or microlesions in the skin**.** In transmission by blood, the tiniest amounts contaminating syringe needles, ear-piercing needles, tattooing instruments, etc. suffice to produce an infection**.** Hepatitis B infections from blood transfusions have been greatly reduced by thorough screening of blood donors for HBs antigens, despite which patients receiving multiple transfusions or dialysis remain a high risk group**.**

Another high-risk group includes all healthcare workers with regular blood contact**.** All blood samples must be considered potentially infectious and handled only with disposable gloves**.** Addicts who inject drugs with needles are also obviously exposed to a very high level of risk**.**

No effective chemotherapy against HBV has been developed to date**.** WHO recommends general hepatitis B prophylaxis in the form of active immunization with HBs antigen**.** In response to a sudden high-level infection risk (accidental inoculation with infectious material), persons whose immune status is uncertain should also be passively immunized with human anti-HBs antiserum—if possible within hours of pathogen contact.

It has not yet proved feasible to grow HBV in vitro. The antigen used in vaccinations can be isolated from human blood. Fear of AIDS infections has resulted in emotionally based, unjustifiable rejection of this vaccine. An alternative vaccine is now available based on developments in genetic engineering: the HBs antigen can now be synthesized by a yeast fungus.

Prevention: hepatitis B booster vaccines

Periodic booster shots, especially for persons at high risk, were recommended for some time to maintain sufficient immune protection. However, since all successfully vaccinated persons build up immunity rapidly following renewed contact with the pathogen (“immunological memory,”), this recommendation has been replaced in a number of countries by the following scheme:

- Following immunization on the classic model (0, 1, and 6 months), the anti-HBs antibody titer is measured within one to three months
- Responders (titer 100 IU/l) require no booster
- In hyporesponders and nonresponders (titer <100 IU/l), an attempt should be made to reach a titer of 100 IU/l with a maximum of three further vaccinations.

RNA viruses

Viruses with Single-Stranded RNA Genomes, Sense-Strand Orientation

- ✓ To date, six viral families are known: **picornavirus, calicivirus, togavirus, coronavirus, flavivirus**, and **retrovirus**
  - Picornaviruses
  - Astroviruses
  - Caliciviruses
  - Hepatitis E Virus
  - Togaviruses
  - Flaviviruses
  - Coronaviruses
  - Retroviruses
  - Human Immune Deficiency Virus (HIV)

Picornaviruses

The picornaviruses are among the most thoroughly studied viruses of all. The name *picorna* is an abbreviation that stands for two characteristics of this family: they are *small (pico)* viruses with an *RNA* genome *(rna*). The RNA is polyadenylated at its 3' end and has no cap at the 5' end, but instead a virus-coded, basic protein, the VPg (virus protein, genome-linked), which functions as a primer for RNA synthesis.

Important human pathogenic genera of picornaviruses are:

- Enteroviruses: polioviruses, cocksackieviruses and echoviruses
- Parechoviruses: types 1 and type 2
- Hepatoviruses: hepatitis A virus
- Rhinoviruses: common cold viruses (cause rhinitis)

Enteroviruses (Poliovirus, Coxsackievirus, Echovirus) and Parechoviruses. The genus Enterovirus, isolated from the intestinal tract, includes

- Poliovirus (poliomyelitis pathogen) with 3 serotypes
- Coxsackievirus, group A, with 22 serotypes
- Coxsackievirus, group B, with 6 serotypes
- Echovirus with 34 serotypes
- Enteroviruses numbers 68–71

The genus Parechovirus includes the species parechovirus types 1 and 2.

Pathogenesis and clinical pictures

The enteroviruses and parechoviruses are transmitted per os and replicate at first in the lymphoid tissue of the pharyngeal space, later mainly in the intestinal wall**.** Then reach their

"target organs" via the bloodstream (e.g., CNS, muscles, heart, liver), followed by manifest organ infection, which, however, only develops in a small percentage of cases. Most infections run an asymptomatic course. Viremia is always present, so that even asymptomatic enterovirus and parechovirus infections confer effective immunity.

Enteroviruses and Parechoviruses: Clinical Syndromes

Virus type	Important syndromes
Polioviruses	Poliomyelitis, paralysis, aseptic meningitis, encephalitis
Coxsackie viruses A & B, echoviruses, enterovirus 68–70	Meningitis, paralysis, pharyngitis (herpangina), pneumonia, hepatitis, maculous and vesicular exanthems, including hand, foot, and mouth disease (HFMD)
Coxsackie virus B	In addition to the above: myalgia, pleurodynia (Bornholm disease), pericarditis and myocarditis, pancreatitis, diabetes
Enterovirus 71	Acute hemorrhagic conjunctivitis, HFMD
Parechovirus 1 and 2	Respiratory and gastrointestinal ("summer diarrhea") infections

Diagnosis

PCR or isolation of the virus is used. Specimens: CSF, pharyngeal smear, or lavage, with the best chances of success from stool. Serodiagnosis plays only a minor role.

Epidemiology and prevention

Humans are the reservoir of the enteroviruses. Transmission is either direct (smear infection) or in food and water. Where hygienic standards are high, droplet infections also play a significant role. Special prophylactic measures to prevent infections with coxsackieviruses or echoviruses are neither practicable nor generally necessary. Salk introduced a dead vaccine in 1954 for poliomyelitis prophylaxis (**IPV, inactivated polio vaccine**) consisting of three poliovirus types inactivated by formalin. Five years later, the live vaccine (**OPV, oral polio vaccine according to Sabin**) was introduced, which contains three live but no longer neurovirulent poliovirus strains, either singly or in combination. The WHO plan to eradicate poliomyelitis worldwide would seem feasible with this vaccine as demonstrated by its eradication in several countries including all of South America.

Hepatoviruses (Hepatitis A Virus)

HAV differs in some characteristics from enteroviruses, to which group it was long considered to belong. Growth in cell cultures requires long adaptation. Only one serotype is known to date.

Pathogenesis and clinical picture

The clinical picture of hepatitis A differs in no major particulars from that of hepatitis B. The disease nearly always takes a benign course. Only a small number of fulminant (and sometimes lethal) or chronic courses have been described. The pathogenic process at first corresponds to that of the enteroviruses, whereby hepatitis A replicates in the intestine and then, after a brief viremic episode, attacks its target organ, the liver. Disease manifestation with this pathogen, unlike most of the enteroviruses but similar to hepatitis B, involves immunological processes.

Diagnosis

- It is based on IgM detection due to the early presence of these antibodies in patient serum**.**
- Lack of hepatitis A antibodies at the onset of clinical manifestations excludes hepatitis A.

Epidemiology and prevention

- Transmission is by food and water or in the form of smear infections.
- Active immunization with an inactivated HAV vaccine is available.

Rhinoviruses

About 117 serotypes found to date. Their genomic organization and replication system generally match those of the enteroviruses. They differ in that they are acid-sensitive and slightly denser.

Pathogenicity and clinical picture

The rhinoviruses are the causative pathogens of the common cold**.** Infect the mucosa of the nasopharyngeal space (nose and throat)**.** They remain strictly localized there and do not cause generalized infections**.** In rare cases, mainly in children, they cause bronchitis or bronchopneumonia. The clinical picture is often worsened by bacterial super infection

Laboratory diagnostics are only required in special cases of rhinovirus infection. The viruses can be grown in cell cultures.

Epidemiology and prevention

Rhinoviruses are transmitted directly by contaminated hands, and partly by droplet infection. Infective contacts between humans appear to involve mechanical inoculation (introduction into the nasopharyngeal space with fingers). Rhinoviruses occur worldwide, with pronounced proliferation in the winter months. The fact that everyone comes down with colds repeatedly is explained by the very brief immunity conferred by infection and the

many different viral types involved. Experiments have shown that the infections are always exogenous, i.e., not reactivations due to cold, wetness, etc. The only conceivable prophylactic measure is to avoid large groups of people.

Astrovirus and Calicivirus; Hepatitis E

Astroviruses and caliciviruses are enteritis pathogens in small children. Human pathogens in these groups include the Norwalk virus and hepatitis E virus (HEV). HEV is transmitted by the fecal-oral route, above all via drinking water, and causes relatively benign infections except in pregnant women. Hepatitis E is considered a traveler's disease.

Astroviruses

Astrovirus owes its name to its starlike appearance. It contains sense RNA with approximately 7500 nucleotides. It appears to have a replication strategy similar to that of the picornaviruses.

Pathogenesis and clinical picture

Astroviruses that are animal and human pathogens are associated with episodes of diarrhea that nearly always run a harmless course. The etiological role of these viruses has still not been clarified. Astroviruses appear to possess only a low level of pathogenicity.

Diagnosis and Epidemiology

Astroviruses are diagnosed by electron microscopy. They occur worldwide. They tend to infect young children and older persons weakened by other diseases.

Caliciviruses

Caliciviruses possess only one capsid protein and a polyadenylated, 7500-nucleotide RNA with a VPg at the 5' end. The surface of the viruses has a characteristic structure with small, regular, calyx like concavities that give the capsid the form of a Star of David. Caliciviruses are classified based on genomic similarities as either human caliciviruses (HuCV) or "small, round-structured viruses," SRSV. This designation stems from their initial identification under the electron microscope as "small, round, virus particles." The SRSV are grouped in two subtypes, I and II. Type I includes the Norwalk virus and a number of similar viruses named for their geographic venues, some with antigenicity differing from the Norwalk type

Clinical picture

Caliciviruses cause enteritis. Together with rotaviruses and adenoviruses, they are the most frequent viral enteritis pathogens in children. Often causing minor epidemics during the winter months ("winter vomiting disease")

Diagnosis and epidemiology

Caliciviruses are diagnosed by EM or antigen assay in stool**.** Two-thirds of the adult population in the temperate zone carries antibodies to the Norwalk virus**.** SRSV are regularly implicated in minor epidemics and family outbreaks. The transmission route are fecal-oral route, water and uncooked foods.

Hepatitis E Viruses

- An infectious inflammation of the liver endemic to Asia, Central America, and parts of Africa
- Apparently transmitted by the fecal-oral route
- The RNA genome of the culprit agent has now been sequenced and the virus in question, the hepatitis E virus, has been classified with the caliciviruses
- It occurs in at least 13 variants divided into three groups
- In-vitro culturing of HEV has not succeeded to date

Pathogenesis and clinical picture

The clinical course of hepatitis E infections tends to be benign and resembles that of hepatitis A. It shows no chronicity. However, infections in the third trimester of pregnancy have a lethality rate of 10–40%.

Diagnosis

Antibodies can be detected by means of an EIA. Due to cross-reactions with other caliciviruses, the specificity of the results is uncertain. A diagnosis is often arrived at based on clinical evidence and medical history (travel to endemic areas).

Epidemiology

HEV causes repeated outbreaks of considerable dimensions. The infections can be traced to contaminated drinking water. No specific prophylactic measures exist.

Togaviruses

The togavirus family (Togaviridae) comprises two genera. Alphavirus infections are transmitted by arthropods and are imported to central Europe mainly by travelers to tropical and subtropical countries. Their clinical pictures are variable, but almost always include joint pain (arthralgias). The most important representative of the genus Rubivirus is the rubella virus, the causative agent in German measles. This harmless childhood disease can cause severe embryopathies during the first trimester of pregnancy.

Possess an icosahedral capsid and a close fitting envelope. Genome is a single-stranded, polyadenylated, sense RNA. Replication not only produces new 40S genomic RNA, but a subgenomic 26S RNA fragment, which codes for the capsid proteins. Viral progeny are released by “budding” at the cell surface.

Togaviruses include

- zoopathic pestiviruses,
- one species of rubivirus; rubella virus
- alphaviruses with 25 species

The alphaviruses most important to travelers are transmitted to humans by bloodsucking mosquitoes

- Chikungunya virus (Africa, Asia),
- Sindbis virus (Africa, Asia, Australia),
- Ross River virus (Australia, Oceania),
- Mayaro virus (South America)

Pathogenesis and clinical picture

The arthropodborne alphaviruses, zoonoses of the tropical and subtropical regions, frequently cause asymptomatic or benign infections with fever, exanthem, and joint pain. Occasionally, however, persistent arthralgia and polyarthritis (lasting months or even years) do occur, sometimes involving joint destruction. Even rarer, sequelae include encephalitis and meningoencephalitis with high lethality rates. "German measles" is a harmless exanthemous infection in children and youths, caused by rubella virus, and transmitted by direct contact. The infections remain inapparent in nearly half the cases.

The virus, at first, replicates in lymphoid organs at the portal of entry and in the nasopharyngeal space, after which a viremia develops before the exanthem manifests.

In pregnant women, the virus takes this route through the placenta to the embryo, where it can cause congenital deformities or embryonic death, especially in the first three months of pregnancy. The organs in the developmental stage in this trimester are most seriously affected by the rubella infection. The most frequent congenital deformities are deafness, cataracts, cardiac defects, microcephaly, and spina bifida. In intrauterine embryo deaths due to rubella infections the immediate cause of death is usually myocardial damage. A measles infection confirmed by IgM detection or a raised antibody count is therefore an indication for a first-trimester abortion.

Diagnosis and prevention

Serodiagnosis is the method of choice in suspected alphavirus and rubivirus infections. EIA methods are also available for IgM detection. There are vaccines to protect against alphavirus infections and rubella. The main aim of rubella prophylaxis is to prevent rubella-caused embryopathies. Since 10–15% of young adults are still susceptible to rubella infections and a live vaccine with few side effects that confers reliable immunity is available, serial vaccination of children (boys and girls!) is done before puberty. The vaccine is tolerated so well that prior immune status checks are not required.

Flaviviruses

Viruses in the flavivirus family (Flaviviridae) include the genera Flavivirus, Hepacivirus, and Pestivirus. Flaviviruses (the prototype being the yellow fever virus [Latin: flavus, yellow]) are transmitted by arthropods. They cause a biphasic infection that can have serious consequences (hemorrhagic fever with a high lethality rate). In southern and eastern countries, these viruses are significant human pathogens. Only one representative of this family, the tickborne encephalitis pathogen, is encountered in Europe.

Hepaciviruses (HCV and HGV) are not arthropodborne. HCV is transmitted mainly in blood (transfusions, blood products, intravenous drug use) and is a frequent cause of chronic disease (70% of cases), including cirrhosis of the liver and hepatocellular carcinoma. HGV is related to HCV and has not been characterized in detail as yet. Pestiviruses are only important in veterinary medicine.

Flaviviruses show morphological uniformity, an icosahedral capsid and closefitting, spiked envelope. The genome of the flaviviruses is a single stranded sense RNA. It codes for three structural and seven nonstructural proteins. The morphogenesis of the virus occurs at the endoplasmic reticulum, into the lumen of which the finished viruses bud. These characteristics have not been directly demonstrated for the hepatitis C virus, which cannot be cultured in vitro. The pestiviruses cause severe animal epidemics (e.g., swine fever). They are not transmitted by arthropods.

Flavivirus (Arthropodborne Yellow Fever Type)

The flavivirus family includes 63 species. Prototypic viruses of the family are the yellow fever virus, and the pathogen that causes European tickborne encephalitis (spring-summer meningoencephalitis, SSME).

Overview of the Most Important Flaviviruses (arthropodborne)

Viral species	Transmitting vector	Geographic spread	Syndrome
Dengue	Mosquito (*Aedes*, *Stegomyia*)	West Africa, Pacific, South and Southeast Asia, Caribbean, Venezuela, Colombia, Brazil	Dengue syndrome, DHF, DSS
Yellow fever	Mosquito (*Aedes*)	West and Central Africa, South and Central America	Hemorrhagic fever
Japanese B encephalitis	Mosquito (*Culex*)	East, Southeast and South Asia, western Pacific	Encephalitis
St. Louis encephalitis	Mosquito (*Culex*)	North and Central America, Brazil, and Argentina	Encephalitis
West Nile fever	Mosquito (*Culex*), ticks (*Argasidae*)	East and West Africa, South and Southeast Asia, Mediterranean countries, recently USA as well	Dengue syndrome, encephalitis
Tickborne encephalitis (Central European* and Russian)	Ticks (*Ixodes*)	Central Europe, Russia	Encephalitis

* Syn. spring-summer meningoencephalitis (SSME)

Pathogenesis and clinical picture

The arthropodborne flaviviruses cause diseases of different levels of severity. The infections are typically biphasic.

An initial phase, not very characteristic phase, includes fever, headache, muscle pain, and in some cases exanthem (Denguelike disease). This phase includes a pronounced viremia. The illness, in this stage often not recognized as a flavivirus infection.

Secondary phase: initial phase may then be over or it may progress after one to three days to a second, severe clinical picture: a hemorrhagic fever with a high lethality rate involving hemorrhages and intravasal coagulation. In Dengue fever, this form is becoming more and more frequent and is called Dengue hemorrhagic fever (DHF) or Dengue shock syndrome (DSS) depending on the predominant characteristics.

Diagnosis

A flavivirus infection always involves viremia (transmission by bloodsucking arthropods!). The viruses can be isolated from blood by inoculating cell cultures or newborn mice. In autopsies of fatal cases, the virus can be isolated from liver tissue. The viruses are labile by nature and identification can take time, for which reason the diagnostic focus is on serology (titer rise or IgM detection).

Epidemiology and prevention

A cycle of infection involving a vertebrate host (mammals, birds) and a transmitting vector (bloodsucking mosquitoes and flies, ticks) has developed for most flavivirus infections. The cycles are efficient for the virus and relatively harmless for the host. The vertebrate host frequently shows few signs of disease and recovers from the infection after a brief viremia. During this period, the bloodsucking vector is infected, which thereafter remains a lifelong salivary secretor and thus infectious.

In ticks, transovarian transmission of the virus is also possible. The human host is a dead end for the virus, not a normal component of the cycle. Exceptions to this are Dengue fever and urban yellow fever. Humans are the only known main hosts for the Dengue virus.

There are two forms of yellow fever:

- Rural or jungle (“sylvatic”) yellow fever with a monkey-mosquito-monkey (sometimes human) cycle.
- Urban yellow fever with humans as the main hosts and Aedes mosquitoes as the transmitting vectors. This form is on the upswing due to increasing numbers of breeding places (e.g., empty tin cans in garbage piles) for the vector.

Another “new” (more accurately: revived) infectious disease is the West Nile viral infection, observed for the first time in the USA (New York) in 1999, apparently introduced into the

area by migrating birds. It is still not known why the geographic distribution of the virus or infected birds changed.

Vaccines are available against yellow fever (live vaccine) and European tickborne encephalitis (dead vaccine).

Hepaciviruses (Hepatitis C and G)

HCV was discovered by molecular biological in 1988. It is designated as "non-A-non-B (NANB) hepatitis". They belong to a flavivirus with approximately 10 kb sense RNA and several genotypes. HGV, another flavivirus, also causes hepatitis.

Pathogenesis and clinical picture

Hepatitis C resembles hepatitis B in many respects. One major difference is that it much more frequently produces a persistent infection (85 %). In 70% of cases, develops into a chronic hepatitis which results in cirrhosis of the liver within 20 years and a hepatocellular carcinoma (HCC) in a further 10 years. The reason for the high level of viral persistence is thought to be a pronounced mutability facilitating evasion of the immune defenses - quasispecies of RNA viruses.

Diagnosis

Antibody EIA using genetically engineered viral proteins and western blot to confirm the result. RNA can be detected by RT-PCR. Course of therapy monitored with quantitative PCR.

Epidemiology and prevention

Transmission is by blood and blood products. High-risk persons include dialysis patients, healthcare staff, and needle-sharing drug consumers. Perinatal transmission is possible, but sexual contact does not appear to be a risk factor. The transmission route is not apparent in many cases, giving rise to the expression "community- acquired infection." Feasible protective measures are the same as in hepatitis B; no immunization by vaccine is available. Especially in combination with ribavirin, therapeutic use of interferon can lead to elimination of the virus in persistent infections and thus to prevention of cirrhosis of the liver and HCC.

Coronaviruses

The Coronaviridae family includes several viral species that can infect vertebrates - dogs, cats, cattle, pigs, rodents, and poultry. The name (corona, as in wreath or crown) refers to the appearance of the viruses. "Spikes", with club or drumstick-like swellings, are located at regular, relatively generous intervals on the pleomorphic envelope.

One coronavirus species (human coronavirus, HuCV) is known to be a human pathogen. It has at least two serotypes and probably a number of serological variants. In November 2002, a new coronavirus emerged in China and, after originally being mistaken as a new

influenza recombinant, was identified as the causative agent of severe acute respiratory syndrome, or SARS, in spring 2003. Its origin, possibly from animals, is not known to date.

Pathogenesis and clinical picture

Common cold-coronaviruses cause an everyday variety of respiratory infections, which are restricted to the ciliated epithelia of the nose and trachea. They are responsible for about 30% of common cold infections.

The immunity conferred by infection, apparently IgA-dependent, is short lived. Reinfections are therefore frequent, whereby the antigenic variability of the virus may be a contributing factor. Various enteral coronaviruses with morphologies similar to the respiratory types have also been described in humans. Their pathogenicity, and hence their contribution to diarrhea, has not been clarified.

The SARS virus is transmitted aerogenically with an incubation time of two to 10 days. Clinically, fever and a marked shortness of breath is noted, developing into a severe atypical pneumonia with new pulmonary infiltrates on chest radiography. Shedding of virus is by respiratory discharges. Whether the virus present in other body fluids and excreta plays a decisive role for virus transmission is not yet clear.

Diagnosis

The common-cold coronavirus can be grown in organ cultures of human tracheal tissue or in human diploid cells. Isolating the viruses for diagnostic purposes is not routine. Serodiagnosis (complement-binding reaction, immunofluorescence or enzyme immunoassay) and electron microscopy are feasible methods. The SARS virus can be identified by PCR or isolated in the Vero cell line.

Epidemiology and prevention

Transmission of the virus is by droplets, but close contact ("household transmission") with possibly other routes of transmission seems important. The only preventive measure to date is exposure prevention. Under therapy with ribavirin and intensive care, mortality of SARS is around 10%.

Reading Assignment!!!

- **SARS-CoV-2 (Aetiology of COVID-19)**

Retroviruses

Retroviridae family is the classification for all RNA viruses with reverse transcription of RNA to DNA in their reproductive cycles (RNA-dependent DNA synthesis). Only zoopathic retroviruses were known for many years. These viruses cause various kinds of tumors in animals. In 1980, retroviruses were also discovered in humans. This virus family includes seven genera, of which, three play significant roles in human medicine.

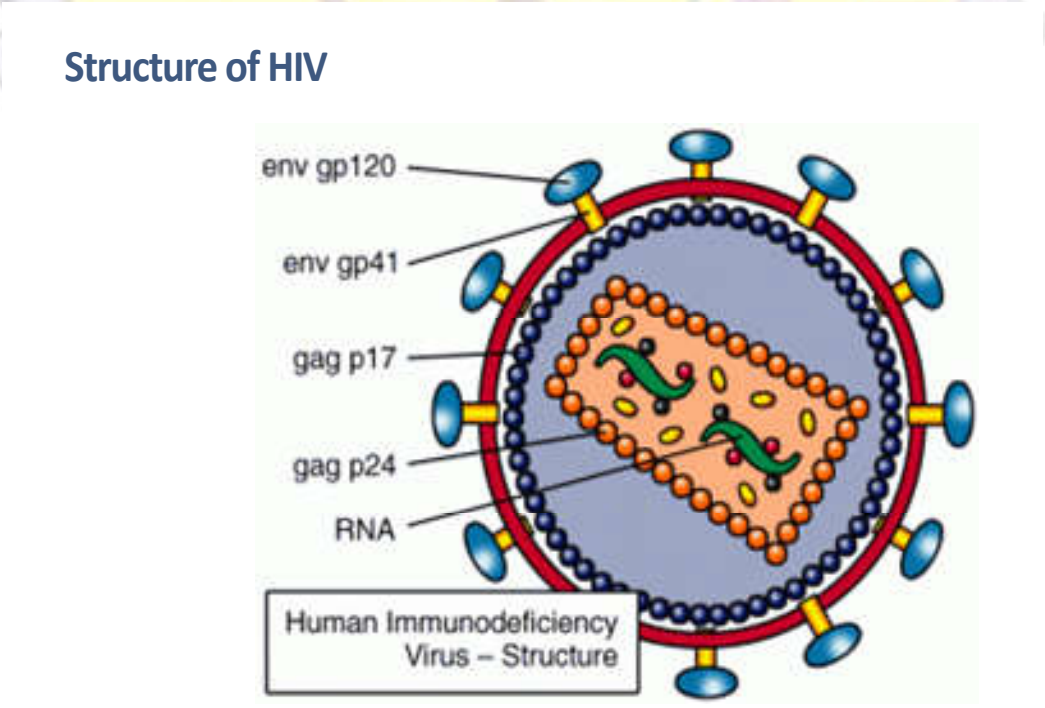
Human retroviruses

HTLV-BLV retroviruses: HTLV types I & II and the bovine leukemia virus. Spumaviruses, which only occur in animals, two of which are (probably) from humans. Lentiviruses with the human pathogens HIV 1 and 2, maedivirus (pneumonia), and visnavirus (encephalomyelitis) in sheep, viruses affecting goats and horses, and animal immune deficiency viruses.

HTLV types I & II

A human pathogen retrovirus was isolated for the first time in 1980 from adults suffering from T-cell leukemias. It was designated as HTLV I (human T-cell leukemia virus). A short time later, a virus was isolated from hairy cell leukemia patients and named HTLV II. HTLV I is found in adults with T-cell malignancy as well as in patients with neurological diseases (myelopathies). HTLV II appears to be associated with T-cell malignancy and other lymphoproliferative diseases. Its own etiological role is still under discussion.

Human Immune Deficiency Virus (HIV)



Types of HIV:

- HIV-1 ➔ related to SIVcpz (chimpanzee SIV) and HIV-2 (more closely related to SIVagm (African green monkey SIV)
- HIV-1:
  - Three groups
  - M (referring for the main group), O (outlier), and N (non-M, non-O)

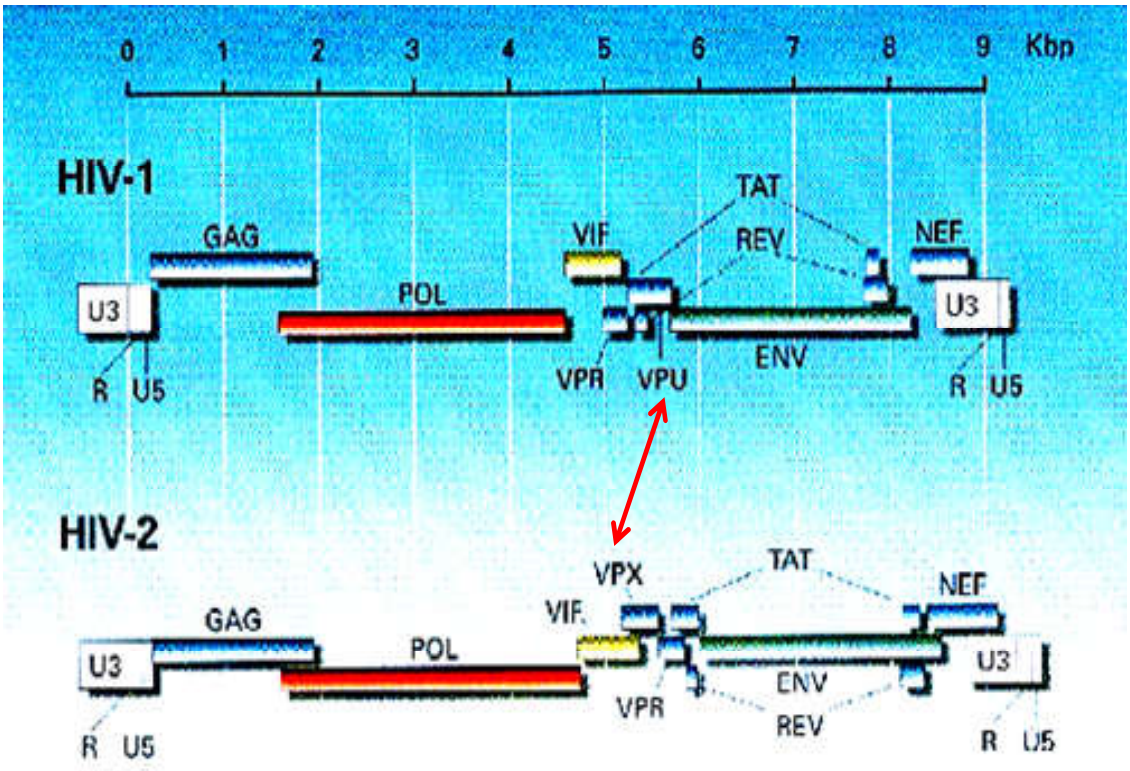
Group M:

- nine subtypes (A-J) excluding I, which has been found to be recombinant between subtypes A and E) and
- About 15 Circulating Recombinant Forms (CRFs)

How many genes does HIV have?

HIV has just nine genes (compared to more than 500 genes in a bacterium, and around 20,000-30,000 in a human). Three of the HIV genes, called gag, pol and env, contain information needed to make structural proteins for new virus particles. The other six genes, known as tat, rev, nef, vif, vpr and vpu, code for proteins that control the ability of HIV to infect a cell, produce new copies of virus, or cause disease. At either end of each strand of RNA there is a sequence called the long terminal repeat which helps to control HIV replication.

Genomic organization of HIV



Genes essential to viral replication:

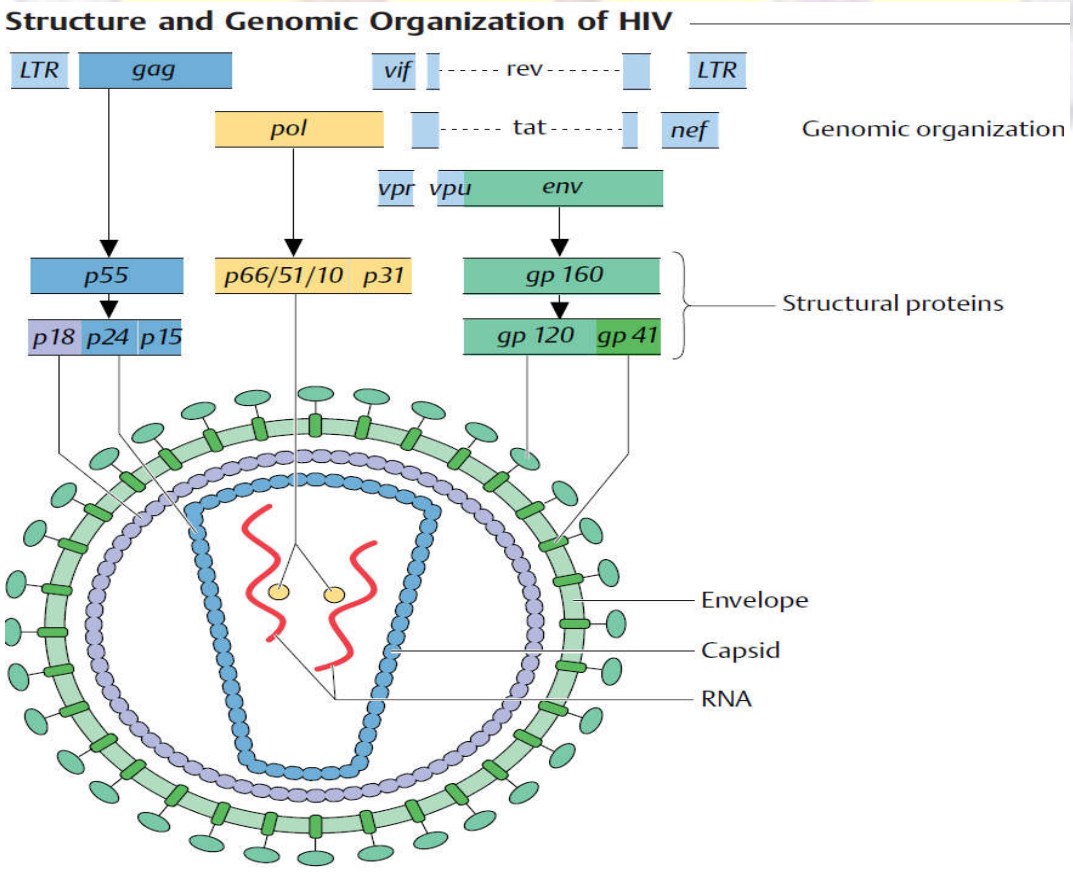
- ***tat*** gene: "transactive transcription," enhances the transcription and thus the expression of viral proteins by binding to the TAR (transactivation responsive region) in the LTR.
- ***rev*** gene: posttranscriptional activator for splicing and transport of viral mRNA (production of structural proteins).
- ***LTR*** sequence: promoter and enhancer elements.

Structural genes:

- ***gag*** gene: group-specific antigen.
- ***pol*** gene: codes for the reverse transcriptase, a protease and the integrase.
- ***env*** gene: envelope glycoprotein (gp).

Genes not essential to viral replication:

- **Virus infection factor (*vif*)**: makes the virus more infectious.
- **"Negative" factor (*nef*)**: inhibits or activates viral transcription as required, influences T-cell activation, reduces CD4 expression.
- ***vpr***: controls rate of replication.
- ***vpx***: only in HIV 2, controls rate of replication.
- ***vpu***: only in HIV 1, contributes to viral release, increases CD4 turnover.



Gene	Gene product	Function
Structural genes:		
gag	p55	p55 Nucleocapsid, precursor of p18, p24, p15
	p18	Matrix protein
	p24	Capsid protein
pol		Polymerase region
	p66/51/10	Reverse transcriptase/RNase/protease
	p31	Integrase
env	gp160	Glycoprotein, precursor of gp120, gp41
	gp120	Surface protein (binds to CD4 molecule of host cell)
	gp41	Transmembrane protein

Major genes and their associated gene products

Classes of protein	Gene	Gene products	Function/Properties
Structural proteins	*Gag* (group-antigen)	P17 (matrix)	Interacts with gp41
		P24 (capsid)	Core protein
		P7 (nucleocapsid)	Nucleocapsid; binds to RNA
	Envelope (env)	gp120	Binding to host CD4 receptor & facilitate Viral entry into cell
		gp41	Trans-membrane protein/cell fusion
Catalytic	*Polymerase (pol)*	Protease	Proteolytic cleavage of Gag and Pol
		Reverse transcriptase/ RNase H)	Transcription of DNA from genomic RNA/ degradation of old RNA
		Integrase	Integration of viral DNA into host chromosome
Regulatory proteins	*tat*	Trans-activation of transcription (Tat)	Major viral trans-activator; by binding to viral RNA together with host factor, it stabilizes viral mRNA and enhances rate of transcription
	rev	Regulator of expression of virion protein (Rev)	Enhances expression of unspliced and singly spliced RNAs
Accessory proteins	*vpu* (in HIV-1 only)	Viral protein U (Vpu)	Enhances virion release from cells
	vif	Virion infectivity protein (Vif)	Enhances cell-free transmission
	vpr	Viral protein R (Vpr)	Enhances viral replication in primary cells
	nef	Negative regulatory factor (Nef)	Inhibits or enhances viral replication depending on strain and cell type

HIV replication

- ✓ HIV can infect T- lymphocytes and other cells bearing the CD4 marker on their surface
- ✓ The CD4 molecule is the main receptor for HIV, or more precisely for its gp120
- ✓ In addition, either the chemokine receptor CCR5 (macrophage- tropic R5 HIV strains) or CXCR4 (T cell-tropic X4 strains) is used as a coreceptor.
- ✓ Persons with (homozygotic) missing CCR5 are highly resistant to HIV infection
- ✓ A number of other coreceptors are also active depending on the viral strain involved
- ✓ HIV is then taken in by the cell.
- ✓ After uncoating, reverse transcription takes place in the cytoplasm.
- ✓ The rest of the viral replication process basically corresponds to the description of retroviral replication on "Introduction section"
- ✓ The interaction of the many different contributing control genes is responsible for the long latency period and subsequent viral replication.

Pathogenesis and clinical picture

- The pathogenicity of the disease is based on suppression of cellular immunity as a result of the loss of the CD4+ T helper cells
- The primary infection either remains inapparent or manifests as "acute retroviral syndrome" with conjunctivitis, pharyngitis, exanthem, and lymphadenopathy, as well as a transitory meningoencephalitis in some cases
- P24 antigen is detectable in serum after about 14 days, i.e., before the antibodies.
- This stage is followed by a long period of clinical latency (the incubation period is described as 10 years), during which the carrier is clinically normal but may be infectious.
- The HIV can persist in a latent state in CD4+ T lymphocytes, macrophages, and the Langerhans cells in the skin
- Apparently, viral replication continues throughout this period, especially in lymphoid organs
- The drop in CD4+ lymphocytes and the rise in the virus count (viral load) in peripheral blood is followed by the lymphadenopathic stage
- Opportunistic infections then set in, frequently combined with lymphomas, the otherwise rare Kaposi sarcoma, or so-called AIDS encephalopathy (subacute AIDS encephalitis, AIDS dementia complex).
- Similar neurological symptoms may also be induced because of HIV-induced immunosuppression

Diagnostic Definitions for AIDS in Adults

$CD4^+$ T cells/µl	Clinical categories		
	A	B	C
>500	A1	B1	**C1**
200–499	A2	B2	**C2**
<200	**A3**	**B3**	**C3**

A3, B3, and C1–3 confirm AIDS diagnosis

Clinical categories

A: Asymptomatic or acute (primary) HIV infection; persistent generalized lymphadenopathy (LAS)

B: Symptoms indicative of weakened cellular immune defenses, but no AIDS-defining diseases.

C: AIDS-defining diseases:

AIDS-defining diseases

Viruses
HSV: chronic ulcer, esophagitis, bronchitis, pneumonia
VZV: generalized zoster
CMV: retinitis, encephalitis, pneumonia, colitis
JC virus: progressive multifocal leukoencephalopathy
HIV: encephalitis
HIV: wasting syndrome

Protozoans
Cryptosporidium: chronic diarrhea
Isospora belli: chronic diarrhea
Toxoplasma gondii: encephalitis

AIDS-defining diseases…

Bacteria
Recurrent salmonellar septicemia
Recurrent pneumonia
Mycobacterial tuberculosis, pulmonary and extrapulmonary forms
Opportunistic mycobacteria (*M. avium*, etc.), disseminated or extrapulmonary

Fungi
Candida: esophagitis, pneumonia, bronchitis
Histoplasma, *Cryptococcus neoformans*, coccidiosis: extrapulmonary, disseminated
Pneumocystis carinii: pneumonia

Malignomas
Kaposi sarcoma
Invasive cervical carcinoma
B-cell lymphoma EBV-positive

LABORATORY DIAGNOSIS

- Serology (ELISA and rapid test algorithms). IgG develops 4-6 weeks post exposure and remains detectable for life. Its presence in serum therefore indicates infection. Exception: uninfected infants of HIV-positive mothers.
- Direct detection of virus
  - p24 antigen ELISA
  - Cell culture
  - PCR – to determine viral load

Prevention

Exposure prophylaxis when contact with blood is involved (drug addicts, healthcare staff) and sexual intercourse. Postexposure prophylaxis and prophylaxis in pregnancy with chemotherapeutics can be used.
Standard vaccines can be used to prevent other infections.

- For example, opportunistic infections in HIV-positive persons, especially children showing no symptoms.
- The dead vaccine type is recommended for polio.
- Live vaccine materials should generally not be used in persons showing AIDS symptoms.

Therapy

- Commonly used antiretroviral drug classes:
  - Nucleoside analogues (against Reverse Transcriptase)
  - Non-Nucleoside analogues (against Reverse Transcriptase)
  - Protease Inhibitors
  - Fusion Inhibitors (against the gp41; recently introduced; not widely used)

- **Nucleoside analogue RT inhibitors**
  - Zidovudin (ZVD)
  - Didanosine (ddI)
  - Zalcitabine (ddC)
  - Stavudine (d4T)
  - Abacavir (ABC)
  - Lamivudine (3TC)
  - Tenofovir (TDF)
- **Nonnucleoside analogue RT inhibitors**
  - Nevirapine
  - Delaviridine
  - Efavirenz
- **Protease Inhibitors**
  - Indinavi
  - Ritonavir
  - Saquinavir
  - Nelfinavir
  - Lopinavir
  - Atazanavir
- **Entry Inhibotor**
  - Enfuvirtide

Antiretroviral mode of action

- Nucleoside reverse transcriptase inhibitors
  - Nucleoside analogue lead to premature termination of HIV DNA chain
  - Not recommended as monotherapy – single drug regimen leads to resistance
- Non-nucleoside reverse transcriptase inhibitors
  - Non-nucleoside analogue Lead to premature termination of HIV DNA chain

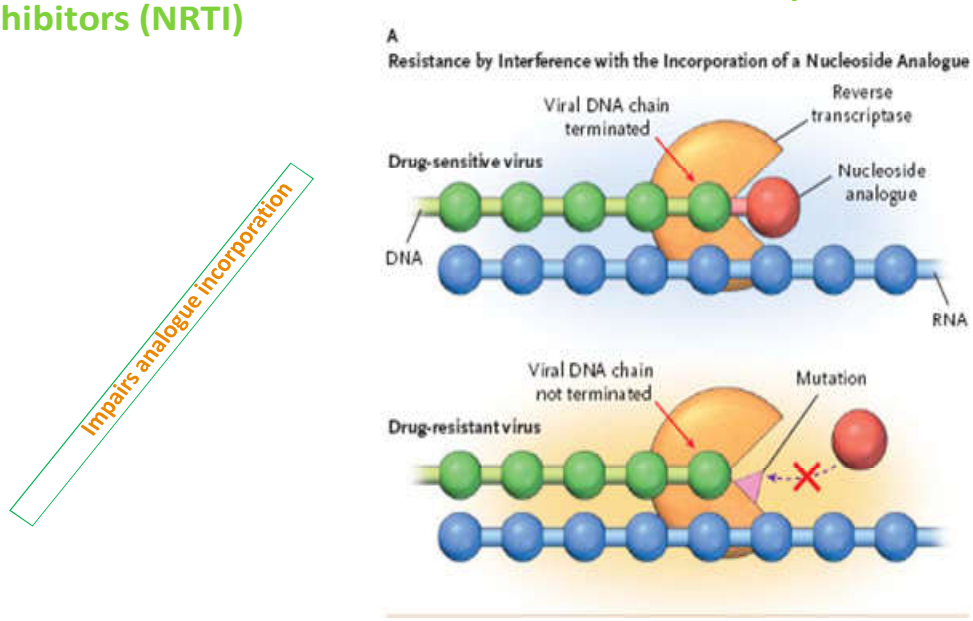
Protease inhibitors (PIs)

- HIV protease enzyme
  - cleaves various polyproteins in the process of producing mature infectious virions
- Protease Inhibitors or PIs
  - interfere with the production of HIV protease
  - lead to reduction of the virus in the body
  - reduction is sometimes significant enough to lead to undetectable levels of virus
  - do not use Pis alone (monotherapy) because rapid resistance will develop—they should be used in combination with other drugs

Drug resistance

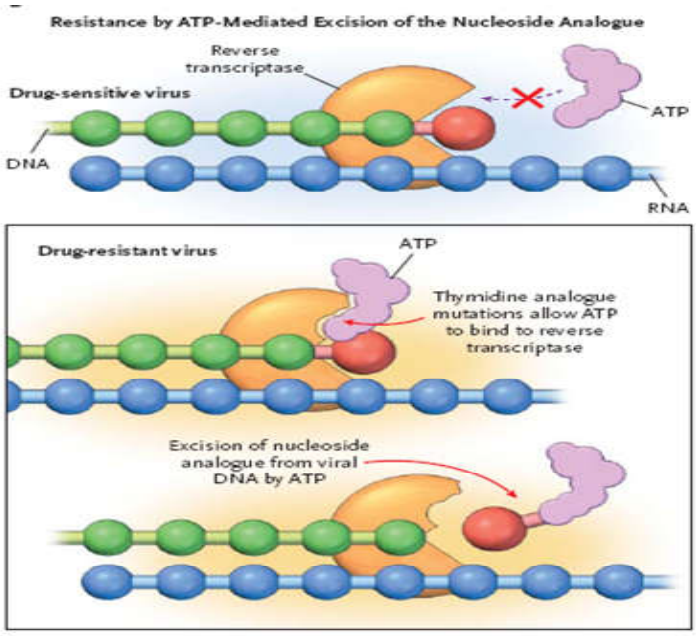
Drug resistance development is a common phenomenon among drug experienced patients. HIV develops resistance against all class of ARV drugs. It does this by undergoing genetic changes on the genes coding for target proteins so that they escape from the drug effect. Drug resistant variants escape drug activity via different mechanisms: changing the gp41 protein sequence for fusion inhibitors.

Chain termination for nucleoside reverse transcriptase inhibitors (NRTI)



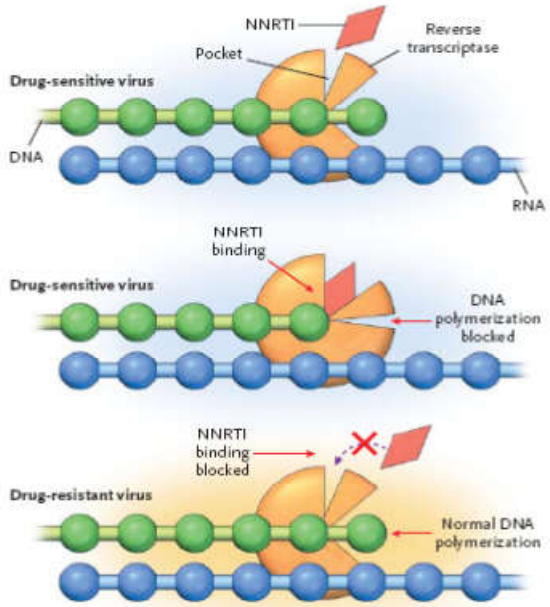
Impairs analogue incorporation

NRTI

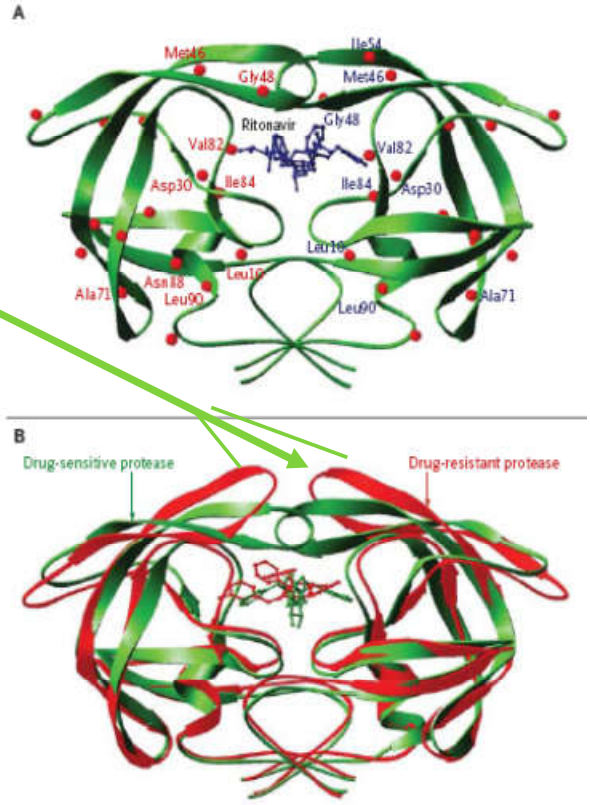


causes analogue removal

Impairing the active pocket (allosteric site) for NNRTI



Changing the shape of the enzyme for Protease Inhibitor



Combination Treatments of HIV Infections:

To avoid development of resistant HIV variants, a combination of at least three drugs from at least two substance classes is usually administered. The following combinations are currently established practice:

a) One PI and two NRTIs

b) One NNRTI and two NRTIs

c) Two PIs and one or two NRTIs

d) One PI and one NNRTI, alternatively with one or two NRTIs as well;

e) Three NRTIs

a) and b) appear to produce the best long-term results.

Viruses with ds-RNA Genomes: Reoviruses

The name reovirus is derived from the abbreviation for respiratory enteric orphan virus, recalling that no diseases were associated with the virus upon its discovery (hence "orphan virus"). All of the viruses in the family Reoviridae possess a double icosahedral capsid. It contains the segmented RNA genome, comprising from 10 to 12 double-stranded subunits depending on the species.

Family Reoviridae

The family Reoviridae includes, in addition to phytopathogenic and zoopathogenic strains, three genera in which human pathogens are classified:

- Coltiviruses include a large number of pathogens significant in veterinary medicine as well as the human pathogen virus that causes Colorado tick fever
- Reoviruses in the narrower sense, with three serogroups
- Rotaviruses, groups A to F, further subdivided into subgroups, serotypes, and electropherotypes

The rotaviral genome consists of eleven segments of double-stranded RNA. Each segment codes for one viral protein. Some segments in other reovirses code for two or three proteins.

Pathogenesis and clinical picture

- **Coltiviruses**:

Colorado tick fever usually runs a mild course with fever, myalgias, nausea, and vomiting, rarely encephalitis

- **Reoviruses**

Implication in diseases is still uncertain. It appears they are capable of infecting the respiratory and intestinal tracts of children. The fact that they are also found very frequently in asymptomatic persons makes it difficult to correlate them with specific clinical pictures.

- **Rotaviruses**

In the mid-seventies these viruses were recognized as diarrhea- causing viruses in infants and small children. They are the most frequent cause of diarrhea in children aged six months to two years. It was recently discovered that they also play a role in infections of the elderly, and above all in immunosuppressed patients (e.g., bone marrow transplant patients), and can cause severe clinical pictures in these groups. Rotaviruses enter the body per os or by droplet infection, replicate in the villi of the small intestine and cause diarrhea, potentially resulting in exsiccosis.

Diagnosis

Colorado tick fever can be diagnosed serologically. Reovirus infections can be diagnosed by isolating in cell cultures.

Rotaviruses do not readily grow in cell cultures for diagnostic purposes. They can be detected more readily under an electron microscope or in antigen assays using commercially available solid phase tests (EIA) or passive agglutination. An elegant typing method for the different rotavirus strains involves analysis of the electrophoretic mobility of the 11 dsRNA strands of the viral genome.

Epidemiology

Humans are the sole natural reservoir of rotaviruses. Generalized contamination is practically 100% when children reach school age, but carriers and reinfections are still possible despite immunity. Diarrheal infections are among the most important causes of death in small children in developing countries; 20% of these infections are due to rotaviruses. Rotaviruses remain viable for long periods on objects and skin (hands!) and are therefore spread rapidly by infected persons and healthy carriers. The most effective prophylactic approach is to practice stringent hygiene.

Rotavirus vaccines

The vaccine is very effective in preventing rotavirus gastroenteritis. CDC recommends routine vaccination of infants with either of the two available vaccines:

- RotaTeq® (RV5), which is given in 3 doses at ages 2 months, 4 months, and 6 months; or
- Rotarix® (RV1), which is given in 2 doses at ages 2 months and 4 months

Both rotavirus vaccines are given orally. The vaccines are very effective (85% to 98%) in preventing severe rotavirus disease. Rotavirus vaccines will not prevent diarrhea or vomiting caused by other viruses, but they are very effective against rotavirus infection.

Rotavirus summary

Rotavirus	
Characteristics	Linear segmented (11 segments) dst RNA Icosahedral capsid Non-enveloped
Reservoirs	Humans (only reservoir)
Transmission	Direct contact Fecal-oral Contaminated water, food and fomites
Diseases	Gastroenteritis Most common cause of gastroenteritis Abdominal pain, vomiting and severe watery diarrhea (> 20 liter per day) May complicate by leading to hypovolemia shock,death My also complicate by Rotavirus viremia, meningoencephalitis Primarily occurs in children
Treatment	Oral fluid and electrolyte replacement
Prevention	Two different rotavirus oral vaccines are currently licensed for infants in the United States. The vaccines are RotaTeq® (RV5) and Rotarix® (RV1) Improved sanitation measures and good hygiene practice is important methods of control

Viruses with Single-Stranded RNA Genomes, Antisense-Strand Orientation

- **Six viral families** have an antisense RNA genome:
  - Orthomyxoviridae,
  - Bunyaviridae,
  - Arenaviridae,
  - Paramyxoviridae,
  - Rhabdoviridae,
  - Filoviridae

Orthomyxoviruses

The family has one genus, Influenza virus. Influenza virus has three types: influenza A, B, and C. Influenza A is the most important and most frequently observed influenza virus. It repeatedly causes epidemics and even pandemics at greater intervals. In contrast, influenza B tends to persist in endemic form and causes few outbreaks. Influenza C is rarely isolated, most frequently in youths. It plays on a minor role as an infective pathogen.

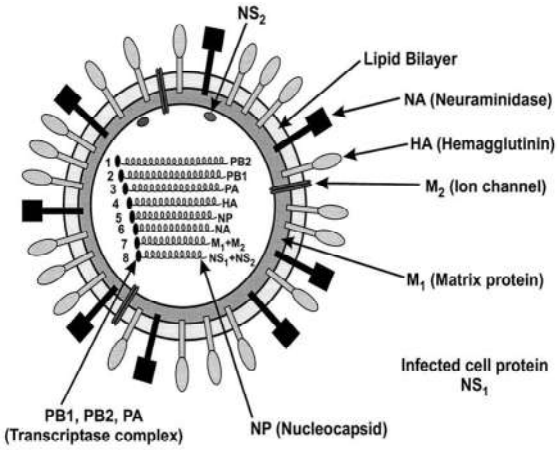
	A	B	C
Severity of illness	**++++**	**++**	**+**
Subtypes	**Yes**	**No**	**No**
Animal reservoir	**Yes**	**No**	**No**
Spread in humans	**Pandemic**	**Epidemic**	**Sporadic**
Antigenic changes	**Shift, drift**	**Drift**	**Drift**

Structure

All influenza viruses show the same and a pronounced pleomorphism. The genome of the influenza viruses is segmented and comprises eight separate antisenses RNA strands each of which codes for one specific protein. Together with the nucleoprotein, they form the helical nucleocapsid. Closely association with this structure is the RNA polymerase complex, which consists of three high-molecular-weight proteins with different functions. The nucleocapsid itself is embedded in a protein (so-called membrane or matrix protein). The virus is enclosed by an envelope made of cell membrane lipids with viral protein inclusions (hemagglutinin and neuraminidase, responsible for infectivity and viral progeny release). Both proteins are seen under the electron microscope as protrusions ("spikes") on the virus surface.

Influenza A Virus Structure

- **Hemagglutinin (HA)**
  - Receptor binding (sialic acid)
  - Membrane fusion
  - Neutralizing antibody target
- **Neuraminidase (NA)**
  - Remove sialic acid residues
  - Virion release
- **Ion channel (M2)**
  - H^+-dependent uncoating
  - Influenza A only
- **Influenza A subtypes based on HA (16) and NA (9)**
  - H1N1, H3N2



Influenza Antigenic Variation

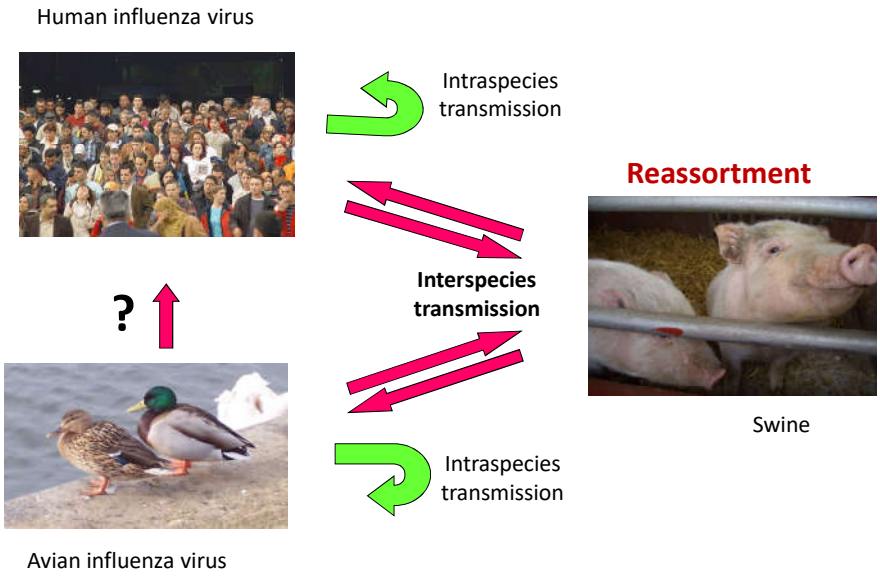
Antigenic drift

Occurs with influenza A, B, and C. Small number of slowly occurring changes (mutations); error-prone viral RNA polymerase. HA changes most prominent, but can occur in any viral gene. It is ppartially responsible for yearly vaccine changes.

Antigenic shift

- Influenza A only
- Large dramatic changes that occur rapidly
- Primarily responsible for pandemics
- Due to gene shuffling and reassortment
- Requirements:
  - ✓ Segmented genome
  - ✓ Multiple HA and NA subtypes
  - ✓ Animal reservoir (wild aquatic birds)
  - ✓ Susceptible species for both avian and human influenza (swine)

Pathways for generation of virulent pandemic influenza viruses



Pathogenesis and clinical picture

Aerogenically transmitted influenza viruses normally replicate in the mucosa of the nasopharynx, resulting in a pharyngitis or at most a tracheobronchitis, after an IP of 24–72 hours. Pulmonary dissemination of the infection can result from an upper respiratory infection or manifest without one, whereby the prognosis in the latter case is less favorable. Pneumonia caused solely by the influenza virus is rare. As a rule, bacterial superinfections with staphylococci, streptococci, pneumococci, or Haemophilus bacteria are responsible. These infections, which used to be the normal cause of influenza deaths (Haemophilus influenzae in the "Spanish flu" of 1918), can be controlled with antibiotics.

Diagnosis

Throat lavages and swabs provide suitable material. The latter must be placed in a suitable transport medium without delay to prevent them from drying out. Influenza viruses can be grown and isolated in cell cultures if the diagnostic specimen is obtained very early, i.e., in the first one or two days of the infection. Identification of the cultured viruses is achieved based on the hemagglutinating properties of the myxoviruses in the hemagglutination inhibition test or by means of immunofluorescence. If the specimen was obtained too late for virus isolation, a diagnosis can be arrived at by serological means, whereby a rise in the antibody titer of patient serum proves infection.

Classification and Antigen Structure of Influenza A Viruses

Viral prototype	Predominance	Antigen formula	
		Hemagglutinin (H)	Neuraminidase (N)
A/WS/33 A/PR8/34	1932–1946	HO	N1
A/Cambridge/46 A/F/M1/47	1946–1957	H1	N1
A/Singapore/57	1957–1968	H2	N2
A/Hong Kong/68	1968	H3	N2
A/USSR/77	1977	H1	N1

Epidemiology

Influenza A viruses are genetically variable. Slight antigenic changes are the general rule (antigenic drift, quasispecies) and are explained by selection of point mutants in the hemagglutinin under immunological pressure. More profound changes (antigenic shifts) explain the periodic occurrence of influenza A epidemics and pandemics.

Prevention and therapy

An inactivated adsorbate vaccine and some split vaccines (new: intranasal application) are available for influenza prophylaxis. The vaccine is recommended especially for persons whose occupation exposes them to such infections as well as persons with cardiovascular problems in their medical histories. The therapeutic options include amantadine, which inhibits the viral uncoating process, and more recently neuraminidase inhibitors. These substances shorten the duration of illness by blocking the release of the viruses from the host cells and their further dissemination in the body.

Bunyaviruses

The family Bunyaviridae comprises over 200 viral species, among them four human pathogen genera: Bunyavirus, Nairovirus, Phlebovirus, and Hantavirus. Bunyaviruses are spherical, 80–110 nm in size, and posses envelopes with spikes formed on membranes of the smooth endoplasmic reticulum. The genome consists of three antisense-strand RNA segments, whereby each segment produces a separate ribonucleoprotein complex, resulting in a unique feature of the virion: it contains three helical nucleocapsids.

Pathogenesis and Clinical Picture

- Genus Bunyavirus:

These viruses are transmitted by arthropods. They cause benign forms of encephalitis e.g California Encephalitis Virus.

- Genus Nairovirus:

  The main human pathogen in this group is the Crimean-Congo hemorrhagic fever virus, with lethality rate as high as 50%. The virus is endemic to southeastern Europe, Central Asia, China, Saudi Arabia, and Africa and is transmitted by ticks as well as by direct contact with infected animals or patients.

  - Genus Phlebovirus:

This group includes the pathogens that cause the benign Pappataci or phlebotomus fever ("sandfly fever"), which occurs in Europe (Italy, Yugoslavia), North Africa, Asia, and South America and is transmitted by the phlebotomus sandfly. Rift Valley fever (RVF), an acute, febrile disease, rarely also involving hemorrhagic fever, is transmitted by mosquitoes and is endemic to Africa, usually following epizooties in livestock, in which case aerosol infection occurs (slaughtering). Epidemics have been reported with over 200,000 cases in Egypt and 25,000 cases in Senegal. Further epidemics have occurred in Somalia, Kenya, and Sudan.

- Genus Hantavirus:

This genus includes several viral species (or serotypes) that can be classified into two groups according to the clinical symptoms they cause. The pathogens of nephropathica epidemica (NE), and hemorrhagic fever with renal syndrome (HFRS) and Hantavirus pulmonary syndrome (HPS).

The sources of infection are rodents (mice and rats). The infection is acquired by inhaling aerosols of urine, feces, and animal saliva. In NE and HFRS, a renal dysfunction follows the influenza like symptoms. HPS infection results in a rapidly progressive, acute dyspnea with pulmonary edema and is lethal in 60% of cases.

Serotypes of Hantaviruses

Serotype	Syndrome	Geographic dissemination
Hantaan	HFRS, severe form	Asia, southeastern Europe
Belgrade	HFRS, severe form	Southeastern Europe
Puumala	NE	Central and northern Europe
Seoul	HFRS, mild form	Worldwide
Sin Nombre, etc.	HPS	US, Canada

Diagnosis

It is possible to isolate the virus from blood, but the procedure is too drawn-out and costly for routine diagnostics. Serology (IgM detection) is the method of choice, although the results can be difficult to interpret with bunyaviruses due to the rapidly changing antigenic variants produced in many of the viral species.

Epidemiology and prevention

The bunyaviruses and phleboviruses are transmitted by bloodsucking arthropods, whereby the cycle involves either human and vector only or, as with the togaviruses and flaviviruses, a mammal- arthropod-mammal cycle actually independent of humans, and in which human victims represent a dead end for the infectious agent. Hantaviruses are transmitted aerogenically to humans from rodents, in which the viruses persist apathogenically for the lifespan of the animal.

Preventive measures include exposure prophylaxis (avoidance of insect bites and contact with rodents). An active vaccination is available for protection against Rift Valley fever.

Arenaviruses

Most members of the Arenaviridae family were first identified in the 1960s. The protoype arenavirus, the pathogen that causes lymphocytic choriomeningitis (LCM), was identified 30 years earlier, 1933. The human pathogens among the arenaviruses are the LCM virus (Europe, America), the Lassa virus (Africa), and the Junin and Machupo viruses (South America). All arenaviruses are spherical to pleomorphic. They consist of a “spiked” envelope derived from the plasma membrane, with an inner structure that appears to be granulated when viewed in ultrathin sections. It is to these granula the viral family owes its name (arenosus = sandy). They are considered to be host-cell ribosomes. The virion contains at least three strands of host RNA in addition to two viral RNA segments.

Ambisense Genome

The genome of the arenaviruses contains genomic components with sense (plus) polarity and others with antisense (minus) polarity (ambisense viruses, see p. 387) and is structured as follows: the smaller S part (S = small) codes in the 3′ part as an antisense-strand RNA for the nucleocapsid protein (NP) and in the 5′ part as sense-strand RNA for a viral glycoprotein. Each protein is translated separately from the subgenomic RNA; the NP, coded with the antisense orientation, is first transcribed into a sense-strand RNA. The L part (l = large) codes at the 3′ end in antisense-strand orientation for the viral polymerase and at the 5′ end in sense-strand orientation for a regulatory RNA-binding protein.

Pathogenesis and clinical picture

The source of nearly all human arenavirus infections is to be found in rodents. The virus enters the body per os, aerogenically or possibly also by skin contact. A pronounced viremia develops at first, followed by organ manifestations. In the case of LCM, these are normally harmless and flulike, although they can also develop into meningitis or encephalitis, in rare cases with a lethal outcome.

The Lassa virus is pantropic. It causes a hemorrhagic fever affecting nearly all inner organs and has a high rate of lethality. Death results from shock and anoxia

The clinical picture resulting from Junin and Machupo virus infections is similar to lassa virus. Compared to Lassa infections, CNS involvement is more frequent and the lethality rate is somewhat lower with these two viruses.

Diagnosis

In the acute stage, arenaviruses can be isolated from the patient's blood. Postmortem isolation is best done from liver tissue. In the hemorrhagic fevers, especially Lassa fever, the blood is highly infectious and handling it requires proper precautions and utmost care (aerosol formation!). Isolation of the virus is relatively easy in cell cultures. For reasons of safety, only special high-security laboratories are qualified to handle these organisms (e.g., at the Centers for Disease Control and Prevention in Atlanta, GA, USA). Serodiagnosis is also feasible using standard serological techniques.

Epidemiology and prevention

All arenaviruses are endemic to rodents and are transmitted to humans by these animals. No specific immunoprophylactic tools have been developed for any of these viruses. As far as exposure prophylaxis is concerned, it must be remembered that the LCM, Junin, and Machupo viruses are not transmitted among humans, but the Lassa virus is transmitted by this route. The most stringent precautions are therefore called for when treating Lassa patients. Healthcare staff must wear special clothing and facemasks and special reduced-pressure plastic tents are recommended as patient cubicles. The therapeutic tools available for treatment are ribavirin and human immunoglobulin.

Paramyxoviruses

This family includes the genera:

- Parmyxovirus with the parainfluenza viruses
- Rubulavirus with the mumps virus
- Morbillivirus with the measles virus
- Pneumovirus with the respiratory syncytial virus (RS)
- Nonclassified paramyxoviruses (Hendra, Nipah)

Family Paramyxoviridae

The family Paramyxoviridae is a heterogeneous, both in its biology and pathogenic properties. It is divided into two subfamilies:

- Paramyxovirinae
- Pneumovirinae

Paramyxovirinae with the genera Paramyxovirus with the human pathogen species parainfluenza virus types 1 and 3. Rubulavirus with the mumps virus and parainfluenza virus types 2 and 4. Morbillivirus with the human pathogen measles virus and several zoopathic species that cause severe respiratory infections in various animal species (dogs cats, cattle, seals, dolphins, turtles).The nonclassified, closely related zoopathic and human pathogen Hendra and Nipah viruses. Pneumovirinae, genus Pneumovirus, probably with several types of RS viruses (respiratory syncytial virus).

Structure of the Paramyxoviruses

All paramyxoviruses have a similar structure, they are pleomorphic. The smallest forms are 120–150 nm in size (with the exception of the somewhat smaller RS virus). They also possess an envelope that encloses the nucleocapsid. The envelope is derived from the cell membrane**.** The genome consists of a continuous, single-stranded antisense RNA. Various viral proteins are integrated in the envelope, visible in the form of spikes.

The generic taxons are based on these spikes:

- parainfluenza and mumps viruses have two types of spikes, one containing the hemagglutinin (i.e., possessing hemagglutination activity) coupled with neuraminidase (HN protein), and the other the so-called fusion (F) protein, responsible for fusion of the envelope with the cell membrane.
- Measles viruses contain no neuraminidase
- Pneumoviruses possess only the F protein

Pathogenesis and clinical picture

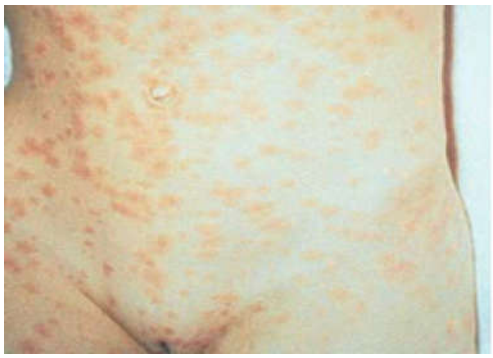
The parainfluenza viruses cause flulike infections, mainly in small children, which occasionally progress to bronchitis or even pneumonia. Occasionally, a dangerous croup syndrome develops. Bacterial superinfections are frequent, as are the usually harmless reinfections.

In mumps virus infections, the virus first replicates in the respiratory tract, then causes a viremia, after which a parotitis is the main development as well as, fairly frequently, mumps meningitis. Complications include infection of various glandular organs. Orchitis can occur in post puberty boys who contract mumps.

The pathogenesis of measles has not been fully explained. It is assumed that the virus, following primary replication in lymphoid tissues, is distributed hematogenously. Thereafter the oral mucosa displays an enanthem and the tiny white “Koplik’s spots”. Then the fever once again rises and the typical measles exanthem manifests. Possible complications include otitis in the form of a bacterial superinfection as well as pneumonia and encephalitis. A rare late sequel of measles (one case per million inhabitants) is subacute sclerosing panencephalitis (SSPE) in which nucleocapsids accummulate in brain cells, whereby few or

no viral progeny are produced for lack of matrix protein. This disease occurs between the ages of one and 20, involves loss of memory and personality changes, and usually results in death within six to 12 months.

Measles Exanthem



The typical exanthem manifests during what is presumably the Second hematogenous disseminative episode of the morbilliviruses

Nipah and Hendra virus infections are zoonoses endemic to Southeast Asia (Nipah) or Australia (Hendra). Both infections result in encephalitis with relatively high lethality rates (up to 40%) and in some cases severe interstitial pneumonias.

RS viruses cause bronchiolitis or pneumonia, mainly in children up to six months of age, or rarely up to two years. Immune status appears to play an important role in the course of the infection. It has been determined that the course of the disease is more severe in children who have received dead vaccine material (similarly to measles). This is presumably due to antibodies, in the case of small children the mother's antibodies acquired by diaplacental transport. Immunosuppressed patients, for instance, bone marrow recipients, are also at risk for RSV.

Diagnosis

- Serodiagnostic methods
- direct detection tests based on immunofluorescence or enzyme immunoassay
- Paramyxoviruses replicate readily in cell cultures from human tissues

Epidemiology

Paramyxoviruses are transmitted by droplet infection. Generalized contamination levels in the population (except for Nipah and Hendra) are already very high in childhood (90% in 10-year-old children for parainfluenza virus types 1–3). Nipah and Hendra viruses are zoonoses

that are transmitted to humans from animals (Nipah: pigs, Hendra: horses). Various different animals can be infected by these pathogens, but bats (Pteropus) appear to be the natural reservoir for both viruses.

Prevention

Attenuated live vaccines are available for measles and mumps. The dead vaccine should not be used due to the aggravating effect mentioned above. No vaccines have as yet been developed for the other parainfluenza viruses.

Rhabdoviruses

The rhabdoviruses of significance in human medicine are classified in seven genotypes. Type 1 is the classic, worldwide type that occurs in two forms: the "street virus" isolated from humans and animals and the "virus fixe" according to Pasteur. In 1882, Pasteur had transmitted the virus intracerebrally to rabbits. Following repeated passages of the virus in the rabbits, he had developed a dead vaccine type. Due to the brain-to-brain passages in the laboratory animals, the "virus fixe" became so highly adapted to brain tissue that it was unable to replicate in extraneural tissues. Types 2–4 were isolated from African bats, types 5 and 6 from European bats, and type 7 from Australian bats.

Rhabdoviruses are rodlike with one end flat and one end rounded ("bulletshaped") and a spiked envelope surrounding a nucleocapsid similar to that of the myxoviruses. The genome consists of antisense strand RNA

Pathogenesis and clinical picture

Rabies viruses are almost always transmitted by the bite, sometimes also the scratch, of a rabid animal. The virus at first replicates at the portal of entry in muscle and connective tissue, then wanders along the nerve cells into the CNS, where more viral replication takes place. Using the same route, the virus then disseminates from the CNS into peripheral organs, above all the salivary glands, cornea, and kidneys. The primary clinical picture is anencephalitis with lethal outcome for humans and animals.

Clinical Course of Rabies

The disease goes through three stages. The initial, or prodromal, stage involves itching and burning at the portal of entry (bite wound), nausea, vomiting, and possibly a melancholy mood. In the second or excitative stage, cramps and spasms of the pharynx and larynx are the main symptoms, rendering swallowing very painful. The spasms can be induced by the mere sight of water ("hydrophobia"). Other mild acoustic and visual stimuli may elicit exaggerated reactions including attacks of cramps and violent anger, hitting, biting, and screaming. Death occurs within three to four days at the earliest. The third, paralytic, stage may develop instead of early death, with ascending paralysis and asphyxia, leading to exitus. Therapy is exclusively symptomatic. Since the patient experiences the disease in a fully conscious state, most of the medication serves to alleviate the pain and anxiety states. The disease runs essentially the same course in humans and animals, whereby the behavior of animals is often radically altered: wild animals lose their fear of humans and tame pets become aggressive. Rabies with the excitative stage is known as "furious rabies," without it as "dumb rabies."

Diagnosis

It is usually made on the basis of clinical signs. There are no tests to confirm rabies with absolute certainty while the patient is still alive. Viruses can be detected by laboratory investigation of the patient's brain after death. The test is not usually carried out in countries with few resources.

Epidemiology

Lyssavirus type 1 is endemic to North America and Europe in wild animals (sylvatic rabies) and in certain tropical areas in domestic pets as well, in particular dogs (urban rabies). The reservoir for the remaining lyssavirus types are bloodsucking (hemovorous) as well as fructivorous and insectivorous bats. The virus is excreted with the saliva of the diseased or terminal incubator animal and enters other animals or humans through scratch or bite wounds. The virus is highly labile, so transmission on contaminated objects is very rare. Human-to-human transmission has not been confirmed with the exception of cases in which rabies in corneal donors had gone unnoticed.

What you should do if someone is bitten by a dog?

- First aid
- Post-exposure prophylaxis

Why first aid and PEP?

Wound care and anti-rabies treatment after a dog bite can reduce the occurrence of rabies in a bitten person by up to 90%.

First aid

Immediately after a dog bite, you should thoroughly clean and flush the wound with soap and water, detergent, or a substance that kills viruses, such as 70% alcohol, tincture of aqueous solution of iodine, or povidone iodine. Continue flushing the wound for at least 15

minutes. The wound should not be sutured (stitched) unless this is essential to stop heavy bleeding. If stitches are required, the wound should not be sutured until after post-exposure prophylaxis has occurred.

Post-exposure prophylaxis

If a person is bitten by a dog in countries where rabies is endemic, there is no way of being certain that the animal is free from rabies. The bitten person should be given post-exposure prophylaxis as soon as possible after the bite. Every year, around 15 million people receive this treatment worldwide, preventing an estimated 327,000 human deaths from rabies

WHO PEP Guidelines

WHO has published the guidelines for PEP following different levels of contact with a suspected/confirmed rabid animal (WHO. Available at http://www.who.ch/programmes/emc/vph.htm)

Category	Type of Contact with a Suspect or Confirmed Rabid Domestic or Wild Animal, or Animal Unavailable for Observation	Recommended Rx
I	Touching or feeding of animals; licks on intact skin	None, if reliable case history is available
II	Nibbling of uncovered skin; minor scratches or abrasions without bleeding; licks on broken skin	Administer vaccine immediately; stop treatment if animal remains healthy throughout an observation Period of **10 days** or if animal is euthanized and found to be negative for rabies by appropriate lab. techniques
III	Single or multiple transdermal bites or scratches; contamination of mucous membrane with saliva (i.e licks)	**Administer rabies Ig** and **vaccine** immediately; stop treatment if animal remains healthy throughout an observation period of **10 days** or if animal is killed humanely and found to be negative for rabies by appropriate lab. techniques

Pre-exposure Prophylaxis

It is indicated for those with a relatively high risk of rabies exposure; includes veterinarians, workers in laboratories using the virus, spelunkers, and those planning to visit countries with a high prevalence of dog rabies for more than 30 days.

Rabies vaccines

- Two types of vaccines exist
- Older type
    - Made from nerve tissue infected with rabies virus
    - Given intramuscularly (IM)
- Newer type
    - Made from virus-infected cells grown (cultured) in the laboratory
    - Safer and more effective
- WHO recommends that the rabies cell-culture vaccine (rabies CCV) should be used in preference to the nerve tissue vaccine wherever possible
- Rabies CCV can be given either by intramuscular injection, or intradermally (ID) into the upper arm
- Intradermal route has been shown to be as safe and effective as the traditional intramuscular route, and is cheaper because it requires less vaccine
- Modern CCVs should be given in four or five doses
- Dosage for each injection depends on the vaccine type and the route of administration
    - Intramuscular dosages are 0.5 ml or 1.0 ml
    - Intradermal dosage is only 0.1 ml
- For rabies PEP, you should tell the patient and their family that it is *essential* to return at fixed intervals for repeat vaccinations in order to prevent rabies
- The intramuscular regimen is given at days 0, 3, 7, 14 and 28 after the exposure
- The intradermal regimen is given at days 0, 3, 7, 28 and 90 after exposure
- There is also a rabies vaccine which is administered at days 0, 7 and 28 days (IM), and rabies duck embryo vaccine, which is administered at day 0, 7 and 21 days (IM or ID)
    - For both these vaccines, a booster dose is given after two to three years

Rabies Ig

- Specific protection in humans with Category III exposure is provided by injecting a human or equine (horse) immunoglobulin at the site of the bite, as soon as possible after exposure to neutralize the virus
- The term 'immunoglobulin' refers to a preparation of antibodies made either in humans or in horses who have been vaccinated against rabies
- Antibodies from their blood which attack the rabies viruses are harvested and stabilized in an injectable liquid
- As much as possible of the dose of rabies immunoglobulin is given into, or as near as possible to, the site of the bite
- Human rabies Ig is given in a single dose of 20 international units (IU) per kilogram (kg) of the person's body weight
- Horse rabies Ig is cheaper, but less effective and more likely to produce adverse allergic reactions; it is given in a single dose of 40 IU/kg

Prevention of rabies

- Prevention is the best alternative
- The main prevention measures
  - Controlling the animals that transmit the virus
  - Educating the community on how to protect themselves from dog bites, and What action to take if they are bitten

Filoviruses (Marburg and Ebola Viruses)

- The Marburg virus was isolated for the first time in 1967 as a result of three simultaneous outbreaks among laboratory staff in Marburg, Frankfurt, and Belgrade.
- The infection victims had been processing the organs of Cercopithecus (African green monkeys) from Uganda
- Both the Marburg and Ebola viruses are threadlike, 14 µm-long viral particles, in some cases branched and 80 nm thick in diameter
- Their surface consists of an envelope of host-cell membrane with viral spikes.
- The genome consists of antisense strand RNA in a helical nucleocapsid 50 nm in diameter.

Pathogenesis and clinical picture

- The Marburg and Ebola viruses cause so called hemorrhagic fevers
- The clinical picture first manifests with fever, headache, and neck pain, conjunctivitis and diarrhea, followed by hepatic, renal, and CNS involvement and finally, as a result of consumption coagulopathy, leads to extensive hemorrhaging and terminal shock
- In terms of the anatomical pathology, nearly all organs show hemorrhages and fibrin deposits.

Diagnosis

- Only designated laboratories with special safety facilities can undertake isolation work on these viruses
- Detection is either in blood with an electron microscope or using immunofluorescence on tissue specimens
- The pathogens can be grown in cell cultures.
- Serodiagnosis is also possible.

Epidemiology and prevention

- The reservoir of the Marburg and Ebola viruses is unknown
- Subsequent to the Marburg outbreak in 1967 among lab personnel in Europe, Marburg viruses have only been found in Africa.
- The Ebola virus, named after a river in Zaire, has caused several outbreaks in Africa since 1976 in which lethality rates of 50–90% were observed.
- Imported Ebola infections have also been seen in monkey colonies in the USA and Italy.
- Protective suits and vacuum-protected plastic tents are no longer recommended for healthcare workers in contact with Marburg and Ebola patients (as with Lassa fever),

since interhuman transmission is by excretions (smear infection) and in blood, but not aerogenic.

- Despite this fact, the high level of infectivity of any aerosols from patient material must be kept in mind during laboratory work and autopsies.