Semester – II

I7MBP201 **VIROLOGY** **4H – 4C**

Instruction Hours / week: L: 4 T: 0 P: 0 **Marks:** Internal: **40** External: **60** Total: **100**

End Semester Exam: 3 Hours

SCOPE

Virologist are highly demanded in the medical research companies, Pharmaceutical companies, governmental agencies, laboratory testing companies, or cancer treatment or research companies depending upon the specialization

OBJECTIVES

Virology, often considered a part of microbiology or of pathology, is the study of biological viruses and virus like agents: their structure, classification and evolution, their ways to infect and exploit cells of virus reproduction, the disease they cause, the techniques to isolate and culture them and their potential uses in research and therapy.

UNIT – I

Historical perspective of virology - Scope of virology -Viral classification and properties of viruses – Replication of viruses, cultivation of viruses (animal inoculation, Embryonated egg and tissue culture) - properties of viroids and Prions.

UNIT – II

Animal viruses- DNA viruses - morphology, replication, pathogenesis and laboratory diagnosis of Pox virus, Adeno virus, Hepatitis viruses – type A,B and D. Herpes simplex viruses, oncogenic viruses- Papova virus,- oncogenes and Oncogenesis.

UNIT – III

Animal viruses - RNA viruses - morphology, replication, pathogenesis and laboratory diagnosis of Poliovirus. Rabies virus, Influenza virus, mumps virus, Measles virus and rubella virus, Retro virus - HIV virus. Dengue and Japanese Encephalitis, SARS, Swine Flu.

UNIT – IV

Plant viruses – RNA viruses – TMV, Cowpea mosaic virus, Bromomosaic viruses, Satellite viruses – Double stranded DNA viruses - CaMV – Single stranded DNA viruses – Gemini virus. Structure and replication of Bacteriophage (T4) – Filamentous phage (ΦX174).

UNIT – V

Nosocomial infections, viral syndromes. Viral vaccines-interferons - Antiviral drugs - strategies to develop AIDS vaccines - Rabies vaccines preparation (animal and cell culture) and their immunization.

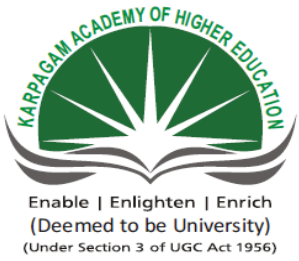
SUGGESTED READINGS

TEXT BOOKS

1. Ananthanarayanan, R., and Panicker, C.K.J., (2005). *Text book of Microbiology*. (7th ed.). Orient Longman, New Delhi.
2. Carter, J., and Saunders, V., (2007). *Virology: Principles and Applications*. (1st ed). Wiley.
3. Chakraborty, P. (2003). *A Text book of Microbiology*. (2nd ed.). New Central Book Agency (P) Ltd, Calcutta.
4. Dubey, R.C., and Maheswari, D.K., (2004). *A Text book of Microbiology*. (1st ed.). S. Chand and Company Ltd, New Delhi.
5. Pelczar, Jr. M.J., Chan, E.C.S., and Kreig, K.R., (2003). *Microbiology.* (5th ed.). Tata McGraw-Hill Publishing Company, New Delhi.

REFERENCES

1. Acheson, N.H. (2006). *Fundamentals of Molecular Virology*. Wiley publication.
2. Cann, A.J. (2005). *Principles of Molecular Virology*, Academic Press.
3. Dimmock, N.J., Easton, A.J., and Leppard, K.N., (2007). *Introduction to Modern Virology*, (6th ed.). Blackwell Scientific Publications, Oxford, UK.
4. Flint, S.J., Racaniello, V.R., Enquist, L.W., Rancaniello, V. R., and Skalka, A. M., (2003). *Principles of Virology: Molecular Biology, Pathogenesis, and Control of Animal Viruses*. American Society Microbiology.
5. Jawetz, E., Melnic, J.L, and Adelberg, E.A., (2001). *Review of Medical Microbiology*. (22nd ed.). Lange Medical Publishers, NY.
6. Levy, J. A., Fraenkel-Conrat, H., and Owens, O. S., (1994).*Virology*. (3rd ed.). Benjamin Cummings.
7. Knipe D.M., Howley P.M., and Griffin D.E., (2006). *Fields Virology*. (5th ed). Vols - I,II. Lippincott, Williams & Wilkins.
8. Prescott, M., Harley, J.P., and Klein, D.A., (2007). *Microbiology*. (7th ed.). McGraw-Hill Inc. New York.
9. White, D. O., and Fenner, F.J., (1994). *Medical Virology*, (4th ed.). Academic Press, New York.



KARPAGAM ACADEMY OF HIGHER EDUCATION

(Deemed to be University Established Under Section 3 of UGC Act 1956)

Coimbatore – 641 021.

LECTURE PLAN
DEPARTMENT OF MICROBIOLOGY

STAFF NAME: Dr. A. A. ARUNKUMAR
SUBJECT NAME: VIROLOGY SUB.CODE:17MBP201
SEMESTER: II CLASS: I M.Sc. (MB)

S. No	Lecture Duration Period	Topics to be Covered	Support Material/Page Nos
		UNIT-I	
1	1	History of virology	T1: 427, W1
2	1	Scope of virology	W1
3	1	Viral classifications and properties	T1: 427 - 430
4	1	Replication of virus	T1: 431 - 434
5	1	Cultivation of virus	T1: 434 – 436
6	1	Animal inoculation	R1- 189
7	1	Embryonated egg	R1: 189 - 190
8	1	Tissue culture	R1: 190
9	1	Properties of viroids and Prions	T1: 442
10	1	Revision	
11	1	Possible Questions discussions	
12	1	One mark discussion	
13	1	Class test	
	Total No of Hours Planned For Unit 1=13		
		UNIT-II	
1	1	Animal DNA Virus	T1: 427
2	1	DNA virus – Morphology	T1: 428
3	1	DNA virus – replication	T1: 431
4	1	DNA virus – pathogenesis	T1: 431
5	1	Laboratory diagnosis of Pox virus	T1: 461
6	1	Laboratory diagnosis of Adenovirus	T1: 480
7	1	Laboratory diagnosis of Hepatitis virus type A, B & D	T1: 540
8		Laboratory diagnosis of Herpes simplex virus	T1: 466
9	1	Laboratory diagnosis of Oncogenic virus, Papova virus	T1: 466
10	1	Oncogenes and Oncogeneisis	T1:564
11	1	Revision, Class test	
12	1	Possible Questions discussions, One mark discussion	
	Total No of Hours Planned For Unit II = 12		

		UNIT-III	
1	1	Animal RNA Virus	T2: 61 – 67
2	1	RNA virus – Morphology	T2: 61 – 67
3	1	RNA virus – replication	T2: 60
4	1	RNA virus – pathogenesis	T2: 68
5	1	Laboratory diagnosis of Polio virus & Rabies virus	J1
6	1	Laboratory diagnosis of Influenza virus & Mumps virus	T2: 183
7	1	Laboratory diagnosis of Measles virus & Rubella virus	W1
8	1	Laboratory diagnosis of Retro virus, HIV virus	T2: 207
9	1	Dengue and Japanese Encephalitis	W1
10	1	SARS and Swine flu	W1
11	1	Revision	
12	1	Possible Questions discussions, One mark discussion	
13	1	Class test	
	Total No of Hours Planned For Unit III=13		
		UNIT-IV	
1	1	Plant virus – RNA virus	R2: 1 – 4
2	1	TMV, Cowpea, mosaic virus	R2: 37, W1
3	1	Bromomosaic, Satellite virus	W1
4	1	Double stranded DNA virus- CAMV	R2: 499
5	1	Single stranded DNA virus – Gemini	R2: 499
6	1	Structure and replication of T4	R2 – 484
7	1	Structure and replication of filamentous phage (ϕx174)	R2: 484
8	1	Revision	
9	1	Possible Questions discussions	
10	1	One mark discussion	
11	1	Class test	
	Total No of Hours Planned For Unit IV=11		
		UNIT-V	
1	1	Nosocomial infections	W1, T2: 285
2	1	Viral syndromes	W1, T2: 297
3	1	Viral vaccines – interferons	W1, T2: 305 – 313
4	1	Antiviral drugs	W1, T2: 315 – 325
5	1	Strategies to develop AIDS vaccines	W1, T2: 322
6	1	Rabies vaccines preparations	W1, T2: 327
7	1	Rabies vaccines immunizations	W1, T2: 329
8	1	Revision	
9	1	Possible Questions discussions	
10	1	One mark discussion	
11	1	Class test	
12	1	Discussion of Previous ESE Question Papers.	
	Total No of Hours Planned for unit V=12		
	Total No of Hours Planned = 60		

TEXT BOOKS

1. Ananthanarayanan, R., and Panicker, C.K.J., (2005). *Text book of Microbiology*. (7th ed.). Orient Longman, New Delhi.
2. Carter, J., and Saunders, V., (2007). *Virology: Principles and Applications*. (1st ed). Wiley.
3. Chakraborty, P. (2003). *A Text book of Microbiology*. (2nd ed.). New Central Book Agency (P) Ltd, Calcutta.
4. Dubey, R.C., and Maheswari, D.K., (2004). *A Text book of Microbiology*. (1st ed.). S. Chand and Company Ltd, New Delhi.
5. Pelczar, Jr. M.J., Chan, E.C.S., and Kreig, K.R., (2003). *Microbiology*. (5th ed.). Tata McGraw-Hill Publishing Company, New Delhi.

REFERENCES

1. Acheson, N.H. (2006). *Fundamentals of Molecular Virology*. Wiley publication.
2. Cann, A.J. (2005). *Principles of Molecular Virology*, Academic Press.
3. Dimmock, N.J., Easton, A.J., and Leppard, K.N., (2007). *Introduction to Modern Virology*, (6th ed.). Blackwell Scientific Publications, Oxford, UK.
4. Flint, S.J., Racaniello, V.R., Enquist, L.W., Rancaniello, V. R., and Skalka, A. M., (2003). *Principles of Virology: Molecular Biology, Pathogenesis, and Control of Animal Viruses*. American Society Microbiology.
5. Jawetz, E., Melnic, J.L, and Adelberg, E.A., (2001). *Review of Medical Microbiology*. (22nd ed.). Lange Medical Publishers, NY.
6. Levy, J. A., Fraenkel-Conrat, H., and Owens, O. S., (1994). *Virology*. (3rd ed.). Benjamin Cummings.
7. Knipe D.M., Howley P.M., and Griffin D.E., (2006). *Fields Virology*. (5th ed). Vols - I, II. Lippincott, Williams & Wilkins.
8. Prescott, M., Harley, J.P., and Klein, D.A., (2007). *Microbiology*. (7th ed.). McGraw-Hill Inc. New York.
9. White, D. O., and Fenner, F.J., (1994). *Medical Virology*, (4th ed.). Academic Press, New York.

WEBSITES

W1: www.wikipedia.org

JOURNALS

J1: Lakshmi et al (2013) Detection of RNA Virus current technologies and future perspectives.

CLASS: I MSc MB **COURSE NAME: VIROLOGY**

COURSE CODE: 17MBP201 **UNIT: I**

Historical perspective of virology - Scope of virology -Viral classification and properties of viruses – Replication of viruses, cultivation of viruses (animal inoculation, Embryonated egg and tissue culture) - properties of viroids and Prions

General Concepts - Virus history

The history of virology goes back to the late 19th century, when German anatomist **Dr Jacob Henle** (discoverer of Henle's loop) hypothesized the existence of infectious agent that were too small to be observed under light microscope. This idea fails to be accepted by the present scientific community in the absence of any direct evidence. At the same time three landmark discoveries came together that formed the founding stone of what we call today as **medical science**. The first discovery came from **Louis Pasture** (1822-1895) who gave the spontaneous generation theory from his famous swan-neck flask experiment. The second discovery came from **Robert Koch** (1843-1910), a student of Jacob Henle, who showed for first time that the anthrax and tuberculosis is caused by a bacillus, and finally **Joseph Lister** (1827-1912) gave the concept of sterility during the surgery and isolation of new organism.

The history of viruses and the field of virology are broadly divided into three phases, namely discovery, early and modern.

The discovery phase (1886-1913)

In 1879, **Adolf Mayer,** a German scientist first observed the dark and light spot on infected leaves of tobacco plant and named it **tobacco mosaic disease**. Although he failed to describe the disease, he showed the infectious nature of the disease after inoculating the juice extract of diseased plant to a healthy one. The next step was taken by a Russian scientist **Dimitri Ivanovsky** in 1890, who demonstrated that sap of the leaves infected with tobacco mosaic disease retains its infectious property even after its filtration through a Chamberland filter. The third scientist who plays an important role in the development of the concept of viruses was **Martinus Beijerinck** (1851-1931), he extended the study done by Adolf Mayer and Dimitri Ivanofsky and showed that filterable agent form the infectious sap could be diluted and further regains its strength after replicating in the living host; he called it as ***"contagium vivum fluidum"***. **Loeffler** and **Frosch** discovered the first animal virus, the foot and mouth disease virus in 1898 and subsequently **Walter Reed** and his team discovered the yellow fever virus, the first human virus from Cuba in1901. Poliovirus was discovered by **Landsteiner** and **Popper** in 1909 and two years later **Rous** discovered the solid tumor virus which he called Rous sarcoma virus.

The early phase (1915-1955)

In 1915, **Frederick W. Twort** discovered the phenomenon of transformation while working with the variants of vaccinia viruses, simultaneously **Felix d'Herelle** discovered **bacteriophage and** developed the assay to titrate the viruses by plaques. **Wendell Stanley** (1935) first crystallized the TMV and the first electron micrograph of the tobacco mosaic virus (TMV) was taken in 1939. In 1933 **Shope** described the first papillomavirus in rabbits. The vaccine against yellow

fever was made in 1938 by **Thieler** and after 45 years of its discovery, polio virus vaccine was made by **Salk** in 1954.

The modern phase (1960-present)

During this phase scientist began to use viruses to understand the basic question of biology. The superhelical nature of polyoma virus DNA was first described by **Weil** and **Vinograd** while **Dulbecco** and **Vogt** showed its closed circular nature in 1963. In the same year **Blumberg** discovered the hepatitis B virus. **Temin** and **Baltimore** discovered the retroviral reverse transcriptase in 1970 while the first human immunodeficiency virus (HIV) was reported in 1983 by **Gallo** and **Montagnier**. The phenomenon of RNA splicing was discovered in Adenoviruses by **Roberts**, **Sharp**, **Chow** and **Broker**. In the year 2005 the complete genome sequence of 1918 influenza virus was done and in the same year hepatitis C virus was successfully propagated into the tissue culture.

Many discoveries are done using viruses as a model. The transcription factor that binds to the promoter during the transcription was first discovered in SV40. The phenomenon of polyadenylation during the mRNA synthesis was first described in poxviruses while its presence was first reported in SV40. Many of our current understanding regarding the translational regulation has been studied in poliovirus. The oncogenes were first reported in Rous sarcoma virus. The p53, a tumor suppressor gene was first reported in SV40.

Important discoveries

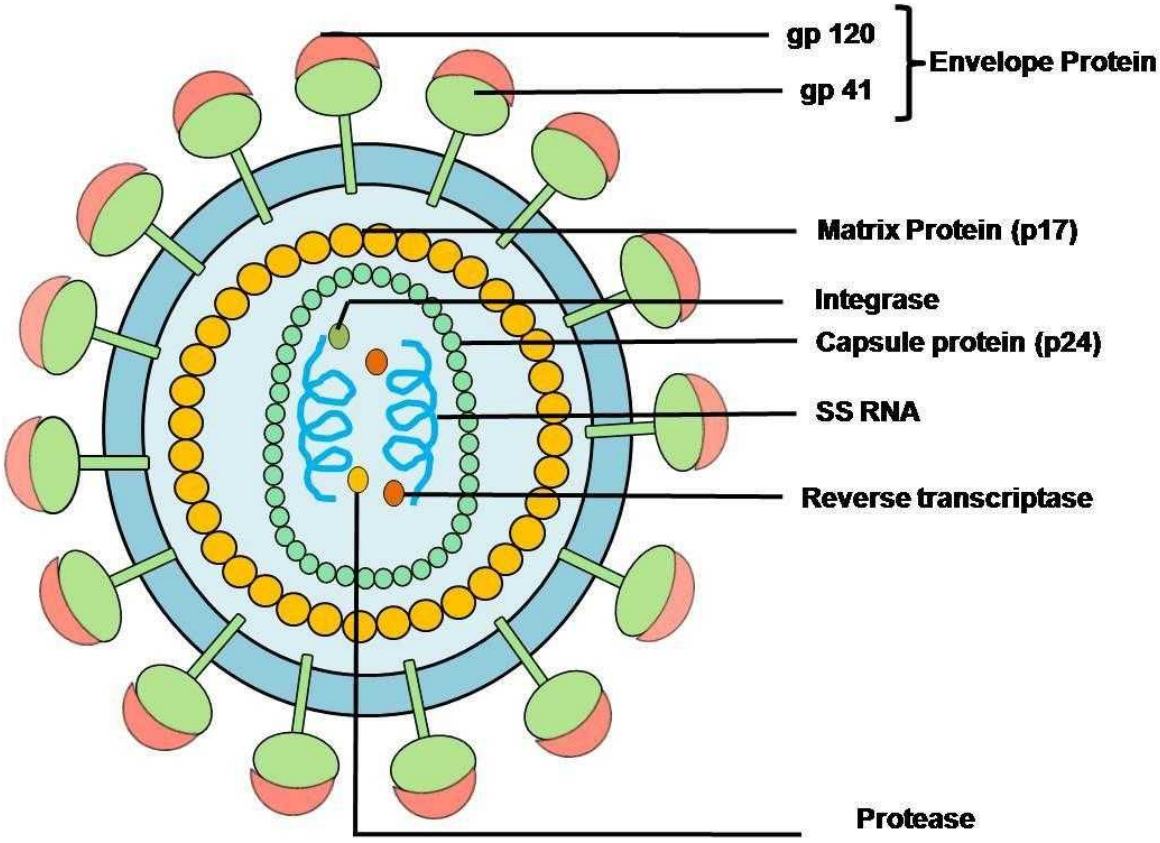
Date	Discovery
1796	Cowpox virus used to vaccinate against smallpox by Jenner.
1892	Description of filterable infectious agent (TMV) by Ivanovsky.
1898	Concept of the virus as a contagious living form by Beijerinck.
1901	First description of a yellow fever virus by Dr Reed and his team.
1909	Identification of poliovirus by Landsteiner and Popper.
1911	Discovery of Rous sarcoma virus.
1931	Virus propagation in embryonated chicken eggs by Woodruff and Goodpasture.
1933	Identification of rabbit papillomavirus.
1936	Induction of carcinomas in other species by rabbit papillomavirus by Rous and Beard.
1948	Poliovirus replication in cell culture by Enders, Weller, and Robbins.
1952	Transduction by Zinder and Lederberg.
1954	Polio vaccine development by Salk.
1958	Bacteriophage lambda regulation paradigm by Pardee, Jacob, and Monod.
1963	Discovery of hepatitis B virus by Blumberg.
1970	Discovery of reverse transcriptase by Temin and Baltimore.
1976	Retroviral oncogenes discovered by Bishop and Varmus.
1977	RNA splicing discovered in adenovirus.
1983	Description of human immunodeficiency virus (HIV) as causative agent of acquired immunodeficiency syndrome (AIDS) by Montagnier, Gallo)
1997	HAART treatment for AIDS.
2003	Severe acute respiratory syndrome (SARS) is caused by a novel coronavirus.
2005	Hepatitis C virus propagation in tissue culture by Chisari, Rice, and Wakita.
2005	1918 influenza virus genome sequencing.

Virus diversity

Viruses are minute, non-living entities that copy themselves once inside the living host cells. All living organisms (animals, plants, fungi, and bacteria) have viruses that infect them. Typically viruses are made up of coat (or capsid) that protects its information molecule (RNA or DNA); these information molecules contain the blue prints for making more virus. The viruses are highly diverse in their shape, size, genetic information, and infectivity. Viruses are all around us, on an average a human body encounters billion virus particles every day. Our intestinal, respiratory, and urogenital tract are reservoirs for many different kinds of viruses, it is astonishing that with such constant exposure, there is little or no impact of these organisms in human health. The host defense mechanism is quite strong to remove all these in normal condition, while they cause many nasty diseases only when the person is **immune-compromised**. Although viruses have a limited host range but sometimes they may jump the species barrier and causes fatal disease, recent spread of **swine influenza** is an ideal example of such kind of spread.
The **epidemic** viruses, such as influenza and severe acute respiratory syndrome (SARS), cause diseases that rapidly spread to a large human population within no time, and seem to attract more scientific and public attention than do **endemic** viruses, which are continually present in a particular population.

Virology as a discipline is merely 100 years old and the way it expanded in this small period of time is rampant. To group the new emerging viruses in a specific group by specifying certain parameters was initiated in 1966 when international committee on the taxonomy of viruses (ICTV) was formed with the aim to classify the viruses. The ICTV has adopted a norm for the description of the viruses. Name for genera, subfamilies, families, and orders must all be a single word, ending with the suffixes **-virus**, **-virinae**, **-viridae**, and **-virales** respectively. In written usage, the name should be capitalized and italicized.
Viruses are **obligate parasite** which means their absolute dependence on living host system. This property of virus made it a valuable tool to study cell functions and its biology. **Adenovirus** is an example of DNA virus that enters the host nucleus but remains separated from the host genome and at the same time use host cell machinery for its replication. On the other hand **influenza** is a RNA virus that carries its own enzyme to replicate its genome while the viral proteins are synthesized by using the host cell machinery. Human immunodeficiency virus (HIV) is a **retrovirus**; it contains RNA as a genetic material but it converts into DNA after entering the host cell by an enzyme called **reverse transcriptase**. It also contains enzymes in its virion namely, **integrase** and viral **protease** which helps HIV during maturation process inside the infected cells. Outer surface of HIV virion contains two surface glycoproteins called as **gp120** and **gp41** which helps in the attachment of virus to the cell surface.

Figure 2.1. Schematic diagram of HIV



Virus Shapes

Early study with tobacco mosaic virus (TMV) strongly suggested that viruses were composed of repeating subunits of protein which was later supported by crystallization of TMV. A major advancement in determining the morphology of virus was the development of negative stain **electron microscopy**. Another modification of classical electron microscopy is **cryo-electron microscopy** where the virus containing samples were rapidly frozen and examined at a very low temperature; this allows us to preserve the native structure of the viruses.

A **virion** is a complete virus particle that is surrounded by the capsid protein and encapsidates the viral genome (DNA or RNA). Sometime structure without nucleic acid can be visible under the electron microscope those structures are called as **empty capsids**. In some of the viruses like paramyxoviruses the nucleic acid is surrounded by the capsid proteins and the composite structures are referred as **nucleocapsid**. Some of the viruses contain the lipid **envelope** which surrounds the nucleocapsids. The envelopes are derived from the **host cell membrane** during the budding process. As the envelopes are derived from host cell membrane they contain many of the surface proteins present in the host cells.

There are two kinds of symmetry found among the viruses: **icosahedral** and **helical**. In theory the icosahedral symmetry may sometime referred as **spherical** based on the external morphology. **Icosahedral** symmetry has **12 vertices**, **30 edges**, and **20 faces**. They also have two, three, or five fold symmetry based on the rotation through axes passing through their edges, faces, and vertices respectively (Figure 3.1). The viruses of this kind look **spherical** in shape. In **helical** symmetry the genomic RNA forms a spiral within the core of the nucleocapsids (Figure 3.2). The viruses of this kind look **rodlike** or **filamentous**. The viruses which contain large DNA genomes are more complex in structure, for example- poxviruses and herpesviruses.

Figure 3.1. An icosahedral virion structure showing two, three, and fivefold symmetry

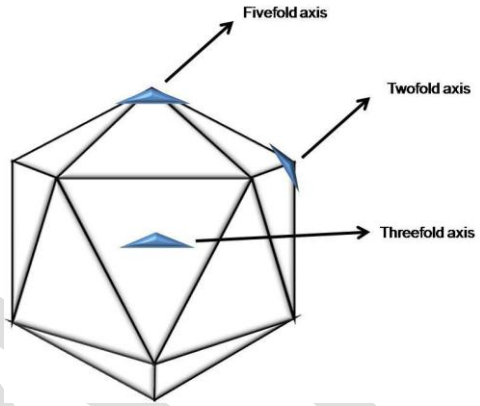


Figure 3.2. Virus structure with helical symmetry.

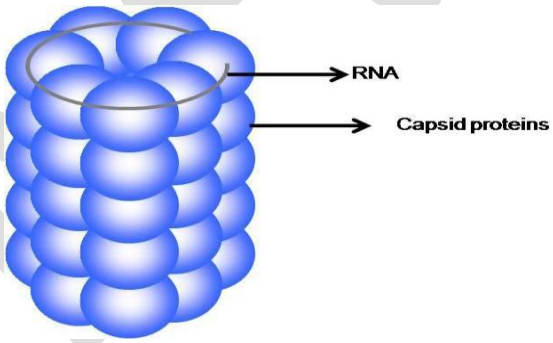


Table 3.1. Shape of viruses belonging to different families

Family	Shape
Poxviridae	Pleomorphic
Iridoviridae	Icosahedral
Asfarviridae	Spherical
Herpesviridae	Icosahedral
Adenoviridae	Icosahedral
Polyomaviridae	Icosahedral
Papillomaviridae	Icosahedral
Hepadnaviridae	Spherical
Circoviridae	Icosahedral
Parvoviridae	Icosahedral
Retroviridae	Spherical
Reoviridae	Icosahedral
Birnaviridae	Icosahedral
Paramyxoviridae	Pleomorphic
Rhabdoviridae	Bullet shaped
Filoviridae	Filamentous
Bornaviridae	Spherical
Orthomyxoviridae	Pleomorphic
Bunyaviridae	Spherical
Arenaviridae	Spherical
Coronaviridae	Spherical
Arteriviridae	Spherical
Picornaviridae	Icosahedral
Caliciviridae	Icosahedral
Astroviridae	Icosahedral
Togaviridae	Spherical
Flaviviridae	Spherical

Virus Size

Viruses are generally much smaller than the bacteria and its average size varies from 25- 300 nm in diameter. They are visible under electron microscope and only the largest and complex viruses are seen under light microscope with high resolution. Among all, the smallest viruses belong to the families ***Circoviridae***, ***Parvoviridae*** and ***Picornaviridae*** which measure about 20 - 30 nm in diameter while the largest one belongs to ***Poxviridae*** that measures around 250-300 nm in diameter. Recently, scientists isolated a new form of virus that infects amoeba and grouped it under a separate family ***Mimiviridae***. The members of the family *Mimiviridae* range from 400-800 nm in diameter. On an average a bacterial cell is about 1400 nm in diameter while an average epithelial cell is about 20,000 nm. Considering both viruses and bacteria to be nearly spherical a bacterial cell has a volume about 30,000 times greater than a virus while an epithelial cell is about 60 million times larger.

Table 4.1. Size of viruses belonging to different families

Family	Size (nm)
Poxviridae	300
Iridoviridae	135-300
Asfarviridae	170-220
Herpesviridae	150
Adenoviridae	80-100
Polyomaviridae	40-50
Papillomaviridae	55
Hepadnaviridae	50
Circoviridae	12-27
Parvoviridae	15-25
Retroviridae	80-100
Reoviridae	60-80
Birnaviridae	60
Paramyxoviridae	150-250
Rhabdoviridae	100
Filoviridae	80
Bornaviridae	80-100
Orthomyxoviridae	80-120
Bunyaviridae	80-120
Arenaviridae	50-280
Coronaviridae	120-150
Arteriviridae	60-70
Picornaviridae	30
Caliciviridae	30-40
Astroviridae	30
Togaviridae	70
Flaviviridae	40-60

Introduction to Virology

Background/Discovery

The concept behind modern virology can be traced back to Adolf Mayer, Dimitri Ivanofsky and Martinus Beijerinck who, independently in the late 1880's, discovered what was later to be called tobacco mosaic virus (TMV). Their discoveries led to the descriptions of filterable agents, too small to be seen with the light microscope, that could be grown in living cells and cause disease. The first filterable agent from animals, foot and mouth disease virus, was described by Loeffler and Frosch in 1898 and the first human filterable agent discovered was yellow fever virus, discovered by Walter Reed in 1901. The term 'virus' derives from the Latin for slimy liquid or poison and was gradually introduced during this period to replace the term 'filterable agents'.

The first virus to be visualized by x-ray crystallography and electron microscopy was TMV, reported in 1941 and 1939, respectively. These advances introduced the notion that viruses were structurally composed of repeating subunits.

Frederick Twort and Felix d'Herelle, working independently, are credited with the discovery of viruses which could infect and lyse bacteria in 1915. D'Herelle introduced the term 'bacteriophages' for these agents and also described the concepts of virus adsorption to its target, cell lysis and release of infectious particles. Over the next 35-40 years, work with phages led to numerous discoveries including how the introduction of DNA into a target cell could reproduce itself and the regulation of cellular macromolecular synthesis directed by viruses. In essence, the field of molecular biology was opened up during this period.

Advances in animal virology were noted throughout the 20th century but the major breakthrough came through the development of tissue culture systems that led, for example, to the isolation of poliovirus by Enders et al. in 1949. This markedly facilitated detailed study of this agent and, most importantly, the development of poliovirus vaccines. The ensuing 60 years have seen diagnostic virology mature as a field with the discovery of new agents and diseases and the parallel determination of the importance of viruses in our understanding of molecular biology and cancer.

History of virology

The history of virology – the scientific study of viruses and the infections they cause – began in the closing years of the 19th century. Although Louis Pasteur and Edward Jenner developed the first vaccines to protect against viral infections, they did not know that viruses existed. The first evidence of the existence of viruses came from experiments with filters that had pores small enough to retain bacteria. In 1892, Dmitry Ivanovsky used one of these filters to show that sap from a diseased tobacco plant remained infectious to healthy tobacco plants despite having been filtered. Martinus Beijerinck called the filtered, infectious substance a "virus" and this discovery is considered to be the beginning of virology. The subsequent discovery and partial characterization of bacteriophages by Felix d'Herelle further catalyzed the field, and by the early 20th century many viruses were discovered.

Pioneers

Despite his other successes, **Louis Pasteur** (1822–1895) was unable to find a causative agent for rabies and speculated about a pathogen too small to be detected using a microscope. In 1884, the French microbiologist **Charles Chamberland** (1851–1931) invented a filter – known today as the Chamberland filter – that had pores smaller than

bacteria. Thus, he could pass a solution containing bacteria through the filter and completely remove them from the solution.

In 1892, the Russian biologist **Dmitry Ivanovsky** (1864–1920) used a Chamberland filter to study what is now known as the tobacco mosaic virus. His experiments showed that crushed leaf extracts from infected tobacco plants remain infectious after filtration. Ivanovsky suggested the infection might be caused by a toxin produced by bacteria, but did not pursue the idea.

In 1898, the Dutch microbiologist **Martinus Beijerinck** (1851–1931) repeated the experiments and became convinced that filtrate contained a new form of infectious agent. He observed that the agent multiplied only in cells that were dividing and he called it a *contagium vivum fluidum* (soluble living germ) and re-introduced the word *virus*. Beijerinck maintained that viruses were liquid in nature, a theory later discredited by the American biochemist and virologist **Wendell Meredith Stanley** (1904–1971), who proved that they were in fact, particles. In the same year **Friedrich Loeffler** (1852–1915) and **Paul Frosch** (1860–1928) passed the first animal virus through a similar filter and discovered the cause of foot-and-mouth disease.

In 1881, **Carlos Finlay** (1833–1915), a Cuban physician, first suggested that mosquitoes were carrying the cause of yellow fever, a theory proved in 1900 by **Walter Reed** (1851–1902). During 1901 and 1902, **William Crawford Gorgas** (1854–1920) organised the destruction of the mosquitoes' breeding habitats in Cuba, which dramatically reduced the prevalence of the disease. Gorgas later organised the elimination of the mosquitoes from Panama, which allowed the Panama Canal to be opened in 1914. The virus was finally isolated by **Max Theiler** (1899–1972) in 1932 who went on to develop a successful vaccine.

By 1928 enough was known about viruses to enable the publication of *Filterable Viruses*, a collection of essays covering all known viruses edited by **Thomas Milton Rivers** (1888–1962). Rivers, a survivor of typhoid fever contracted at the age of twelve, went on to have a distinguished career in virology. In 1926, he was invited to speak at a meeting organised by the Society of American Bacteriology where he said for the first time, "Viruses appear to be obligate parasites in the sense that their reproduction is dependent on living cells."

The notion that viruses were particles was not considered unnatural and fitted in nicely with the germ theory. It is assumed that **Dr. J. Buist** of Edinburgh was the first person to see virus particles in 1886, when he reported seeing "micrococci" in vaccine lymph, though he had probably observed clumps of vaccinia. In the years that followed, as optical microscopes were improved "inclusion bodies" were seen in many virus-infected cells, but these aggregates of virus particles were still too small to reveal any detailed structure. It was not until the invention of the electron microscope in 1931 by the German engineers **Ernst Ruska** (1906–1988) and **Max Knoll** (1887–1969), that virus particles, especially bacteriophages, were shown to have complex structures. The sizes of viruses determined using this new microscope fitted in well with those estimated by filtration experiments. Viruses were expected to be small, but the range of sizes came as a surprise. Some were only a little smaller than the smallest known bacteria, and the smaller viruses were of similar sizes to complex organic molecules.

In 1935, **Wendell Stanley** examined the tobacco mosaic virus and found it was mostly made of protein. In 1939, **Stanley** and **Max Lauffer** (1914) separated the virus into protein and RNA parts. The discovery of RNA in the particles was important

because in 1928, **Fred Griffith** (c.1879–1941) provided the first evidence that its "cousin", DNA, formed genes.

In Pasteur's day, and for many years after his death, the word "virus" was used to describe any cause of infectious disease. Many bacteriologists soon discovered the cause of numerous infections. However, some infections remained, many of them horrendous, for which no bacterial cause could be found. These agents were invisible and could only be grown in living animals. The discovery of viruses was the key that unlocked the door that withheld the secrets of the cause of these mysterious infections. And, although Koch's postulates could not be fulfilled for many of these infections, this did not stop the pioneer virologists from looking for viruses in infections for which no other cause could be found.[17]

Bacteriophages

Discovery

Bacteriophages are the viruses that infect and replicate in bacteria. They were discovered in the early 20th century, by the English bacteriologist **Frederick Twort** (1877–1950). But before this time, in 1896, the bacteriologist **Ernest Hanbury Hankin** (1865–1939) reported that something in the waters of the River Ganges could kill *Vibrio cholerae* – the cause of cholera. Whatever it was in the water could be passed through filters that remove bacteria but was destroyed by boiling. Twort discovered the action of bacteriophages on staphylococci bacteria. He noticed that when grown on nutrient agar some colonies of the bacteria became watery or "glassy". He collected some of these watery colonies and passed them through a Chamberland filter to remove the bacteria and discovered that when the filtrate was added to fresh cultures of bacteria, they in turn became watery. He proposed that the agent might be "an amoeba, an ultramicroscopic virus, living protoplasm, or an enzyme with the power of growth".

Félix d'Herelle (1873–1949) was a mainly self-taught French-Canadian microbiologist. In 1917 he discovered that "an invisible antagonist", when added to bacteria on agar, would produce areas of dead bacteria. The antagonist, now known to be a bacteriophage could pass through a Chamberland filter. He accurately diluted a suspension of these viruses and discovered that the highest dilutions (lowest virus concentrations), rather than killing all the bacteria, formed discrete areas of dead organisms. Counting these areas and multiplying by the dilution factor allowed him to calculate the number of viruses in the original suspension. He realised that he had discovered a new form of virus and later coined the term "bacteriophage". Between 1918 and 1921 d'Herelle discovered different types of bacteriophages that could infect several other species of bacteria including *Vibrio cholerae*. Bacteriophages were heralded as a potential treatment for diseases such as typhoid and cholera, but their promise was forgotten with the development of penicillin. Since the early 1970s, bacteria have continued to develop resistance to antibiotics such as penicillin, and this has led to a renewed interest in the use of bacteriophages to treat serious infections.

Early research 1920–1940

D'Herelle travelled widely to promote the use of bacteriophages in the treatment of bacterial infections. In 1928, he became professor of biology at Yale and founded several research institutes. He was convinced that bacteriophages were viruses despite opposition from established bacteriologists such as the Nobel Prize winner **Jules Bordet (**1870–1961). Bordet argued that bacteriophages were not viruses but just enzymes released from "lysogenic" bacteria. He said "the invisible world of d'Herelle does not exist". But in the 1930s, the proof that bacteriophages were viruses was

provided by **Christopher Andrewes** (1896–1988) and others. They showed that these viruses differed in size and in their chemical and serological properties. In 1940, the first electron micrograph of a bacteriophage was published and this silenced sceptics who had argued that bacteriophages were relatively simple enzymes and not viruses. Numerous other types of bacteriophages were quickly discovered and were shown to infect bacteria wherever they are found. But this early research was interrupted by World War II. Even d'Herelle was silenced. Despite his Canadian citizenship, he was interned by the Vichy Government until the end of the war.

Modern era

Knowledge of bacteriophages increased in the 1940s following the formation of the Phage Group by scientists throughout the US. Among the members were **Max Delbrück**(1906–1981) who founded a course on bacteriophages at Cold Spring Harbor Laboratory. Other key members of the Phage Group included Salvador Luria (1912–1991) and **Alfred Hershey** (1908–1997). During the 1950s, Hershey and Chase made important discoveries on the replication of DNA during their studies on a bacteriophage called T2. Together with Delbruck they were jointly awarded the 1969 Nobel Prize in Physiology or Medicine "for their discoveries concerning the replication mechanism and the genetic structure of viruses".[29] Since then, the study of bacteriophages has provided insights into the switching on and off of genes, and a useful mechanism for introducing foreign genes into bacteria and many other fundamental mechanisms of molecular biology

Plant viruses

In 1882, **Adolf Mayer** (1843–1942) described a condition of tobacco plants, which he called "mosaic disease" ("mozaïkziekte"). The diseased plants had variegated leaves that were mottled. He excluded the possibility of a fungal infection and could not detect any bacterium and speculated that a "soluble, enzyme-like infectious principle was involved". He did not pursue his idea any further, and it was the filtration experiments of Ivanovsky and Beijerinck that suggested the cause was a previously unrecognised infectious agent. After tobacco mosaic was recognized as a virus disease, virus infections of many other plants were discovered.

The importance of tobacco mosaic virus in the history of viruses cannot be overstated. It was the first virus to be discovered, and the first to be crystallised and its structure shown in detail. The first X-ray diffraction pictures of the crystallised virus were obtained by **Bernal and Fankuchen** in 1941. On the basis of her pictures, **Rosalind Franklin** discovered the full structure of the virus in 1955. In the same year, **Heinz Fraenkel-Conrat and Robley Williams** showed that purified tobacco mosaic virus RNA and its coat protein can assemble by themselves to form functional viruses, suggesting that this simple mechanism was probably the means through which viruses were created within their host cells.

By 1935 many plant diseases were thought to be caused by viruses. In 1922, **John Kunkel** Small (1869–1938) discovered that insects could act as vectors and transmit virus to plants. In the following decade many diseases of plants were shown to be caused by viruses that were carried by insects and in 1939, **Francis Holmes,** a pioneer in plant virology described 129 viruses that caused disease of plants. Modern, intensiagriculture provides a rich environment for many plant viruses. In 1948, in Kansas, US, 7% of the wheat crop was destroyed by wheat streak mosaic virus. The virus was spread by mites called *Aceria tulipae*.

In 1970, the Russian plant virologist **Joseph Atabekov** discovered that many plant viruses only infect a single species of host plant. The International Committee on Taxonomy of Viruses now recognises over 900 plant viruses.

20th century

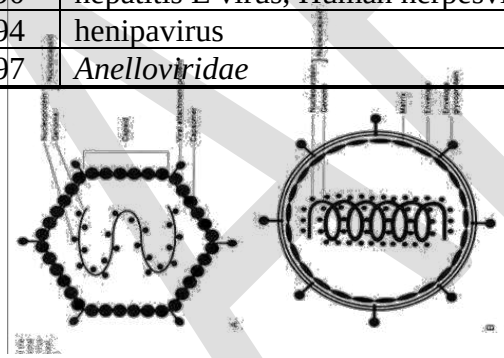
By the end of the 19th century, viruses were defined in terms of their infectivity, their ability to be filtered, and their requirement for living hosts. Up until this time, viruses had only been grown in plants and animals, but in 1906, **Ross Granville Harrison** (1870–1959) invented a method for growing tissue in lymph and, in 1913, **E Steinhardt**, **C Israeli**, and **RA Lambert** used this method to grow vaccinia virus in fragments of guinea pig corneal tissue. In 1928, **HB** and **MC Maitland** grew vaccinia virus in suspensions of minced hens' kidneys. Their method was not widely adopted until the 1950s, when poliovirus was grown on a large scale for vaccine production. In 1941–42, **George Hirst** (1909–94) developed assays based on haemagglutination to quantify a wide range of viruses as well as virus-specific antibodies in serum.

Late 20th century

The second half of the 20th century was the golden age of virus discovery and most of the 2,000 recognized species of animal, plant, and bacterial viruses were discovered during these years. In 1946, Bovine virus diarrhea was discovered, which is still possibly the commonest pathogen of cattle throughout the world and in 1957, equine arteri virus was discovered. In the 1950s, improvements in virus isolation and detection methods resulted in the discovery of several important human viruses including Varicella zoster virus, the paramyxoviruses,– which include measles virus and respiratory syncytial virus and the rhinoviruses that cause the common cold. In the 1960s more viruses were discovered. In 1963, the hepatitis B virus was discovered by Baruch Blumberg (b. 1925) and in 1965, Howard Temin (1934–1994) described the first retrovirus. Reverse transcriptase, the key enzyme that retroviruses use to translate their RNA into DNA, was first described in 1970, independently by Howard Temin and David Baltimore (b. 1938). This was important to the development of antiviral drugs – a key turning-point in the history of viral infections. In 1983 **Luc Montagnier** (b. 1932) and his team at the Pasteur Institute in France, first isolated the retrovirus now called HIV. New viruses and strains of viruses were discovered in every decade of the second half of the 20th century. These discoveries have continued in the 21st century as new viral diseases such as SARS and nipah virus have emerged. Despite scientists' achievements over the past one hundred years, viruses continue to pose new threats and challenges.

Some of the many viruses discovered in the 20th century	
Year	Virus
1908	poliovirus
1911	Rous sarcoma virus
1915	bacteriophage of staphylococci
1917	bacteriophage of shigellae
1918	bacteriophage of salmonellae
1927	yellow fever virus
1930	western equine encephalitis virus
1933	eastern equine encephalitis virus
1934	mumps virus
1935	Japanese encephalitis virus
1943	Dengue virus

1949	enteroviruses
1952	Varicella zoster virus
1953	adenovirus
1954	measles virus
1956	paramyxoviruses, rhinovirus
1958	monkeypox
1962	rubella virus
1963	hepatitis B virus
1964	Epstein–Barr virus
1965	retroviruses
1966	Lassa fever virus
1967	Marburg virus
1972	norovirus
1973	rotavirus, hepatitis A virus
1975	parvovirus B19
1976	Ebola virus
1980	human T-lymphotropic virus 1
1982	human T-lymphotropic virus 2
1983	HIV
1986	human herpesvirus 6
1989	hepatitis C virus
1990	hepatitis E virus, Human herpesvirus 7
1994	henipavirus
1997	*Anelloviridae*



Classification

Viral classification has been confusing and oft-changing over the years. In the past, viruses were often classified by host, target organ or vector and these are still used vernacularly (e.g., the hepatitis viruses). Modern classification is based on the following three characteristics:

Type of viral nucleic acid (RNA or DNA, single-stranded or double-stranded) and its replication strategy.

Capsid symmetry (icosahedral or helical).

Presence and absence of envelope

Pathogenesis of Viral Diseases

As with other infectious agents which cause human disease, the outcome of the interaction of a particular virus with the human host is dependent on both pathogen and host factors. Viral strains within a genus may have differential cell tropisms, replication capacities and cytopathogenic effects. As an example, strains of HIV may preferentially

target monocyte/macrophages or T-lymphocytes, may use different co-receptors (e.g., the chemokine receptors, CCR5 or CXCR4) on the cell surface, may replicate to different levels and may induce different degrees of cell killing. These traits have direct clinical correlates for HIV infected persons with respect to the rates of CD4 cell decline and progression to clinical AIDS. On the host side, the nature of the exposure and the host immune status are probably the two most important determinants of outcome. Thus, the key elements of the virus-host interaction are:

1. Viral strain.
2. Inoculum size.
3. Route of exposure.
4. Susceptibility of host (i.e., is there pre-existent immunity from past exposure or vaccination?).
5. Immune status and age of host.

The net result of this interaction may be:

1. No infection.
2. Abortive infection with limited viral replication.
2. Asymptomatic infection.
3. Symptomatic infection.
4. Depending upon the agent and the immune status of the host, persistent/latent or self-limited infection.

Pathogenetic Steps in Human Infection

A generalized schema of viral infection leading to disease in the human host is as follows:

Depending upon the agent, the virus enters through the skin, mucous membranes, respiratory tract, gastrointestinal tract, via a transfusion or transplanted organ or via maternal-fetal transmission.

There is local replication at the site of the inoculation. Certain agents exhibit pathology at the skin or mucous membrane surface – e.g., herpes simplex virus, human papillomavirus.

For some neurotropic viruses there may be spread along peripheral nerve routes to ganglia (e.g., herpes simplex virus) or the central nervous system (e.g., rabies virus). For other neurotropic agents, the central nervous system is seeded following viremia.

For many agents, there is replication in regional lymph nodes with subsequent viremia and spread to target organs. Some viruses travel in the bloodstream free in plasma (e.g., picornaviruses); others are cell associated (e.g., cytomegalovirus).

Replication in target organs may lead to local damage and further rounds of viremia..

Non-specific and specific host immune responses come into play to try to control and downregulate the viral replicative process.

Immune Responses to Viral Infections

Innate (non-specific) immunity refers to those elements of the immune system that can clear virus or virus infected cells immediately upon or shortly after viral exposure and which are not dependent upon immunologic memory. Non-specific immunity may include:

a. Phagocytic cells (neutrophils and monocyte/macrophages).
b. Cytokines (e.g., interferons) and chemokines.
c. Natural killer cells.
d. Poorly defined antiviral factors that may exist in blood or body fluids.

Adaptive (specific) immunity refers to antigen specific B and T cell responses that lead to the development of antibodies, cytotoxic T cells and antibody dependent cellular cytotoxicity.

In some instances, an intense immunologic reaction to a viral agent can result in immunopathology and a serious clinical syndrome. A prime example is dengue hemorrhagic fever which is likely due to antibody dependent enhancement and T cell activation on re-exposure to dengue virus.

Mechanisms of Viral Persistence

Viruses may cause chronic, persistent infection with continuous viral replication in the face of an immune response. Examples include HIV, hepatitis B virus and hepatitis C virus. Some viruses may demonstrate persistent infection in immune compromised hosts. These include the herpesviruses, human papillomavirus and rubella virus, among others.

Some viruses are able to cause latent infection. Latency is characterized by a quiescent or minimally transcriptionally active viral genome with periods of reactivation. Latent viruses include the herpesviruses (cytomegalovirus, Epstein-Barr virus, herpes simplex virus, varicella-zoster virus), human papillomavirus, human retroviruses. Recurrent herpes labialis or genital herpes due to HSV or herpes zoster due to varicella zoster virus are classic examples of latency and reactivation. Viruses which exhibit latency may also exhibit chronic, persistent replication in the setting of immune compromise of the host. Mechanisms of persistence of viruses which produce chronic infections include antigenic variation to escape antibody or cytotoxic T cell responses, downregulation of class I major histocompatibility antigens resulting in diminished recognition by cytotoxic T cells and modulation of apoptosis. Viruses which establish latent infection escape recognition by the immune system through decreased viral antigen expression and presentation.Sites of persistence include the nervous system (herpes simplex virus, varicella zoster virus, measles virus, poliovirus, JC virus), the liver (hepatitis B virus, hepatitis C virus), and leukocytes (HIV, cytomegalovirus, Epstein-Barr virus).

Oncogenesis

Several viruses are associated with human cancers. These include: Epstein-Barr virus with lymphoma, nasopharyngeal carcinoma and leiomyosarcoma; herpesvirus 8 with Kaposi's sarcoma and body cavity B-cell lymphoma; hepatitis B and C viruses with hepatocellular carcinoma; and human papillomavirus with cervical cancer and anogenital carcinoma. Mechanisms of oncogenesis can include transformation (Epstein-Barr virus and herpesvirus 8) and binding of tumor suppressor proteins (human papillomavirus), among others

Diagnosis of Viral Infections

The diagnosis of viral infections relies first on the recognition of a distinct clinical syndrome (e.g., herpes zoster infection) or a consideration of the viral infection in the differential diagnosis of a presenting syndrome (e.g., aseptic meningitis). The second consideration is the knowledge of the appropriate specimens to send to the laboratory (blood, body fluids, lesion scraping, tissue) to diagnose a particular infection. One general point to remember is that the isolation of viruses relies on the use of proper viral transport medium and quick delivery to the laboratory. A variety of methods exist to diagnose viral infections with the recent trend being toward molecular diagnostics. These methods include: Isolation of virus in tissue culture, animals, embryonated eggs. Most diagnostic laboratories only use tissue

culture for virus isolation. A specific cytopathic effect or induction of a characteristic function (e.g., hemagglutination) can indicate the growth of viruses in tissue culture. This can be confirmed with virus specific antisera applied to the tissue monolayer to neutralize the cytopathic effect or the hemagglutination reaction. Antigen detection in body fluids (e.g., respiratory tract for respiratory viruses) or blood (e.g., cytomegalovirus) or lesion scrapings (e.g, for herpes simplex virus or varicella-zoster virus) with specific immune sera linked to fluorescence or enzyme immunoassay detection.

PCR amplification and/or nucleic acid probes to detect viral nucleic acid in body fluids or tissues.

Antibody detection. IgM antibody detection can assist with acute diagnosis. Four-fold rises in IgG specific antibody or conversion from seronegative status to seropositive status can secure a diagnosis but this may not be helpful in the acute setting.Examination of tissue samples by light microscopy for viral induced cytopathology and antigen detection by immunohistochemical staining. Examination of body fluids or tissues by electron microscopy. This is not an efficient method and is dependent upon sufficient numbers of virions being present to permit detection.

Prevention and Therapy

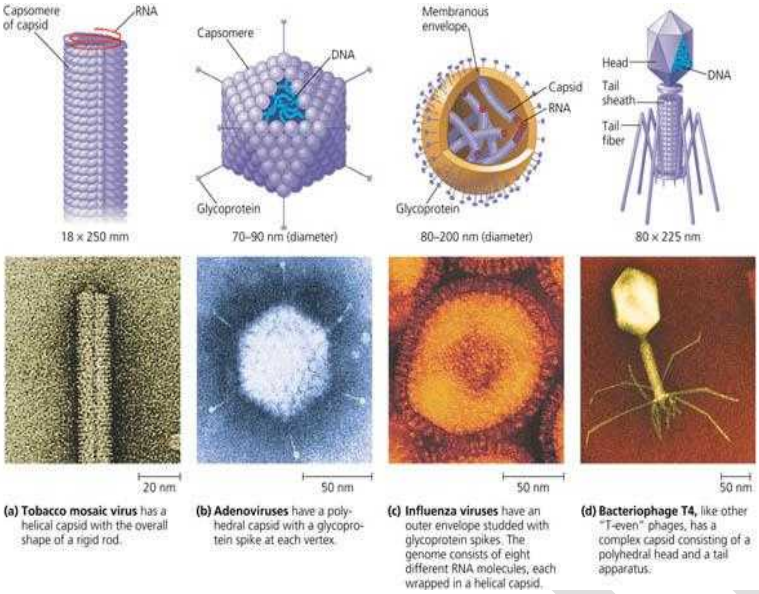
Vaccines for the prevention of life threatening viral infections are one of the most significant advances in human health. The eradication of smallpox is the hallmark example of the effectiveness of a viral vaccine. Effective vaccines exist for polio, mumps, measles, rubella, influenza, hepatitis A, hepatitis B, varicella-zoster, rabies, adenovirus, Japanese B encephalitis and yellow fever.

Immune globulin can prevent or ameliorate clinical disease due to certain viral agents. Examples include varicella-zoster immune globulin for exposure in immune compromised hosts, rabies immune globulin (administered with rabies vaccine) following an exposure, cytomegalovirus immune globulin for transplant recipients, respiratory syncytial virus immune globulin and immune serum globulin for hepatitis A.

Screening of blood for prevention of transmission of HIV, hepatitis B, hepatitis C and in certain transplant situations, cytomegalovirus.

Safe sexual practices for the prevention of HIV, hepatitis B and human papillomavirus infections.

Advances in specific antiviral therapy over the past 30 years have been marked. Effective therapy exists for herpes simplex virus, varicella-zoster virus, cytomegalovirus, HIV, influenza virus, respiratory syncytial virus, hepatitis B and hepatitis C.



Viruses contribute significantly to the global burden of infectious diseases. Most of the diseases are mild, but viruses may cause severe diseases in susceptible individuals, such as the mal-nourished, immuno-compromised, the very old and the very young.

What is a virus? Very simple structures consisting essentially of a nucleic acid genome, protected by a shell of protein. May or may not have a lipoprotein envelope. Has no organelle. Very small, sizes range 20 - 200 nm, beyond the resolving power of the light microscope. Metabolically inert and can only replicate inside a host cell. Genome consists of ONLY one type of nucleic acid; either RNA or DNA.

Viral genome codes for the few proteins necessary for replication: some proteins are non-structural e.g. polymerase, and some are structural, i.e. form part of the virion structure.

Type of infection Virus replicates initially at the site of entry, but then enters the blood (viraemia) or lymphatics and spreads throughout the body .Other viruses may replicate locally initially, and then enter nerve endings and travel up the axon to infect the central nervous system.

Incubation period

Time from exposure to an organism to the onset of clinical disease. Viruses that cause localized infections have short incubation periods (<7 days), while in disseminated infections, the incubation period tends to be longer.

Immune response

Viruses replicate intracellularly, so recovery from a viral infection requires the action of specific cytotoxic T lymphocytes. Virus-specific antibody levels rise during the course of the infection, but antibody plays only a limited role in recovery. Specific antibodies play a very important role in preventing reinfection of the host with the same virus. Certain viruses are able to evade the immune response and establish persistent infections in their host.

DNA Viruses

SS, non-enveloped, 18-25 nm	Parvoviridae	*Human parvovirus B19*	Erythema infectiosum, fifth disease (from a 1905 list of skin rash diseases: 1. measles 2. scarlet fever 3. rubella 4. Filatow-Dukes disease 5. erythema infectiosum 6. Roseola infantum) Mild flu-like symptoms, facial rash, maculopapular rash on trunk and limbs.
DS non-enveloped 70-90 nm	Adenoviridae	*Mastadenovirus*	Respiratory infections in humans, some cause tumors in animals.
40-57 nm	Papovaviridae	*Papillomavirus* (HPV-16)	Warts, some sexually transmitted. HPV-16 associated with close to 90% of cervical cancers, especially serious in South Carolina.
		Polyomavirus	Polyoma and simian viruses cause tumors in animals.
DS enveloped 200-350 nm	Poxviridae	*Variola* major	Smallpox (pox – vesicopustular skin eruptions)
		Vaccina	Cowpox
150-200 nm	Herpesviridae	*Simplexvirus* (Herpes simplex 1 and 2; Human herpes virus, HHV-1 and HHV-2)	HSV 1 – usually oral transmission, lesions on upper body (cold sores); HSV 2 – usually transmitted genitally, infections of lower body. Lesions appear as sores after cell lysis. Virus persists in latent state and is fairly easily reactivated (UV exposure, fever, radiation, stress).
		Varicella zoster (HHV-3)	Primary infection is chicken pox, may be accompanied by pneumonia and encephalitis in immuno-compromised children; more severe in adults, usually accompanied by pneumonia. Shingles (zoster) - virus remains dormant in dorsal root or cranial nerve ganglia, reactivated by stress, travels down nerve fiber and causes painful blisters in the relevant dermatome. Unexposed people can contract chicken pox from zoster lesions but not vice versa; primary exposure imparts immunity to exogenous infection.
		Lymphocryptovirus (HHV-4; Epstein-Barr)	Infectious mononucleosis – malaise and lethargy, pharyngitis, lymph node enlargement, spleenomegaly, fever. Infects B cells and is associated with Burkitt's lymphoma (lymphoma of head and neck) and nasopharyngeal carcinoma.

		Cytomegalovirus (HHV-5)	Usually inapparent, chronic, latent. Estimated 80% of the population carries the virus. Disease appears usually when host is immunocompromised and severity of disease correlates with severity of immunosuppression. Symptoms include pneumonia, hepatitis, mononucleosis, and arthritis. Risk of graft rejection increases significantly with CMV infection.
		Roseolovirus (HHV-6)	Roseola infantum (sixth disease, exanthem subitum) High fever, generalized rash, rapid and complete recovery
		HHV-7	Infects most infants, causes measleslike rashes
		HHV-8	Causes Kaposi's sarcoma (seen in immunocompromised individuals, primarily AIDS patients)
42 nm	Hepadnaviridae	*Hepadnavirus* (Hepatitis B virus)	Serum hepatitis – hepatitis B, may cause hepatocellular carcinoma

RNA Viruses

Genome	Family	Virus	Disease
SS RNA, + strand Non-enveloped 28-30 nm	Picornaviridae	*Poliovirus*	Polio; loss of anterior horn cells (motor neurons), flaccid paralysis, sometimes of diaphragm → iron lung
		Coxsackie B virus	Post-viral fatigue syndrome (PVFS), chronic fatigue syndrome
		Hepatitis A virus	Acute hepatitis, 90% recovery, fecal-oral inoculation
		Rhinovirus	Common cold
35-40 nm	Calciviridae	*Norovirus*	Gastroenteritis
		Hepatitis E virus	Enterically transmitted non-A, non-B hepatitis
SS RNA, + strand enveloped 60-70 nm	Togaviridae	*Alphavirus*	Transmitted by arthropods, eastern and western equine encephalitis.
		Rubivirus (rubella)	German measles; respiratory transmission, causes rash, imparts long-lasting immunity. Especially dangerous to 1st trimester fetuses.
40-50 nm	Flaviviridae	*Flavivirus* (an arbovirus; transmitted by mosquito bites)	Yellow Fever: a classic viral hemorrhagic fever. Hepatic necrosis, jaundice, fever, vomiting, diarrhea, mortality rate of 80%; Yellow fever prohibited significant colonization of large parts of South and Central America until controlled (elimination of mosquito population); Panama canal was discontinued until the disease was controlled. Vaccine is now available.
		Hepatitis C virus	Blood-borne non-A non-B hepatitis
Nidovirales 80-160 nm	Coronaviridae	*Coronavirus*	Upper respiratory infections, common cold.

Mono-negavirales SS RNA - strand enveloped 70-180 nm	Rhabdoviridae	*Lyssavirus* (rabiesvirus)	Rabies: Zoonotic, transmission by contact with infected animals. Virus spreads from wound to brain along neurons. Incubation is 1 week to 1 year depending on site of wound. Symptoms include cerebral hyperirritability, rage, pharyngeal muscle spasm, alternating mania and coma until death, usually by respiratory failure (destruction of respiratory center. Vaccine available, treatment includes injection with immune globulin and vaccine.
80-14,000 nm	Filioviridae	*Filovirus* (Marburg virus, Ebola virus)	Viral hemorrhagic fever, both initiate from contact with infected monkeys or tissues, may be passed secondarily by contact with secretions or unsterilized instruments. Human-human contact inefficient. Acute fever, muscle pain, abdominal pain, rash, severe gastrointestinal bleeding, generalized hemorrhage, shock, death. Ebola has a mortality rate of close to 90%.
150-300 nm	Paramyxoviridae	Mumps virus	Mumps. Half of infections are unapparent. Invades upper respiratory tract and lymph nodes, spreads to target organs (most common is parotid gland). Can cause orchitis (testicular inflammation) in post-pubescent males, may result in sterility. Vaccine available.
		Measles virus	Red measles. Transmission by inhalation usually, spreads to lymph nodes, infects T-cells. Antibody titer rises, rash appears (probably immune complex mediated hypersensitivity), fever, cough, conjunctivitis. Recovery is usually rapid, complete, and imparts lifelong immunity.
RNA – strand, 1 strand 32 nm	Deltaviridae	Hepatitis D	Depends on co-infection with Hepatitis B virus –formerly known as the delta agent and thought to be a defective virus the disease is actually caused by a viroid enclosed in a hepatitis B viral coat
90-120 nm	Bunyaviridae	*Bunyavirus* *Hantaviruis*	Hantaviruses, cause viral hemorrhagic fevers
110-130 nm	Arenaviridae	*Arenavirus*	Venezuelan hemorrhagic fever, Lassa fever
RNA – strand, multiple strands segmented 80-200 nm	Orthomyxoviridae	Influenza viruses	Influenza: Types A, B, and C. Segmented genome allows extensive recombination leading to antigenic changes. Transmitted by inhalation, infects respiratory mucosa, allows secondary bacterial infections to occur after epithelial denudation.
DS RNA + strands	Retroviridae	Oncoviruses: HTLV I & II	Leukemia
		Lentivirus (HIV)	AIDS
DS RNA non-enveloped 60-80 nm	Reoviridae	*Reovirus* *Rotavirus*	Respiratory infections Gastroenteritis

Viral cell interactions

When an intact of infectious virus particles makes contacts with a susceptible host cell may develop a number of reactions at the cell surface lead to release of the genetic material at the virus within the cell. This is immediately followed by a series of biosynthetic processes lead to formation of new virus like e.g.

1. Defective virus:- viruses that have lost ability to perform any one of the essential steps required for successful replication.
2. Incomplete virus:- abnormal viruses produced due to inoculation of high titer virus solution in limited number of host susceptible cells like inoculums containing a high rating of infective units to cells this called Von Magnus phenomena. That the produced viruses without nucleic acid e.g. influenza virus.

Interferon

Soluble substance produced by living cells of many different types in cell cultures, embryonated eggs, in lab. Animals when infected by some animal viruses either DNA or RNA and can inhibit multiplication of active virus e.g. influenza virus.

Characteristics of interferon molecules:-

1. It is small protein without nucleic acid.
2. Low molecular weight of about 25- 45000 Dalton.
3. Thermo stable at 4 C° and resist heating at 50 C° for I hour.
4. Interferon is active through a wide range of pH values (2-12).
5. It is relatively non-toxic, weakly antigenic and cannot neutralized by the specific antiserum.
6. Inactivated by protolytic enzymes such as trypsin.
7. Not affected with RNase & DNase.
8. Interferon specific to animal species but not to viruses species i.e.: it act against wide variety of viruses.

Viral culture

Viral culture is a laboratory test in which samples are placed with a cell type that the virus being tested for is able to infect. If the cells show changes, known as cytopathic effects, then the culture is positive.[1]

Traditional viral culture has been generally superseded by shell vial culture, in which the sample is centrifuged onto a single layer of cells and viral growth is measured by antigen detection methods. This greatly reduces the time to detection for slow growing viruses such as cytomegalovirus, for which the method was developed.[2] In addition, the centrifugation step in shell viral culture enhances the sensitivity of this method because after centrifugation, the viral particles of the sample are in close proximity to the cells.

Human and monkey cells are used in both traditional viral culture and shell vial culture. Human virus types that can be identified by viral culture include adenovirus, cytomegalovirus, entero viruses, herpes simplex virus, influenza virus, para-influenza virus, rhinovirus, respiratory syncytial virus, varicella zoster virus, measles and mumps.[3] For these, the final identification method is generally by immunofluorescence, with exception of cytomegalovirus and rhinoviruses, whose identification in a viral culture are determined by cytopathic effects.

General Characteristics of Viruses

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

Characteristics

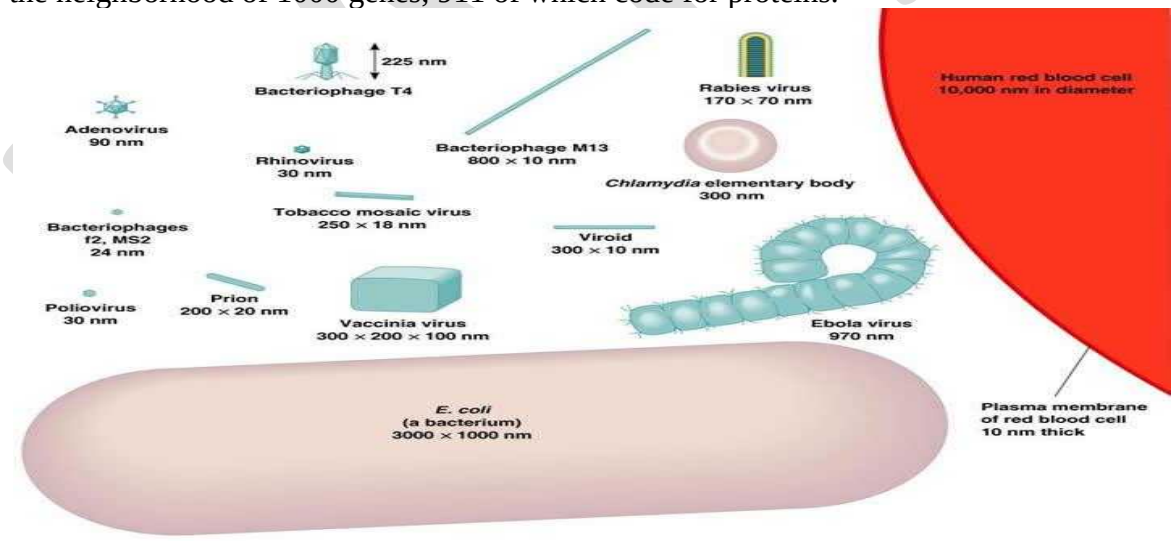
Single type of nucleic acid - DNA or RNA
Protein coat, or capsid, some has envelopes
Multiply inside of living cells using the host cell machinery
Direct the synthesis of structures to transfer viral nucleic acid to other cells

Host Range

The specific types of cells a virus can infect in its host species represent the host range of the virus.
Usually species specific Classification:
Animal virus
Plant virus
Bacterial virus (bacteriophage)
Host range is determined by attachment sites (receptors).
Anti-bacterial therapy - phage therapy
Anti-tumor therapy - oncolytic viruses

Viral Size

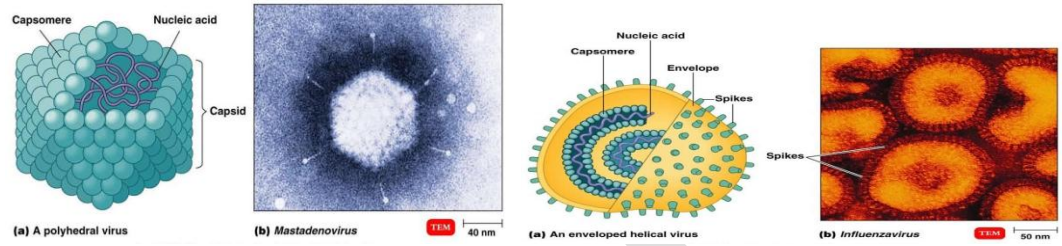
Determined by electron microscopy.
Ranges from 20 to 14,000 nm in length.
There is also a group of giant viruses, including the giant mimi virus, which is something like 800 nm in diameter and has a genome with 1.2Mbp base pairs carrying somewhere in the neighborhood of 1000 genes, 911 of which code for proteins.



Viral Structure

Virions are complete, fully developed viral particles composed of nucleic acid surrounded by a protein coat. Some viruses have an envelope composed of a phospholipid bilayer with viral glycoproteins.
1. Nucleic acid
Viral genomes are either DNA or RNA (not both).
Nucleic acid may be single- or double-stranded

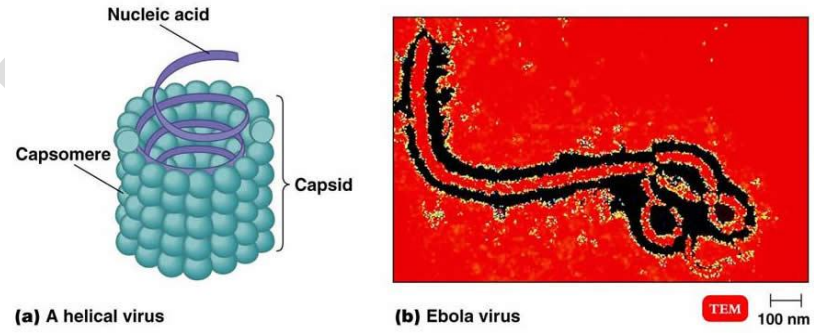
Nucleic acid may be circular or linear or separate molecules.
Nucleic acid:protein ranges from about 1% - 50%.
2. Capsid
Capsid - protein coat
Capsomeres are subunits of the capsid
Protomeres are capsomere subunits.



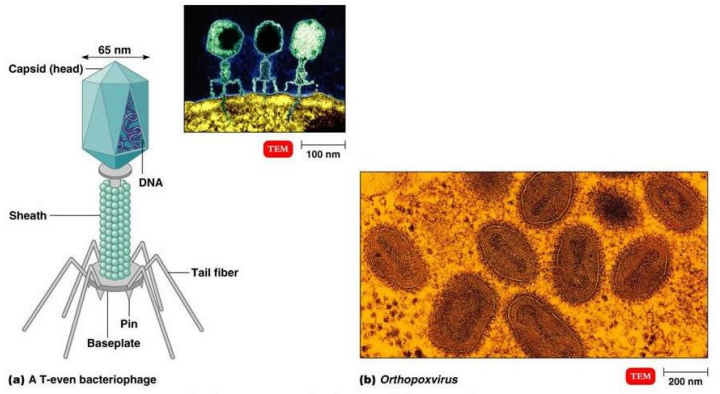
3. Envelope – the outer covering of some viruses, the envelope is derived from the host cell plasma membrane when the virus buds out. Some enveloped viruses have spikes, which are viral glycoproteins that project from the envelope.
Influenzavirus has two kinds of spikes, H (hemagglutinin) and N (neuraminidase). The H spike allows the virus to attach to host cells (and red blood cells), the N spike is an enzyme that allows the mature viral particles to escape from the host cell
Non-enveloped or naked viruses are protected by their capsid alone.

General Morphology

Based on capsid architecture, although enveloped viruses end up being approximately spherical.
1. Helical, non-enveloped
2. Helical, enveloped



3. Polyhedral, non-enveloped
4. Polyhedral, enveloped
Polyhedral means many sides (most are icosahedral - 20 triangular faces and 12 corners)
5. Complex viruses are, well, complex.



Taxonomy of Viruses

Classification of viruses is based on type of nucleic acid, strategy for replication, and morphology.

Virus family names end in *–viridae*; genus names end in *–virus*, order names end in *-ales*.

A viral species is a group of viruses sharing the same genetic information and ecological niche. There is no specific epithet used, common names that are descriptive are used; subspecies are designated with a number.

Families of viruses that affect humans:

The Isolation, Cultivation and Identification of Viruses

Viruses must be grown in living cells. They can't be grown in culture media or on agar plates alone, they must have living cells to support their replication.

The easiest viruses to grow are bacteriophages (because the easiest cells to grow in the lab are bacteria).

Growing Bacteriophages In The Laboratory

Once viruses have replicated and been harvested the concentration of viral particles (virions) in the viral stock solution must be determined. One of the easiest ways to determine the concentration of a stock solution of bacteriophages is to use the plaque method.

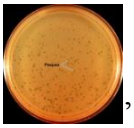
The plaque method:

Virus, bacteria, and agar mixed, plated and incubated.

After replication the virus lyses the bacteria, forming plaques, or clear zones.

Each plaque is assumed to come from a single viral particle.

The titer (concentration of the stock solution) of the virus is given in plaque forming units.

 ,

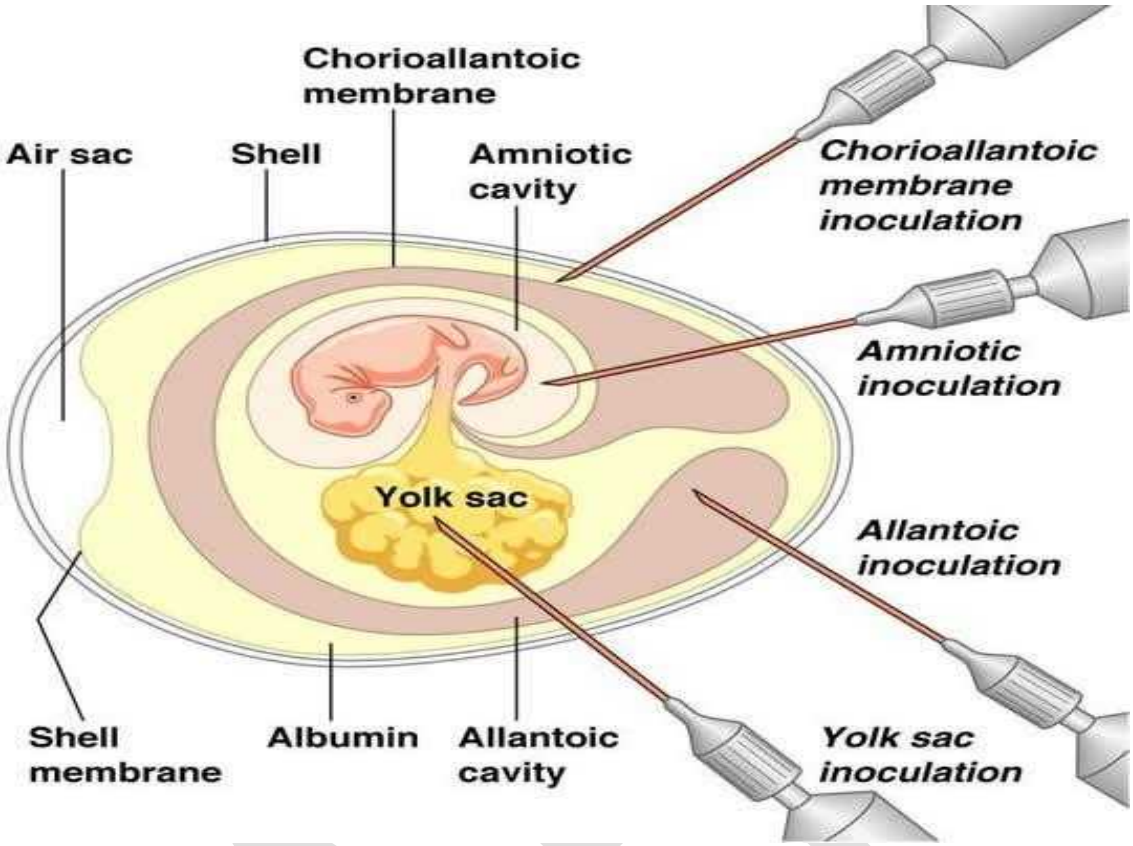
Growing Animal Viruses In The Laboratory

1. Live animal cultures have to be used for some animal viruses.

Simian AIDS and feline AIDS provide models for studying human AIDS.

2. Embryonated eggs can serve as substitutes for some viruses.

Can inoculate membrane that best supports specific virus (allantoic, amniotic, chorioallantoic, or yolk sac).



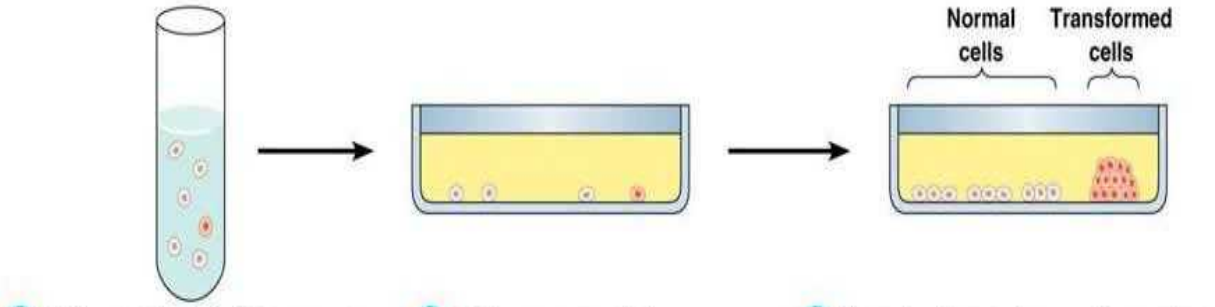
3. Cell culture is a lot cheaper and easier to work with (contamination can be a problem however).

Primary cell lines have a short lifespan in culture – a few generations before reaching senescence.

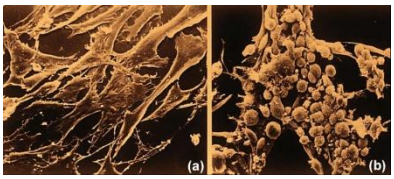
Diploid cell lines are derived from embryos and can grow for up to 100 population doublings before senescence.

Continuous cell lines are derived from ***transformed*** cells and grow indefinitely in culture.

Hela cells – 1st continuous cell line, derived from Helen Lane (fictional name - actually named Henrietta Lacks), a cervical cancer patient who died in 1951. This is the oldest continuous cell line and was first used to culture and identify polio virus.



Transformed Cells in Culture



Viral growth can cause cytopathic effects in the cell culture.
Cytopathic effects can appear early or late in the course of the viral infection.
Cytopathic effects may be cytocidal (cell death) or non-cytocidal.
Non-cytocidal effects include acidophilic or basophilic inclusion bodies in the nucleus, cytoplasm, or both; cell fusion, and transformation.
Cytopathic effects can be so characteristic of individual viruses that they can often be used to identify viruses.

Viral Identification
Serological methods
Western blotting
Cytopathic effects
Diagnostic inclusion bodies are associated with rabies virus, measles virus, vaccinia virus, smallpox virus, herpesvirus, and adenoviruses.
Molecular methods include PCR and RFLPs.
PCR was used to identify the West Nile virus and the SARS-associated coronavirus

Study of Viruses

The study of viruses is known as virology. Viruses can be studied in two ways. The first way is through isolation and cultivation, and the second way through detection, identification and diagnosis. For isolation and cultivation, animals, plants, chick embryo and tissue culture are used. For detection, identification and diagnosis, there are several methods. These methods include tissue culture methods, physical methods, serological methods, immunological methods, and others and molecular biology.

Techniques of Virus Cultivation

Viruses are obligate intracellular parasites so they depend on host for their survival. They cannot be grown in non-living culture media or on agar plates alone, they must require living cells to support their replication.

The primary purpose of virus cultivation is:

1. To isolate and identify viruses in clinical samples.
2. To do research on viral structure, replication, genetics and effects on host cell.
3. To prepare viruses for vaccine production.

Cultivation of viruses can be discussed under following headings:

1. Animal Inoculation
2. Inoculation into embryonated egg
3. Cell Culture

1. Animal Inoculation

- Viruses which are not cultivated in embryonated egg and tissue culture are cultivated in laboratory animals such as mice, guinea pig, hamster, rabbits and primates are used.
- The selected animals should be healthy and free from any communicable diseases.
- Suckling mice(less than 48 hours old) are most commonly used.

- Suckling mice are susceptible to togavirus and coxsackie virues, which are inoculated by intracerebral and intranasal route.
- Viruses can also be inoculated by intraperitoneal and subcutaneous route.
- After inoculation, virus multiply in host and develops disease. The animals are observed for symptoms of disease and death.
- Then the virus is isolated and purified from the tissue of these animals.
- Live inoculation was first used on human volunteers for the study of yellow fever virus.

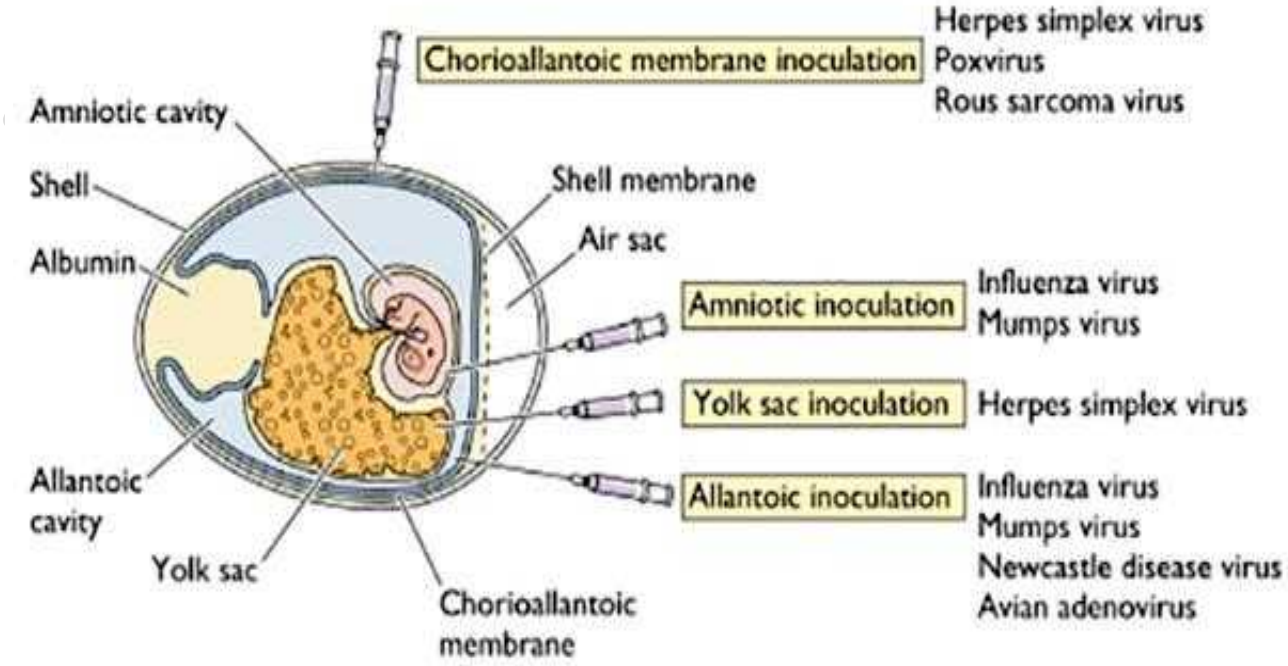
Advantages of Animal Inoculation

1. Diagnosis, Pathogenesis and clinical symptoms are determined.
2. Production of antibodies can be identified.
3. Primary isolation of certain viruses.
4. Mice provide a reliable model for studying viral replication.
5. Used for the study of immune responses, epidemiology and oncogenesis.

Disadvantages of Animal Inoculation

1. Expensive and difficulties in maintenance of animals.
2. Difficulty in choosing of animals for particular virus
3. Some human viruses cannot be grown in animals or can be grown but do not cause disease.
4. Mice do not provide models for vaccine development.
5. It will lead to generation of escape mutants
6. Issues related to animal welfare systems

2. Inoculation into embryonated egg



- Good pasture in 1931 first used the embryonated hen's egg for the cultivation of virus.
- The process of cultivation of viruses in embryonated eggs depends on the type of egg which is used.

- Viruses are inoculated into chick embryo of 7-12 days old.
- For inoculation, eggs are first prepared for cultivation, the shell surface is first disinfected with iodine and penetrated with a small sterile drill.
- After inoculation, the opening is sealed with gelatin or paraffin and incubated at 36°c for 2-3 days.
- After incubation, the egg is broken and virus is isolated from tissue of egg.
- Viral growth and multiplication in the egg embryo is indicated by the death of the embryo, by embryo cell damage, or by the formation of typical pocks or lesions on the egg membranes
- Viruses can be cultivated in various parts of egg like chorioallantoic membrane, allantoic cavity, amniotic sac and yolk sac.

1. Chorioallantoic Membrane (CAM):

- Inoculation is mainly for growing poxvirus.
- After incubation and incubation, visible lesions called pocks are observed, which is grey white area in transparent CAM.
- Herpes simplex virus is also grown.
- Single virus gives single pocks
- This method is suitable for plaque studies.

2. Allantoic cavity:

- Inoculation is mainly done for production of vaccine of influenza virus, yellow fever, rabies.
- Most of avian viruses can be isolated using this method.

3. Amniotic sac:

- Inoculation is mainly done for primary isolation of influenza virus and the mumps virus.
- Growth and replication of virus in egg embryo can be detected by haemagglutination assay.

4. Yolk sac inoculation:

- It is also a simplest method for growth and multiplication of virus.
- It is inoculated for cultivation of some viruses and some bacteria (Chlamydia, Rickettsiae)
- Immune interference mechanism can be detected in most of avian viruses.

Advantages of Inoculation into embryonated egg

1. Widely used method for the isolation of virus and growth.
2. Ideal substrate for the viral growth and replication.
3. Isolation and cultivation of many avian and few mammalian viruses.
4. Cost effective and maintenance is much easier.
5. Less labor is needed.
6. The embryonated eggs are readily available.
7. Sterile and wide range of tissues and fluids
8. They are free from contaminating bacteria and many latent viruses.
9. Specific and non specific factors of defense are not involved in embryonated eggs.
10. Widely used method to grow virus for some vaccine production.

Disadvantages of Inoculation into embryonated egg

1. The site of inoculation for varies with different virus. That is, each virus have different sites for their growth and replication.

3. Cell Culture (Tissue Culture)

There are three types of tissue culture; organ culture, explant culture and cell culture.

Organ cultures are mainly done for highly specialized parasites of certain organs e.g. tracheal ring culture is done for isolation of coronavirus.

Explant culture is rarely done.

Cell culture is mostly used for identification and cultivation of viruses.

- Cell culture is the process by which cells are grown under controlled conditions.
- Cells are grown in vitro on glass or a treated plastic surface in a suitable growth medium.
- At first growth medium, usually balanced salt solution containing 13 amino acids, sugar, proteins, salts, calf serum, buffer, antibiotics and phenol red are taken and the host tissue or cell is inoculated.
- On incubation the cell divide and spread out on the glass surface to form a confluent monolayer.

Types of cell culture

1. Primary cell culture:

- These are normal cells derived from animal or human cells.
- They are able to grow only for limited time and cannot be maintained in serial culture.
- They are used for the primary isolation of viruses and production of vaccine.
- Examples: Monkey kidney cell culture, Human amnion cell culture

2. Diploid cell culture (Semi-continuous cell lines):

- They are diploid and contain the same number of chromosomes as the parent cells.
- They can be sub-cultured up to 50 times by serial transfer following senescence and the cell strain is lost.
- They are used for the isolation of some fastidious viruses and production of viral vaccines.
- Examples: Human embryonic lung strain, Rhesus embryo cell strain

3. Heteroploid cultures (Continuous cell lines):

- They are derived from cancer cells.
- They can be serially cultured indefinitely so named as continuous cell lines
- They can be maintained either by serial subculture or by storing in deep freeze at -70°c.
- Due to derivation from cancer cells they are not useful for vaccine production.
- Examples: HeLa (Human Carcinoma of cervix cell line), HEP-2 (Humman Epithelioma of larynx cell line), Vero (Vervet monkey) kidney cell lines, BHK-21 (Baby Hamster Kidney cell line).

Susceptible Cell Lines

1. **Herpes Simplex** Vero Hep-2, human diploid (HEK and HEL),human amnion
2. **VZV** human diploid (HEL, HEK)
3. **CMV** human diploid fibroblasts
4. **Adenovirus** Hep2, HEK,
5. **Poliovirus** MK, BGM, LLC-MK2, human diploid, Vero, Hep-2,Rhadomyosarcoma
6. **Coxsackie B** MK, BGM, LLC-MK2, vero, hep-2
7. **Echo** MK, BGM, LLC-MK2, human diploid, Rd
8. **Influenza A** MK, LLC-MK2, MDCK
9. **Influenza B** MK, LLC-MK2, MDCK
10. **Parainfluenza** MK, LLC-MK2
11. **Mumps** MK, LLC-MK2, HEK, Vero

12. **RSV** Hep-2, Vero
13. **Rhinovirus** human diploid (HEK, HEL)
14. **Measles** MK, HEK
15. **Rubella** Vero, RK13

Advantages of cell culture

1. Relative ease, broad spectrum, cheaper and sensitivity

Disadvantage of cell culture

1. The process requires trained technicians with experience in working on a full time basis.
2. State health laboratories and hospital laboratories do not isolate and identify viruses in clinical work.
3. Tissue or serum for analysis is sent to central laboratories to identify virus.

Cultivation of plant viruses and bacteriophages

Cultivation of plant viruses

There are some methods of Cultivation of plant viruses such as plant tissue cultures, cultures of separated cells, or cultures of protoplasts, etc. viruses can be grown in whole plants.

Leaves are mechanically inoculated by rubbing with a mixture of viruses and an abrasive. When the cell wall is broken by the abrasive, the viruses directly contact the plasma membrane and infect the exposed host cells. A localized necrotic lesion often develops due to the rapid death of cells in the infected area. Some plant viruses can be transmitted only if a diseased part is grafted onto a healthy plant.

Cultivation of bacteriophages

Bacteriophages are cultivated in either broth or agar cultures of young, actively growing bacterial cells.

Viral Multiplication

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

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

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

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

Burst time is the time from phage adsorption to release.

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

Multiplication of Bacteriophages

The virus may cause lysis or lysogeny.

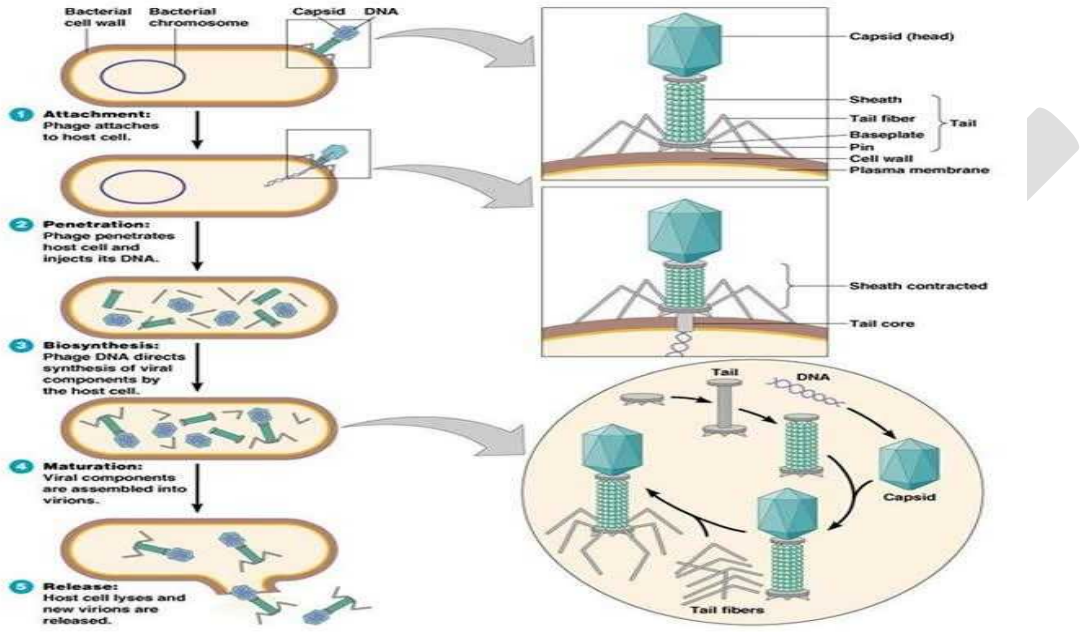
Events of the **lytic cycle**:

Attachment or adsorption

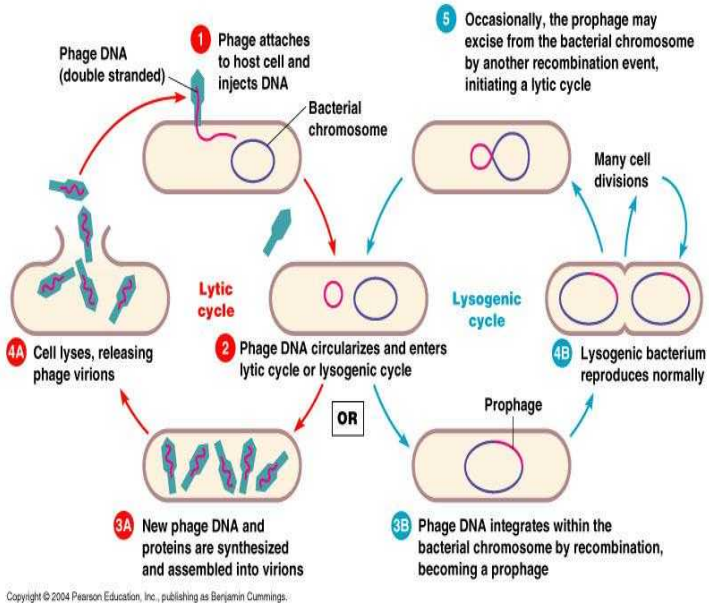
Requires a receptor

Penetration

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



Lysogeny is a cycle in which the phage DNA recombines with the bacterial chromosome.
The incorporated viral DNA is now a prophage.
The prophage genes are regulated by a repressor coded for by the prophage, the prophage is replicated each time the host DNA is replicated.
Exposure to mutagens can lead to excision of the prophage and initiation of the lytic cycle.

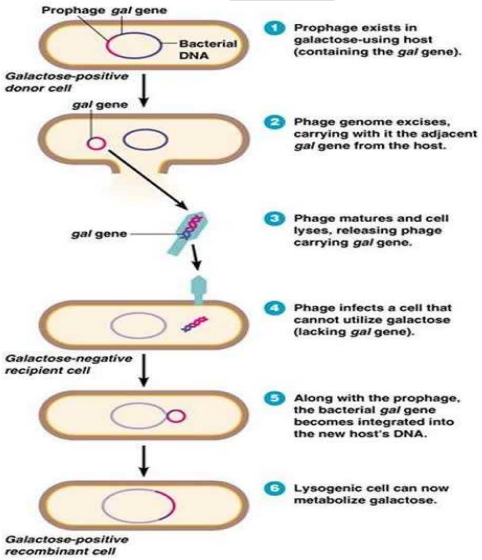


Outcomes of lysogeny

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

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

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

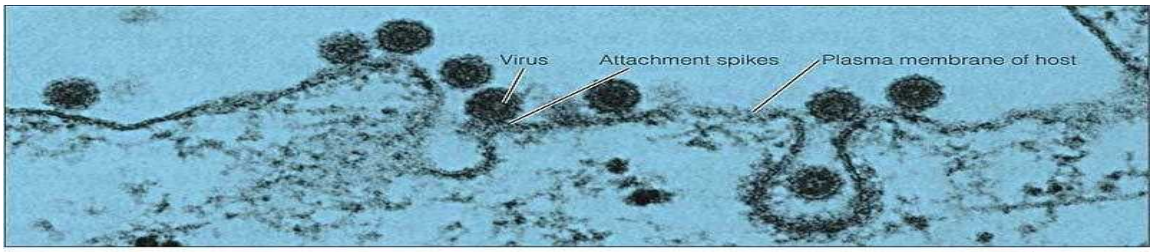


Attachment or adsorption

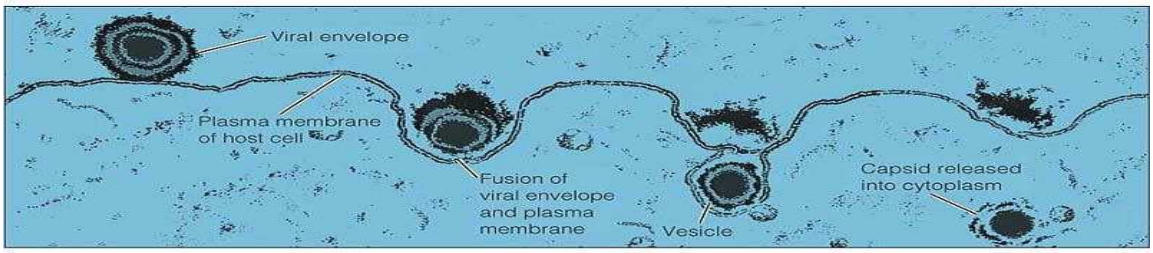
Penetration

a) Endocytosis (pinocytosis) - togavirus

b) Fusion - herpesvirus



(a) Entry of togavirus



(b) Entry of herpesvirus

Uncoating

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

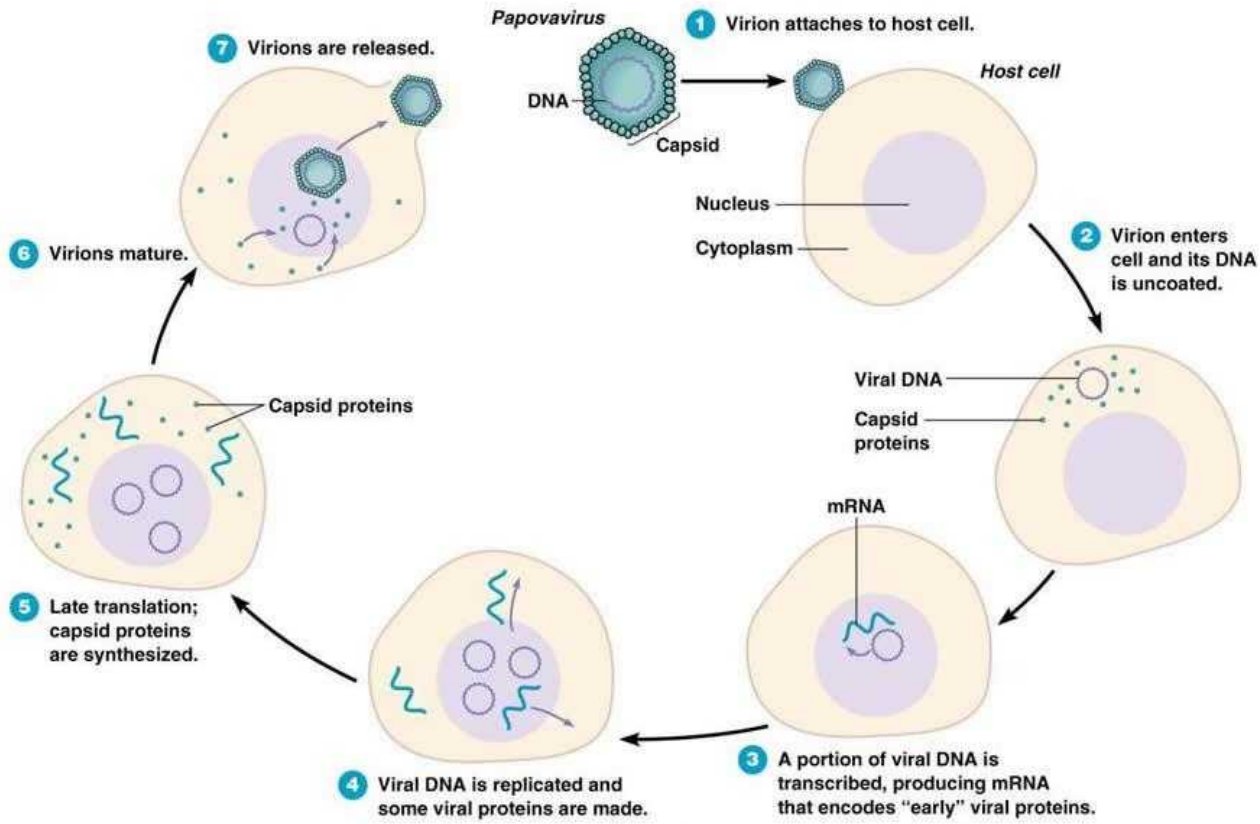
Biosynthesis

Biosynthesis of DNA viruses

TABLE 13.4 The Biosynthesis of DNA and RNA Viruses Compared

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

DNA of most DNA viruses is released into the nucleus of the host cell.
Transcription and translation of early genes produces enzymes to reproduce viral DNA
Transcription and translation of late genes produces capsid proteins in the cytoplasm.

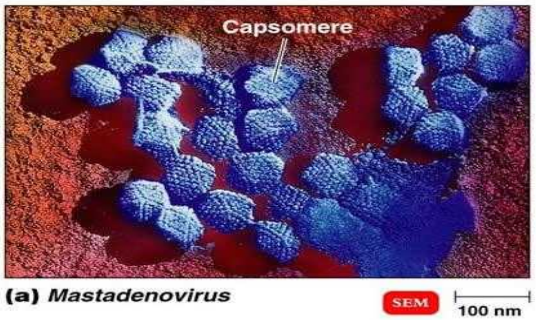


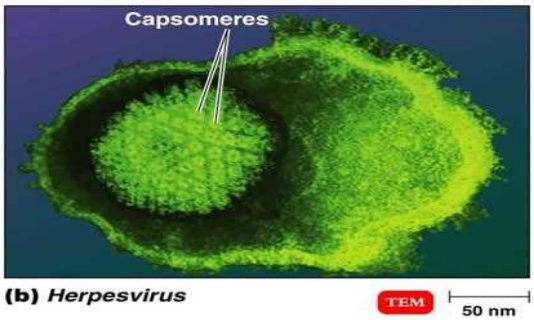
Advantages and Limits

Lytic cycle
- Replication of new viruses is fast
- However, the host is also immediately killed preventing the viral genome from passing onto the next generation of host cells

Lysogenic cycle
- Many more viruses can be made because the viral genome is passed onto future generation of host cells
- However, replication is takes longer because it is dependent on the host cell's replication

DNA-containing animal viruses





Some examples of DNA viruses:
Adenoviridae - from adenoids, cause respiratory diseases.
Poxviridae - pox refers to the pus-filled lesions that accompanies the diseases caused by these viruses

Herpesviridae - named after spreading (herpetic) appearance of cold sores
Papoviridae - named for ***pa***pillomas (warts), ***po***lyomas (tumors), and ***va***cuolation (cytoplasmic vacuoles)
Hepadnaviridae - name comes from the fact that they cause ***hepa***titis and contain ***DNA***.

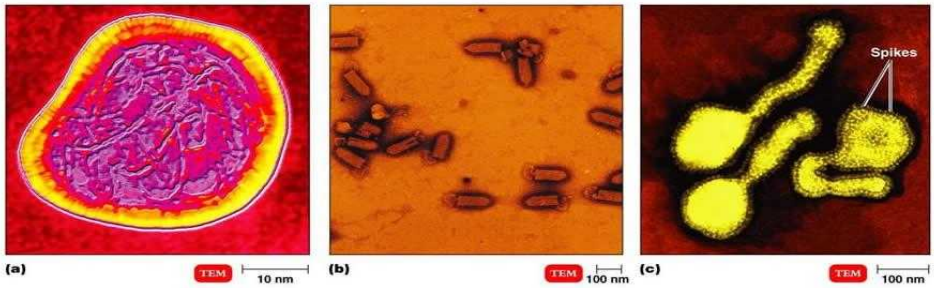
Biosynthesis of RNA Viruses
RNA viruses multiply in the cytoplasm. RNA-dependent RNA polymerase synthesizes a double-stranded RNA.
Sense strand (+ strand) can act as mRNA directly and as a template for antisense strand (- strand) synthesis.

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

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

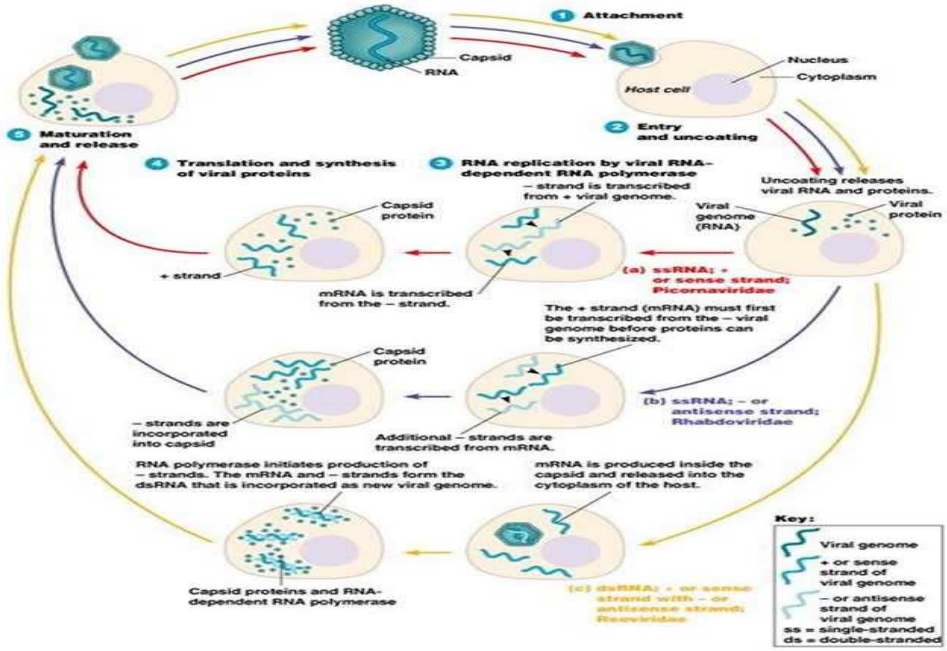
ds +/- RNA viral replication: transcription of - strand makes more copies of the + strand, which serves as mRNA. Transcription of the + strand provides viral protiens (including RNA-directed RNA polymerase) and more copies of - strand, which is packaged along with the complementary + strands in the capsid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

RNA-containing animal viruses



Picornaviridae - some of the smallest viruses (pico-); contain RNA, name comes from pico + RNA. Single stranded + strand viruses.
Example: poliovirus
Togaviridae - enveloped, name comes from toga (covering). Single stranded + strand viruses - transcription of a - strand serves as a template, the + strands transcribed from the - strand template are produced as a short strand mRNA that codes for envelope proteins and a long strand mRNA that codes for capsid proteins.
Examples: *Art*hropod-*bo*rne *arbo*viruses or alphaviruses which cause viral encephalitis.
Rhabdoviridae - Rhabdo- is from the Greek for rod (they're really more bullet shaped). Single stranded - strand viruses.
Example: *Lyssavirus* (rabiesvirus)
Reoviridae - named for habitat, respiratory and enteric tract. Before they were associated with disease they were considered orphan viruses, name comes from ***r***espiratory, ***e***nteric, and ***o***rphan. Double stranded RNA viruses. Example: Rotavirus

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

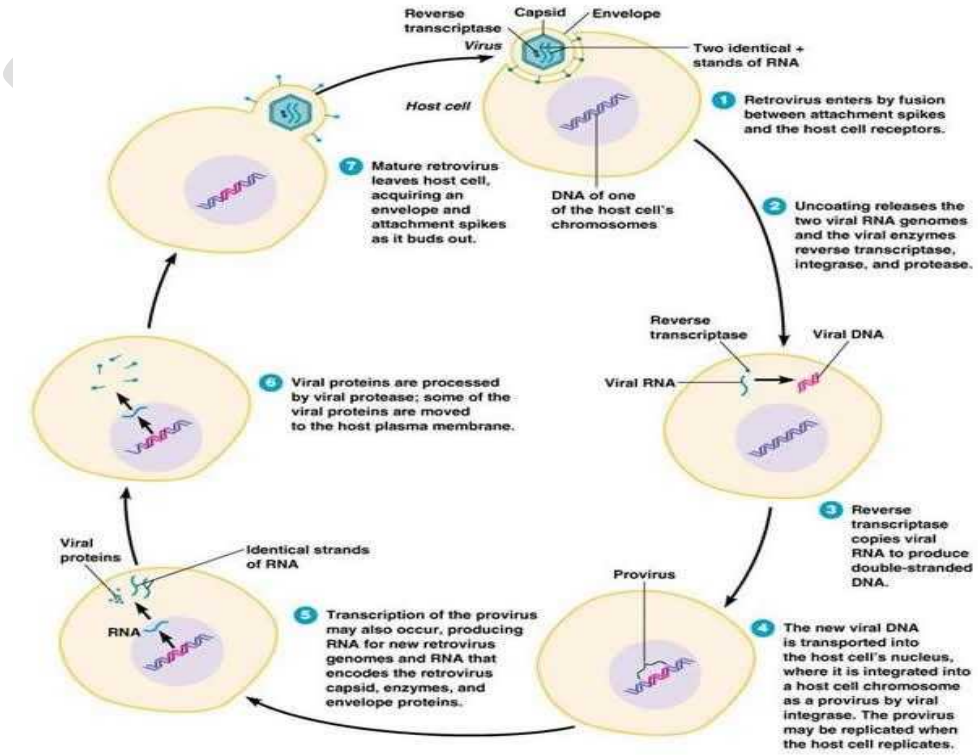


Multiplication of Retroviruses

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

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

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



CLASS: I MSc MB **COURSE NAME: VIROLOGY**

COURSE CODE: 17MBP201 **UNIT: II**

Animal viruses- DNA viruses - morphology, replication, pathogenesis and laboratory diagnosis of Pox virus, Adeno virus, Hepatitis viruses – type A,B and D. Herpes simplex viruses, oncogenic viruses- Papova virus,- oncogenes and Oncogenesis.

DNA viruses

Small DNA viruses: parvo- and polyomaviruses

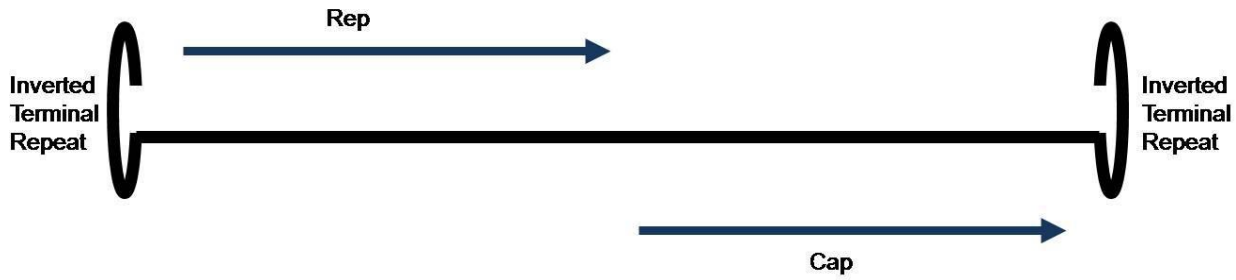
Cellular DNA synthesis occurs only during the S phase of the cell cycle, so the viruses which depend on host cell DNA polymerase must either wait for cells to enter S phase or express some protein early during infection to regulate the cell cycle (many small DNA viruses).

Parvoviruses

Parvo in latin stands for small. The virion is icosahedral, non-enveloped, and around 25 nm in diameter. These are the smallest of all animal viruses. They do not contain any viral or host enzyme and virion is made up of 80% protein and 20% DNA by weight.

The genome of parvovirus is linear, ssDNA which contains approximately 4500 to 5500 nucleotides. All parvoviruses contain terminal palindromic sequences at their 5' and 3' ends which allow the formation of Y or T shaped structures. In addition, they also contain inverted repeats at 3' and 5' ends which allow the circularization of the genome. **Rep** gene is required for replication of DNA while **cap** gene forms the capsid. The virion also contains 3 coat proteins namely, VP1, VP2, and VP3. VP3 is the most abundant among all three and it is made by the proteolytic cleavage of VP2. VP2 constitutes about 80% of capsid protein.

Figure 35.1 Genome map of parvoviruses:

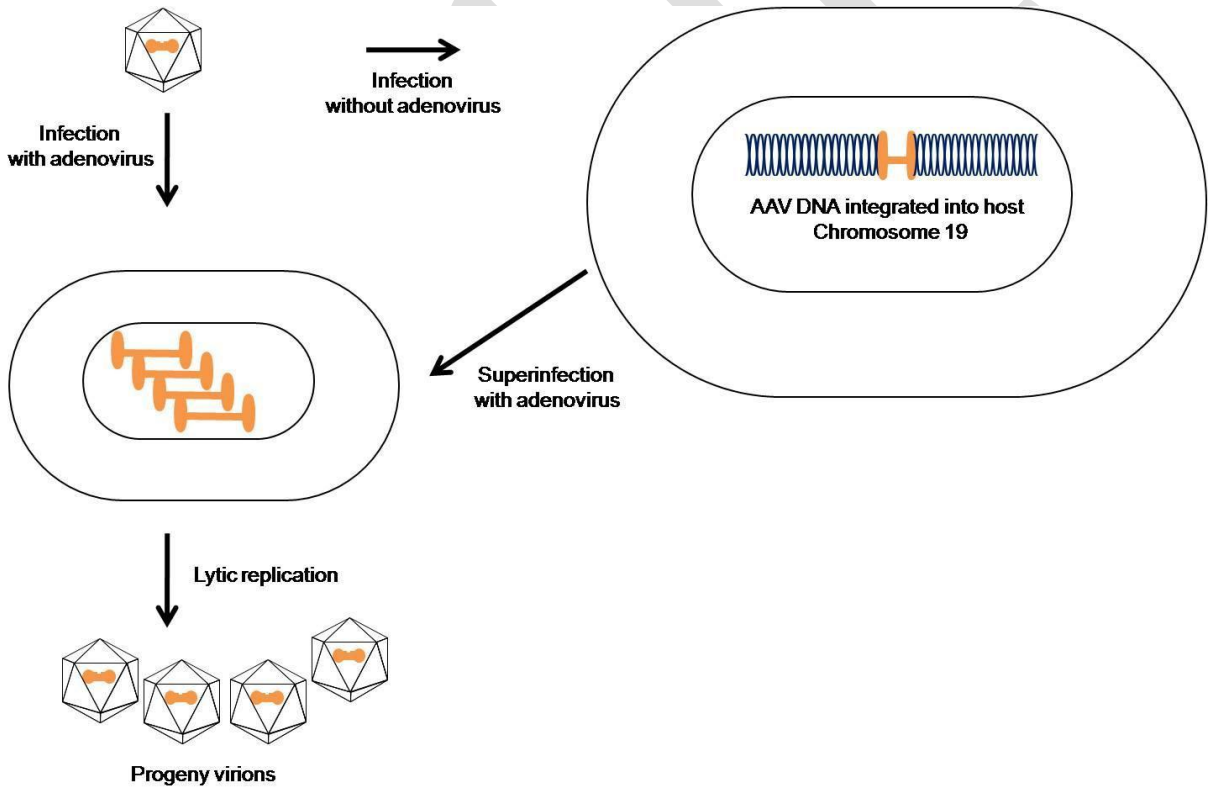


There are 3 genomes of Parvoviruses.-

1) Parvovirus
2) Dependovirus
3) Densovirus

1) **Parvovirus** – They only replicate in actively dividing cells. These viruses are highly resistant to heat, nucleases, detergents, proteases, and mild acid. They generally spread through body secretions. The most common strain of parvovirus that infects humans is B19.
2) **Dependovirus** – They are also called as adeno associated virus (AAV). They require adenoviruses to replicate. Upon infection in absence of helper virus they can establish latent infection by integrating into the host genome. They are one of the very important tools for targeted gene therapy viral vector.

Figure 35.2 Helper dependent replication strategy of adeno-associated viruses:



3) **Densovirus** – They infect only invertebrates.

Replication

The receptor for parvovirus has not been identified yet. It replicates with the help of host DNA polymerase and its assembly occurs in nucleus. Palindromic sequences at

the termini are the initiation sites for DNA replication. Following replication, transcription is carried out by host cell RNA polymerase II. The genome of parvovirus codes for two non- structural proteins NS1 and NS2 apart from 3 coat proteins VP1, VP2 and VP3. Translation of the mRNA occurs in the cytoplasm and protein enters into the nucleus

where assembly occurs. The virion exits the cell by lysis and the whole process is completed in 24hrs.

Papovaviruses

The name is derived from ***pa*pillomas** (warts), **polyomas** (multifocal tumors), and **vacuoles** in infected cells. These viruses contain dsDNA and are non- enveloped and spherical. They replicate and assemble in the nucleus of the infected cell and are released out following the lysis of the cell.

There are two genera

1) Papilloma
2) Polyoma

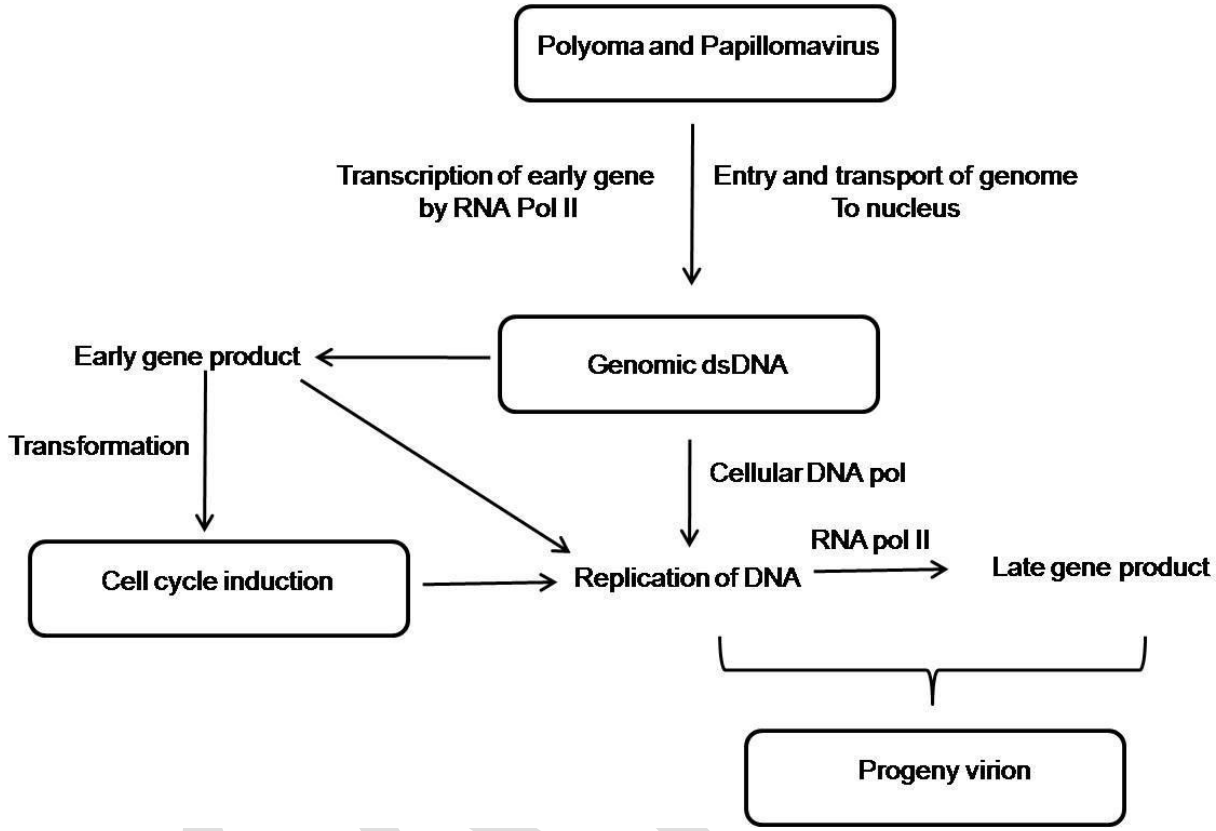
Papilloma - Genome is around 8000 bp long. They depend upon the replication machinery of the host cell. They often infect basal cell layers of the skin and hence are associated with warts. Papilloma viruses do not grow in tissue culture.

Polyoma - Genome is around 6000 bp long. They are associated with leukoencephalopathy and immunosuppression in humans. The most common virus SV40 that has been used to study mammalian replication belongs to this group. SV 40 makes large and small T-antigens which are required for viral DNA replication as it binds to origin of replication and is known to possess helicase and ATPase activity.

Replication

I. Adsorption of virions to the cell surface and entry by endocytosis.
II. Transport to the cell nucleus and uncoating
III. Transcription to produce early gene mRNAs and translation to produce early proteins (T antigens).
IV. Viral DNA replication and transcription of late gene mRNAs.
V. Translation to produce late proteins (capsid proteins) and assembly of progeny virions in the nucleus.
VI. Release of virions from the cell

Figure 35.3 General replication strategies of polyoma and papillomaviruses:



Large DNA viruses

The viruses with large DNA have different strategies for their genome replication. The virus having intermediate size DNA (Adenoviruses) carry their own DNA replication machinery including DNA polymerase and other regulatory proteins. However, they still depend on the host cell RNA pol-II for the transcription of the viral RNA. The virus having large genome size like pox and herpesviruses have their own machinery to fulfill the requirement of RNA transcription and genomic DNA replication.

Virus type	Genome	Replication	Example
Adenovirus	dsDNA, 35 kb	Nucleus with DNA polymerase	Many serotypes infecting humans as well as animals
Herpesvirus	dsDNA, 120-230 kbp	Nucleus with DNA polymerase	HSV-1 and -2, Varicella/Zoster, HHV-5, 6, &7, Epstein-Barr

Poxvirus	dsDNA, 200 kbp	cytoplasm with DNA polymerase	Smallpox, Sheeppox, ORF, cowpox, vaccinia

Figure 36.1 General replication strategies of Adenoviruses:

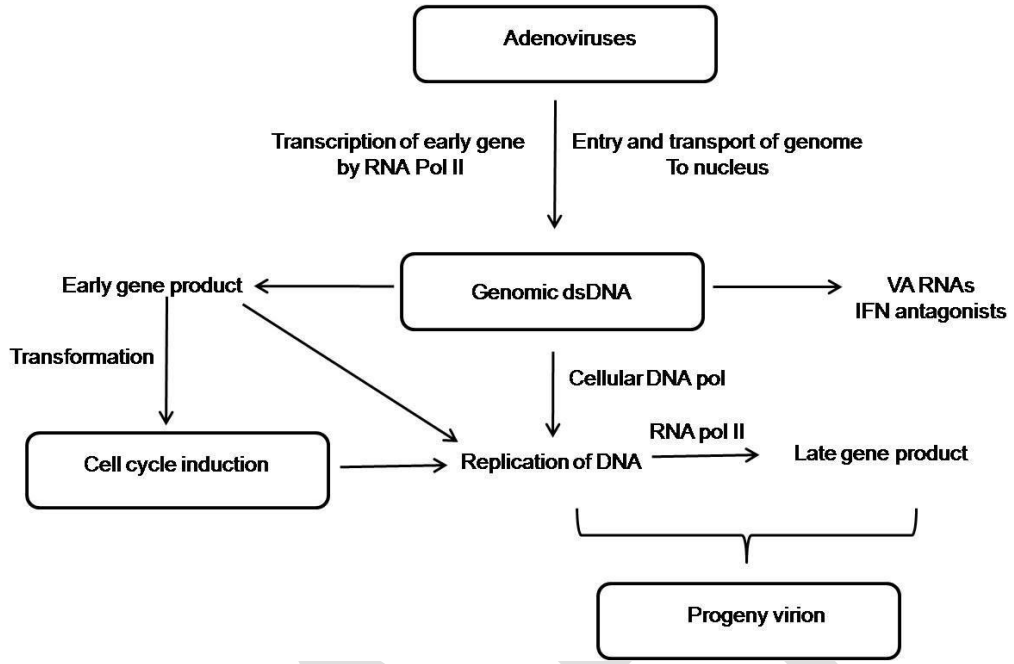
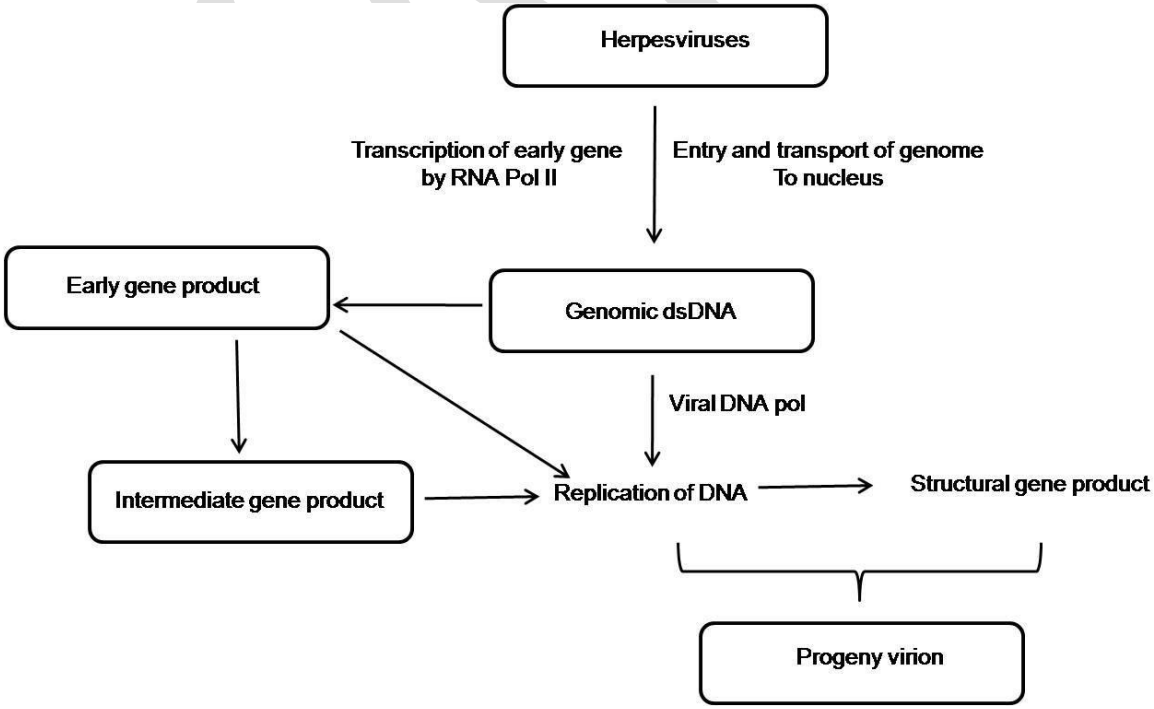


Figure 36.2 General replication strategies of herpesviruses:



Oncogenic DNA viruses

Epstein-Barr virus, *Hepatitis B* virus, papillomaviruses and Human Herpesvirus type

8 (HHV-8) or Kaposi's sarcoma are associated with one or other type of cancer in humans. In addition, HIV induces severe immunosuppression and facilitates development of cancer by other persisting infections, especially by HHV-8, Epstein-Barr virus and human papillomaviruses. Thus these agents contribute indirectly to human cancer. The mechanism about how these DNA viruses induce cancer is quite well known now. E6 and E7 gene of human papillomavirus modulate large array of cellular gene making cells vulnerable to undergo uncontrolled multiplication. Similarly, Epstein-Barr virus nuclear antigen 2 (EBNA-2) results in the induction of viral oncogenes that modulate many cell proteins. Many liver associated malignancies are caused by Hepatitis B virus. Bovine papillomaviruses can induce tumors (sarcoids) in horses and donkeys.

Herpesviruses

The herpes name is derived from the Greek word *herpein*, meaning to creep. Many of the herpesviruses were isolated from different species of animal and at least eight from human. One of the important characteristics of this virus is to cause latency in the infected individuals.

Herpesvirus virion

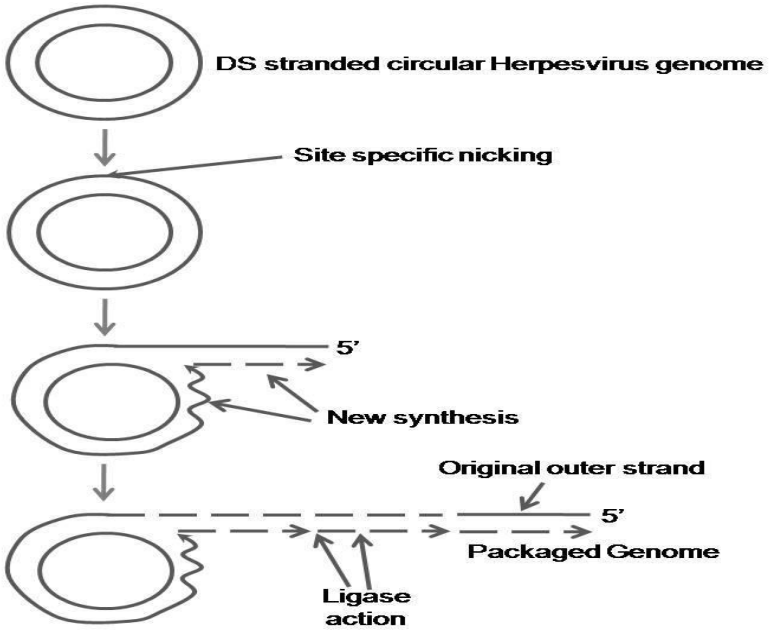
Herpesviruses have a complex structure because of its large size, multiple proteins, and tegument. The viral genome is a linear dsDNA of about 125-250 Kbp. The DNA is encapsidated inside an icosahedral capsid which is surrounded by a tegument. The tegument of herpesvirus contains many proteins while envelope is composed of 10 or more glycoproteins. The structural proteins of the herpesviruses are called as viral protein (VP) and VP5 is the most abundant protein present in the capsid. The envelope glycoproteins such as gB, gC and gD are the antigenic determinants and are involved in mounting the host immune response. In addition, the virion also contains the hexon and penton fibers. Genome of the herpesvirus contains two unique sequences; large and small and both are flanked by the repeat sequences. The genome encodes more than 75 proteins and many mRNA subspecies. Both strands of the DNA are used for the coding purpose. As some genes are present in the inverted repeats so the genome contains a pair of those genes in each strand.

Herpesvirus replication

Herpesvirus largely infects humans, many animals and lab animal in research laboratories. The virus binds first to **heparan sulfate** and then to cell adhesion molecules such as **nectins**. The virus then fuses with the cell membrane and enters the cell following endocytosis. The nucleocapsid and the tegument are released into the cytoplasm. The replication of the genome occurs in the nucleus and therefore nucleocapsid is first transported into the nucleus. The linear DNA molecule is converted into closed and circular in the nucleus of the host cells. The closed circular DNA then binds with the histones. The major tegument protein VP16 helps in modulating the viral gene expression and is transported along with the viral DNA to the nucleus. The herpesvirus genes express as immediate early (IE), early (E), and late (L). VP16 acts as a

transcription factor to recruit RNA polymerase II to activate the immediate early genes. Early proteins have their role in viral DNA replication and late proteins are formed during the assembly of the virion. Most of the late proteins are structural proteins. DNA replication in herpesvirus starts with a θ mode and later switches to rolling circle mode. Rolling circle mode of replication is the major form observed in the herpesviruses.

Figure 37.1 Rolling circle replication in herpesviruses:



Latency during herpesvirus infections

I. After entry virus releases many factors that initiate infection. The factors include **virion host shut-off** (VHS), **α-trans inducing factor** (αTIF) and many others. α-TIF- helps in the synthesis of 5α mRNA.

VHS- favors to shut off host protein synthesis by degrading cellular mRNA.

II. The viral genome and αTIF migrates to the nucleus where viral gene expression begins.

III. New viruses bud out from the cell and infect other neighboring epithelial cells.

IV. Some newly formed virions cross the synapses and travel downwards the axon to the nerve cells towards peripheral ganglion.

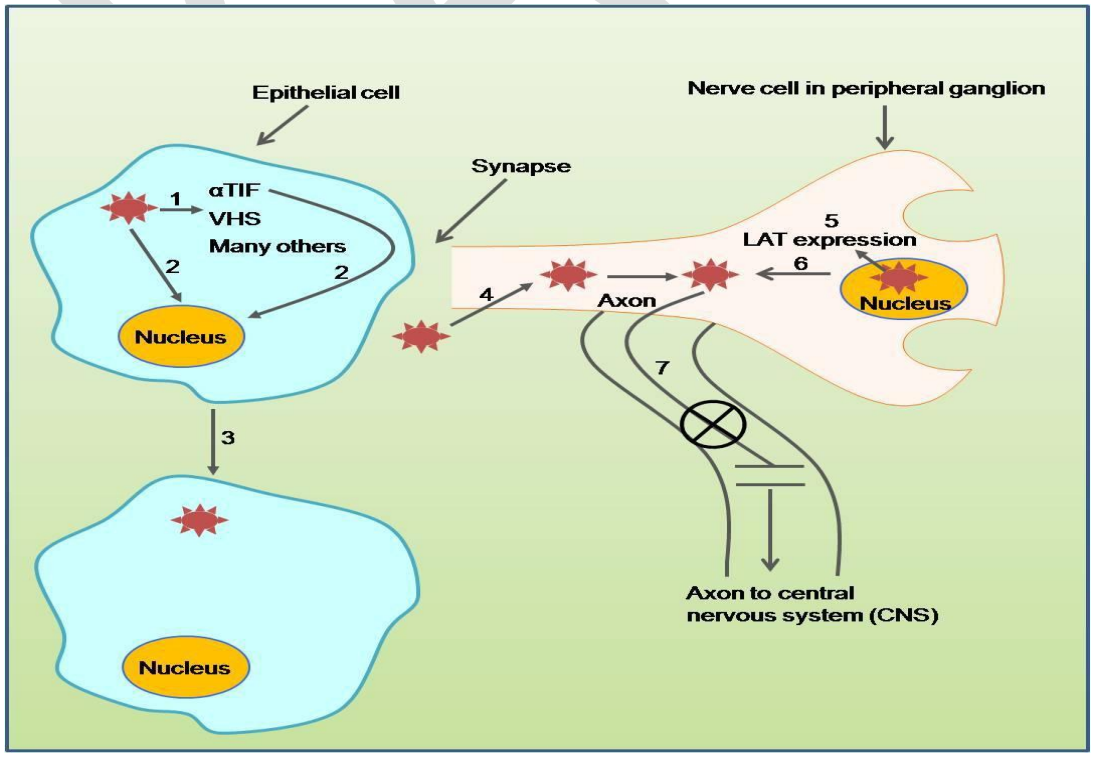
V. The virions become latent inside neurons with the expression of **Latency associated transcript** (LAT).

LAT- It is an mRNA made during latency by viruses.

VI. During the process of reactivation new viruses are made in the nerve cell. They travel back downwards the axon to infect the epithelial cells again.

VII. Nerve cells in the ganglion are well connected to the brain but virus rarely goes in that direction.

Figure 37.2 Latency in herpesviruses:



Important herpesviruses

Herpes simplex viruses 1 and 2

Herpes simplex viruses 1 and 2 (HSV-1 and HSV-2) infect epithelial cells of the buccal cavity, genital mucosa membrane, skin and cornea. Generally the virus migrates to central nervous system via neurons and initiates a latent infection. HSV-1 is mostly transmitted by lips and nasal contacts mostly to the young ones (1-2 years). HSV-2 is mostly transmitted by sexual contact and hence called as **genital herpes**.

Varicella-zoster virus

Varicella-zoster virus leads to a condition commonly called as **chicken pox** (varicella) where virus spreads to the skin and produces rashes. The rashes are mostly towards the face and trunk area. It may spread to CNS to produce a latent infection and reactivates during stress or administration of corticosteroids leading to a condition called as **shingles**. The symptoms include rashes in different body parts, fever, headache, joint pain, and swollen lymph nodes.

Epstein-Barr virus

Epstein-Barr virus is generally transmitted by saliva from an infected individual and spread in the body by its multiplication in the B cells. The virus infects the young ones with asymptomatic infection which activates during adolescence. The virus leads to a condition called as **infectious mononucleosis** or glandular fever. Epstein-Barr virus is also associated with different kind of cancers in humans. In medical science the infection is referred as **kissing disease** since it is transmitted by kissing through saliva.

Human cytomegalovirus

Human cytomegalovirus is transmitted vertically from mother to foetus. The infection at birth can cause reduced brain size and enlargement of the liver and spleen. During the later phase of life virus can cause hearing loss and mental retardation. HIV positive patients who are immunocompromised can easily be infected by cytomegalovirus which terminates into life threatening pneumonia or hepatitis.

Adenoviruses

Adenoviruses are one of the major causative agents of upper respiratory tract or common cold infection in humans. In addition, they also cause conjunctivitis (eye inflammation), tonsillitis (inflammation of tonsils), gastroenteritis (inflammation of intestine), urinary tract infections, and infection to brain.

There are four genera of adenoviruses

1. Aviadenovirus- Infecting to avian species
2. Mastadenovirus- Infecting to mammals
3. Atadenovirus- Infecting to avian and humans
4. Siadenovirus- Infecting to avian, mammals, and reptiles.

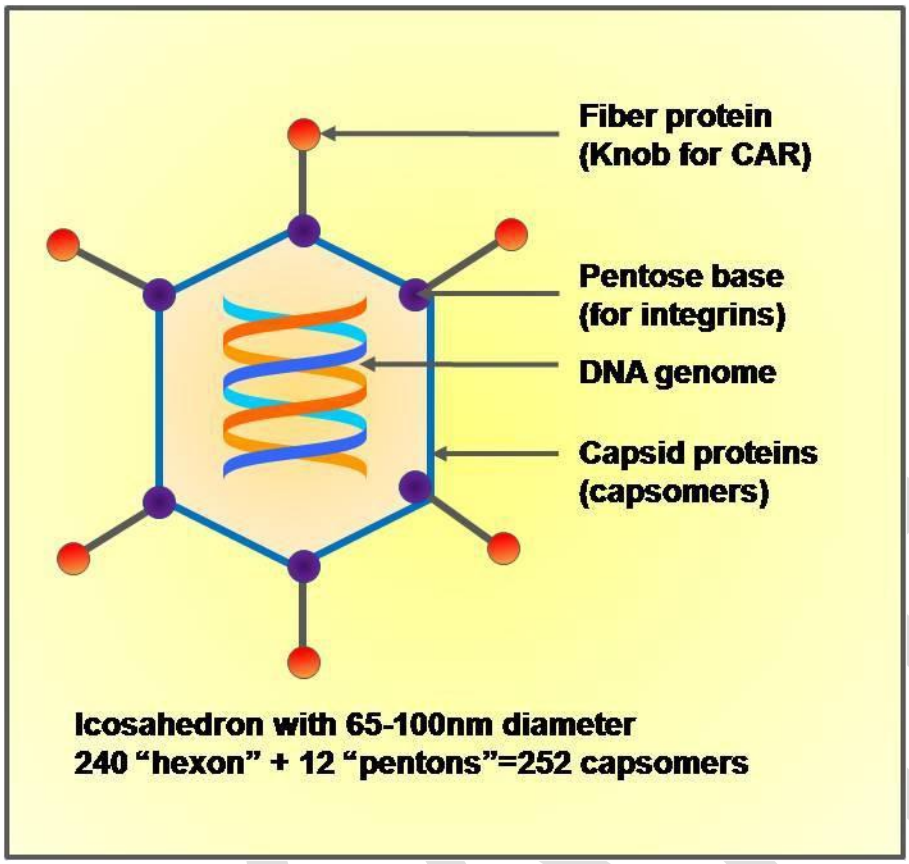
Adenovirus structure and genome organization

Adenoviruses are non-enveloped and icosahedral particles. They are 60-90 nm in diameter and contain 252 capsomers (240 hexons and 12 pentons) in the vertices of the icosahedrons.

The **hexons** on the virions are involved in the stabilization and assembly of the viral particle.

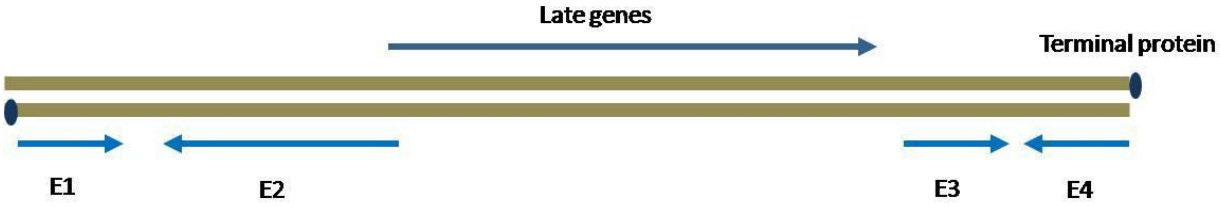
They contain a **penton** fiber that projects from each apex from the virion surface. The penton fiber consists of a shaft and a globular head. They are involved in the attachment of the virus to the surface of the host cell. They are very fragile and usually detached during preparation for electron microscopy.

Figure 38.1 Schematic representation of adenovirus virion:



Genome of adenovirus contains linear dsDNA of about 35 kb which encodes approximately 40 different proteins. The genome has inverted terminal repeats which are required during the replication process. The adenovirus DNA contains terminal protein at its 5' end. The early genes (E1-E4) are present towards either of the ends and are required to control the transcription and viral DNA replication. The late genes are generally associated with the viral structural proteins.

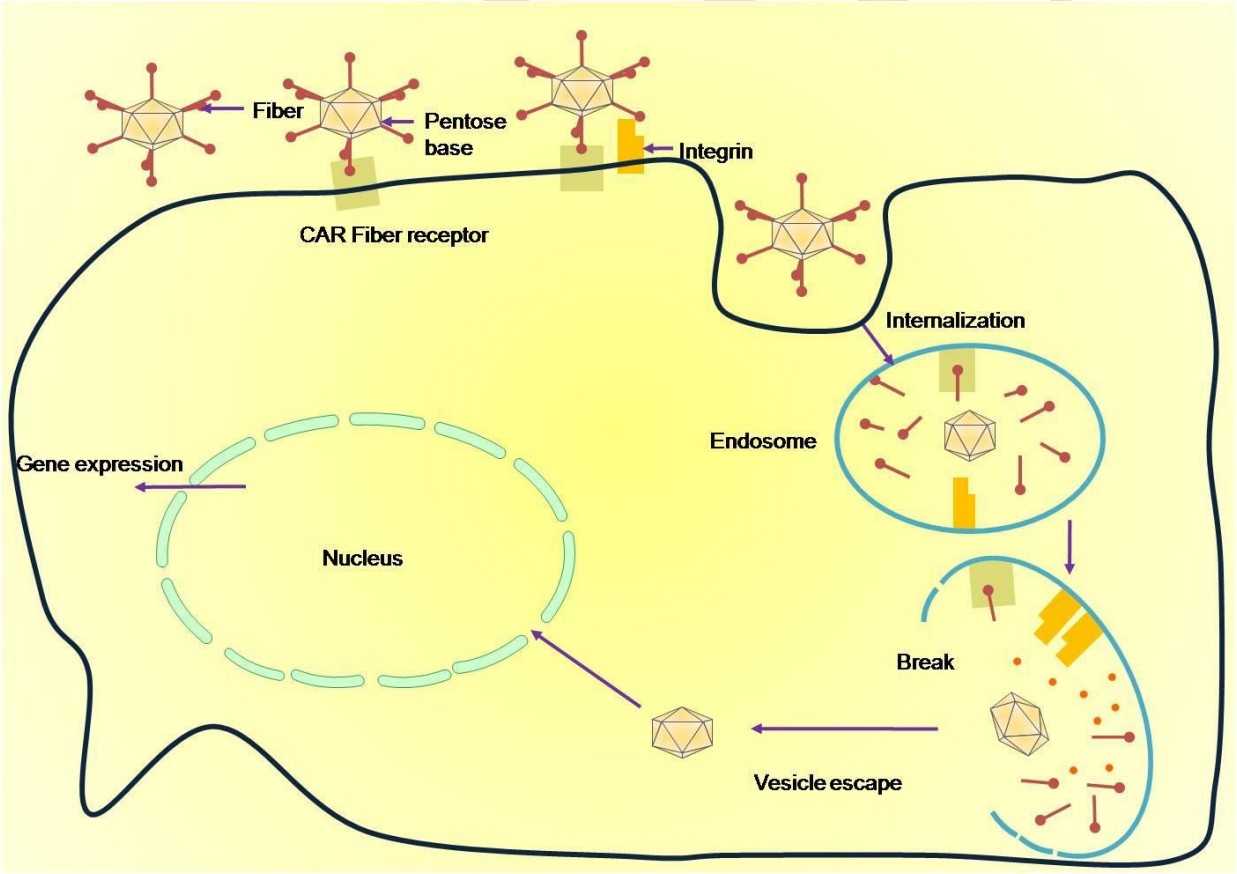
Figure 38.2 Adenovirus genome:



Adenovirus replication

To enter the cells they use a receptor present in the host cell called as **CAR** (coxsackie and adenovirus receptor). The internalization of the virus particles occur through receptor mediated endocytosis. After entry the endosome containing the virus particle migrates to nucleus and the genetic information of the virus is released into the nucleus. Transcription of the first gene is done by the terminal protein attached with the viral DNA. Viral mRNA is then transported to the cytoplasm and translated into the viral proteins. Virus assembly takes place in the cytoplasm and the mature viral particles get released from the infected cells after killing them by accumulated adenoviral death proteins.

Figure 38.3 Adenovirus life cycle:



Adenovirus associated diseases

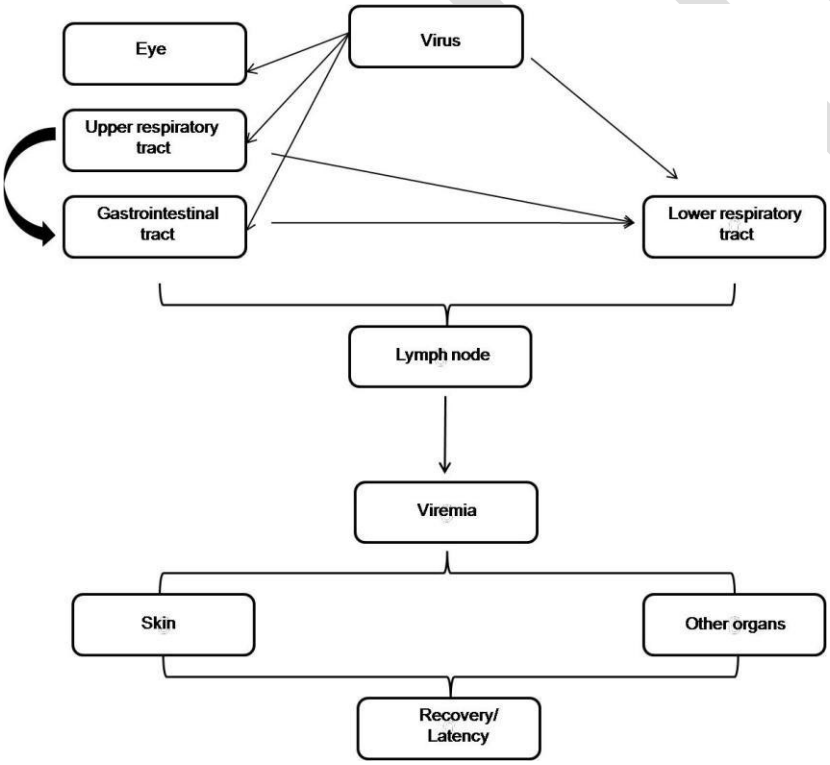
Respiratory diseases

In young children and infants it causes an acute febrile upper respiratory tract infection. It may progress to pneumonia and pharyngeal infection in untreated cases and in immuno-compromised individuals. In adults the symptoms include fever with pneumonia and pharyngitis.

Other diseases

In children the virus can cause acute gastroenteritis and hemorrhagic cystitis (inflammation of urinary bladder). Occasionally they may cause condition like meningoencephalitis in immuno-compromised patients. Sometimes they infect liver and eye leading to hepatitis and keratoconjunctivitis, respectively.

Adenovirus pathogenesis



Prevention and control

Currently no vaccine is available to protect against adenoviruses.

Good hygienic practices can prevent the infection.

Hand washing is still the best way to avoid adenovirus infection.

Wear protective clothing.

Heat and bleach will kill adenoviruses.

Adenoviruses are unusually stable to chemicals, physical agents, and adverse pH, causing them to survive longer in environment.

Poxviruses

Poxviruses belong to family *Poxviridae* and are among the complex viruses in the field of virology. The disease has a great historical impact; the first case of the poxvirus was reported about 2000 years ago in China. The virus produces a characteristic pock like lesions in the body (small pox). Last naturally occurring outbreak was reported in Somalia in 1977.

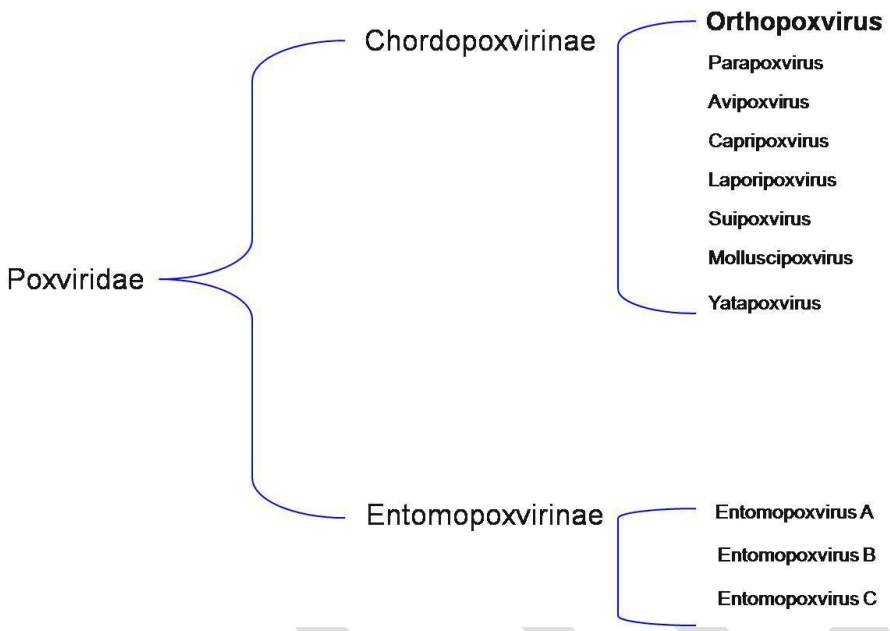
What characteristics of small pox made its eradication possible?

- Short incubation period
- No animal reservoir
- High morbidity and mortality
- Clinically apparent disease
- Mode of transmission
- An effective vaccine
- Social and economic factors

39.1 Classification

All human pox viruses are in the Chordopoxovirinae subfamily, and most of them belong to either the Orthopoxvirus (variola, vaccinia, cow pox) or the Parapoxvirus (Orf virus) genus.

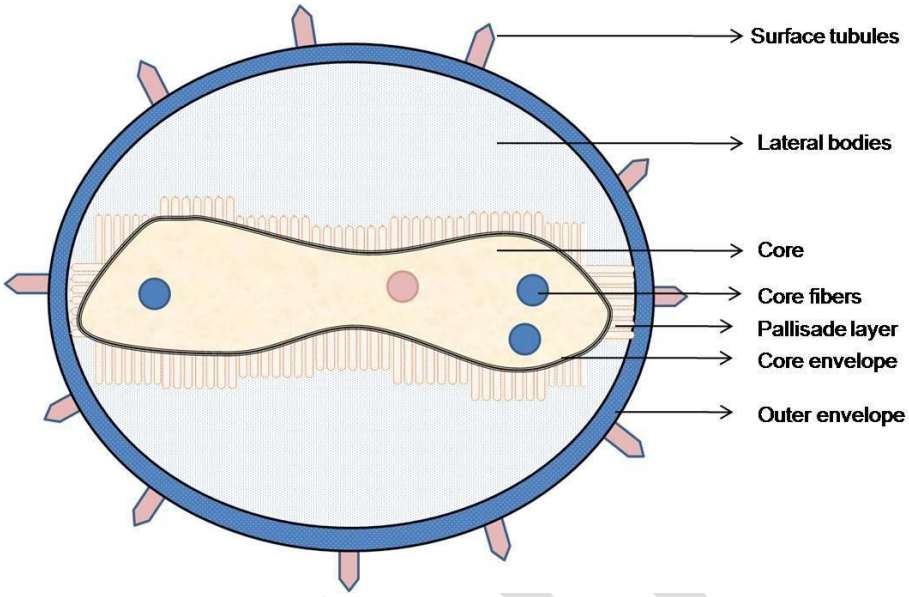
Figure 39.1 Classification of poxviruses:



39.2 Morphology

Virus is brick or oval shaped and around 300-400 nm in diameter. The viruses contain many proteins and are highly complex. The virus contains a lipid envelope that surrounds the core which is dumbbell shaped or biconcave. The virion may be beaded or smooth based on the presence or absence of surface tubules. Beaded form is converted into smooth form by the treatment of non-ionic detergent. The virus is present in both extracellular and intracellular form. The intracellular form contains a single envelope and is called as intracellular envelope virion (IEV) while the extracellular form has two envelopes and is called as extracellular envelope virion (EEV). Either side of the core (dumbbell shape) contains lateral bodies. The core is compactly packed with the genomic DNA. Antigenically, poxviruses are complex and produce a strong antibody response together with a long lasting memory. The genome of the virus contains dsDNA of about 130-300kbp. The terminal end of the viral genome contains inverted terminal repeats. More than 200 genes have been identified for the poxviruses; many of the essential genes are located in the center of the genome while non-essentials lie towards the ends.

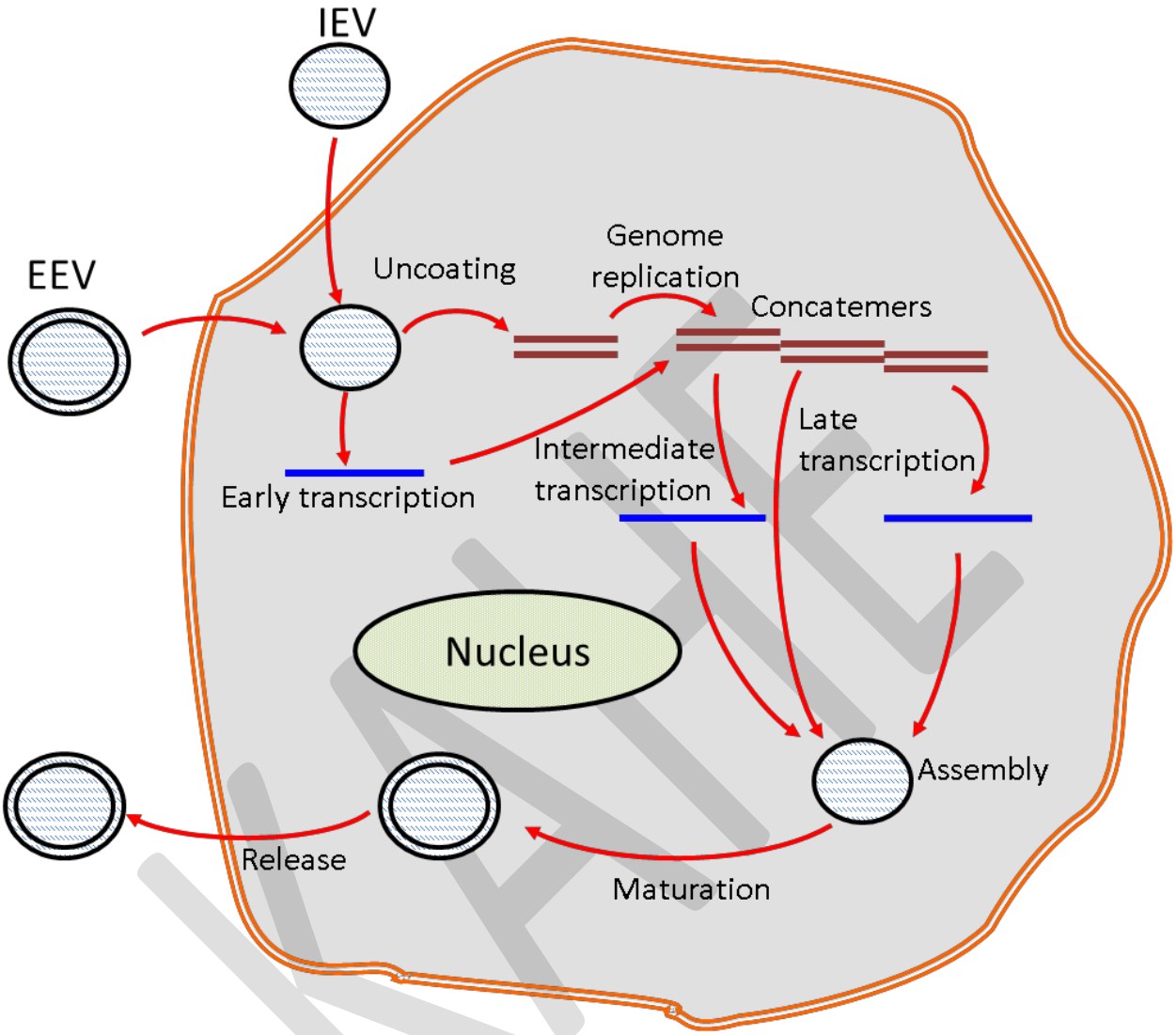
Figure 39.2 Poxvirus virion:



39.3 Replication

The replication of the genomes occurs in the cytoplasm. Many of the poxviruses attach to the cells with the help of **epidermal growth factor** as a receptor. Uncoating of the outer membrane occurs in the cytoplasm and genomic DNA is released into the cytoplasm. The virus contains both early and late gene based on its transcription preference. More than 50% of the early genes are transcribed before the DNA replication while late genes are transcribed after the completion of DNA replication. Many virus encoded enzymes help in the replication of DNA, concatemers are formed during the replication that later on cleave to form viral genome.

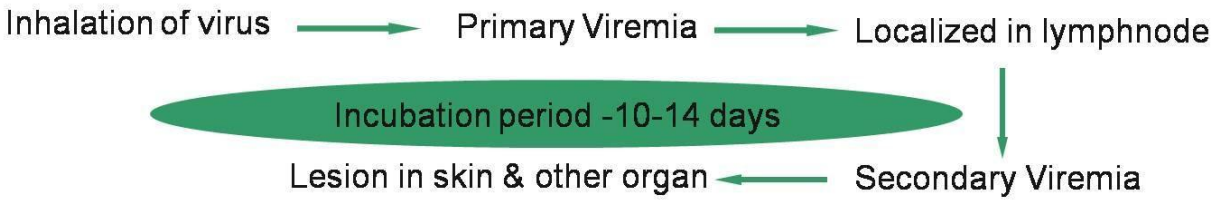
Figure 39.3 Life cycle of poxviruses in infected cell:



39.4 Transmission

In poxviruses, transmission is through direct contact. In case of small pox, the virus is found in lesions in the upper respiratory tract, which can be transmitted to others in droplet secretions, and in skin lesions. Route of transmission makes its spread relatively slow. The mechanical transmission of the virus by flies is also reported.

39.5 Pathogenicity



39.6 Important poxviruses

39.6.1 Vaccinia virus:

The virus causes a wide spread infection in animal and humans. The causative agent is an Orthopoxvirus. Symptoms of the disease includes pustular lesion in the teat and udder of the dairy cattle. Outbreaks in human produce lesions in hands and face of milkers who are not protected from smallpox.

39.6.2 Monkeypox virus

Monkey pox virus is a zoonotic agent with a wide host range. The virus was first reported in Democratic Republic of Congo. The signs of the disease include pustular rashes in the body, high fever and enlargement of lymph nodes.

Miscellaneous viruses

Infectious diseases have played a significant role throughout the history of mankind. Investigation of diseases dates back to ancient times and the query to understand it through science has lead to the discovery of viruses and bacteria as the causative agents of various types of infection and illness. Pathogenicity of viruses and susceptibility of host to infectious agents have constantly appeared through the emergence of new diseases and reappearance of pre-existing diseases. **Emerging infectious viral** diseases are those that have recently appeared in a population as a result of a new virus or the recognition of a previously undetected virus and are often zoonotic. Emergence of an infectious viral disease may occur due to the extension of the geographic or host range of the virus. Recently, bats have been implicated as an important reservoir and source of many emerging viruses. As new technology for detection of viruses becomes increasingly available, more viruses are likely to be detected. Enhanced molecular biology techniques will allow faster and more complete

characterization of new and miscellaneous viruses.

40.1 Bat paramyxoviruses

Bats have been shown to be the reservoir hosts of a variety of viruses responsible for severe disease outbreaks in humans and animals, including filoviruses, coronaviruses and paramyxoviruses. Recently Hendra and Nipah viruses were also isolated from bats.

Hendra and Nipah viruses are zoonotic viruses of the genus *Henipavirus* under the family *Paramyxoviridae.* The natural reservoirs for both the viruses are fruit bats or flying foxes of the genus *Pteropus.* Hendra virus was first isolated from an acute febrile illness in horses and subsequently in humans with a sign of fatal encephalitis. The first known human infections with Nipah virus were detected during an outbreak of severe febrile encephalitis in peninsular Malaysia and Singapore.

Menangle virus was isolated during an outbreak of reproductive disease in pigs in New South Wales, Australia in 1997. Symptoms of the disease included malaise, chills, fever, sweating, headache, weight loss and decrease in farrowing rate (birth giving process in pigs).

Tioman virus was isolated from the urine of fruit bats (*Pteropus hypomelanus*). Tioman virus is lethal in suckling mice 8-12 days post intracerebral inoculation. There role in human and animal infection is still under debate.

Mapuera virus was isolated from the salivary glands of an asymptomatic fruit bat (Sturnira lilium) in 1979 in Brazil.

40.2 Canine Distemper Virus

Canine distemper virus is an important pathogen which naturally infects a broad range of terrestrial and marine carnivores. Canine distemper virus is a member of genus *Morbillivirus* of the family *Paramyxoviridae*. The disease is characterized by skin rash, fever, gastrointestinal and respiratory signs, and a profound immune-suppression as well as by frequent neurological complications.

40.3 Rift Valley Fever Virus

Rift Valley Fever Virus (RVFV) is a member of the genus *Phlebovirus* (family *Bunyaviridae*). The RVFV is transmitted by the bite of mosquitoes. The disease was first reported in sheep in Kenya in 1918. Infection of RVFV is characterized by febrile illness with hemorrhages and inflammation of brain.

40.4 Hantavirus

Hantavirus belongs to the family *Bunyaviridae* (negative-sense, single-stranded RNA viruses). Hantavirus is transmitted by rodents (deer mice) via their urine and feces. Hantavirus is a cause of hemorrhagic fever with a renal (Kidney) syndrome. The early symptoms of the disease are similar to flu and include fever, chills, cough and muscle ache. The disease can progress to Hantavirus pulmonary syndrome.

40.5 Ebola Virus

Ebola virus belongs to family of RNA viruses called the *Filoviridae*. The virus leads to fatal hemorrhagic disease in humans and nonhuman primates. The transmission of the virus occurs by direct contact with the blood and/or secretions of an infected person. Sudden onset of illness is characterized by fever, sore throat, headache, joint and muscle pain, and weakness, followed by diarrhea, vomiting, and abdominal pain. In highly fatal cases internal and external bleeding may be seen in the patients.

40.6 Arenaviruses

The family *Arenaviridae* contains the viruses which are usually associated with rodent-transmitted disease in humans. The virus particles are spherical with a diameter of around 110-130 nm. The virus contains negative strand RNA as a genetic material. Infection of Arenaviruses leads to hemorrhagic disease in humans that are often fatal.

Table 40.1 Different Arenavirus diseases:

Virus	Disease
Junin virus	Argentine hemorrhagic fever
Lassa virus	Lassa fever
Guanarito virus	Venezuelan hemorrhagic fever
Machupo virus	Bolivian hemorrhagic fever

40.7 Prions

Prions are the infectious agents made up of only proteins (No DNA or RNA) and were discovered by **Stanley Prusiner.** Prions are propagated by transmitting the misfolded form of the protein. Prion diseases or transmissible spongiform encephalopathies (TSEs) are a family of rare progressive neurodegenerative disorders of humans and animals. They are characterized by long incubation periods, spongiform changes in brain, and a failure to induce inflammatory response.

Table 40.2 Different prion diseases in human and animals:

Human Prion disease	Animal Prion disease
Creutzfeldt- Jakob Disease (CJD)	Scrapie
Kuru	Mad Cow Disease (Bovine Spongiform Encephalopathy)
Gerstmann- Straussler- Scheinker Syndrome	Chronic Wasting Disease
Transmissible mink encephalopathy	

40.8 Viroids

Viroids are plant pathogen that contains circular single stranded RNA as a genetic material. They are discovered by **Theodor Diener** in 1971. Viroids contain small RNA of around 250 to 500 nt and do not encode any proteins. The **Potato spindle tuber viroid** was the first viroid to be identified.

CLASS: I MSc MB **COURSE NAME: VIROLOGY**

COURSE CODE: 17MBP201 **UNIT: III**

Animal viruses - RNA viruses - morphology, replication, pathogenesis and laboratory diagnosis of Poliovirus. Rabies virus, Influenza virus, mumps virus, Measles virus and rubella virus, Retro virus - HIV virus. Dengue and Japanese Encephalitis, SARS, Swine Flu.

Bacteriophage

A **bacteriophage** /ˈbækˈtɪər.i.oʊˌfeɪdʒ/ (informally, *phage* /ˈfeɪdʒ/) is a virus that infects and replicates within a bacterium. The term is derived from "bacteria" and the Greek: φαγεῖν (*phagein*), "to devour". Bacteriophages are composed of proteins that encapsulate a DNA or RNA genome, and may have relatively simple or elaborate structures. Their genomes may encode as few as four genes, and as many as hundreds of genes. Phages replicate within the bacterium following the injection of their genome into its cytoplasm. Bacteriophages are among the most common and diverse entities in the biosphere.

Phages are widely distributed in locations populated by bacterial hosts, such as soil or the intestines of animals. One of the densest natural sources for phages and other viruses is sea water, where up to 9×10^8 virions per milliliter have been found in microbial mats at the surface, and up to 70% of marine bacteria may be infected by phages. They have been used for over 90 years as an alternative to antibiotics in the former Soviet Union and Central Europe, as well as in France. They are seen as a possible therapy against multi-drug-resistant strains of many bacteria. Nevertheless, phages of Inoviridae have been shown to complicate biofilms involved in pneumonia and cystic fibrosis, shelter the bacteria from drugs meant to eradicate disease and promote persistent infection.

Classification

Bacteriophages occur abundantly in the biosphere, with different virions, genomes, and lifestyles. Phages are classified by the International Committee on Taxonomy of Viruses (ICTV) according to morphology and nucleic acid.

Nineteen families are currently recognized by the ICTV that infect bacteria and archaea. Of these, only two families have RNA genomes, and only five families are enveloped. Of the viral families with DNA genomes, only two have single-stranded genomes. Eight of the viral families with DNA genomes have circular genomes while nine have linear genomes. Nine families infect bacteria only, nine infect archaea only, and one (*Tectiviridae*) infects both bacteria and archaea.

Bacteriophage P22, a member of the *Podoviridae* by morphology due to its short, non-contractile tail.

ICTV classification of prokaryotic (bacterial and archaeal) viruses[1]

Order	Family	Morphology	Nucleic acid	Examples
Caudovirales	*Myoviridae*	Nonenveloped, contractile tail	Linear dsDNA	T4 phage, Mu, PBSX, P1Puna-like, P2, I3, Bcep 1, Bcep 43, Bcep 78
	Siphoviridae	Nonenveloped, noncontractile tail (long)	Linear dsDNA	λ phage, T5 phage, phi, C2, L5, HK97, N15
	Podoviridae	Nonenveloped, noncontractile tail (short)	Linear dsDNA	T7 phage, T3 phage, Φ29, P22, P37
Ligamenvirales	*Lipothrixviridae*	Enveloped, rod-shaped	Linear dsDNA	Acidianus filamentous virus 1
	Rudiviridae	Nonenveloped, rod-shaped	Linear dsDNA	Sulfolobus islandicus rod-shaped virus 1
Unassigned	*Ampullaviridae*	Enveloped, bottle-shaped	Linear dsDNA	
	Bicaudaviridae	Nonenveloped, lemon-shaped	Circular dsDNA	
	Clavaviridae	Nonenveloped, rod-shaped	Circular dsDNA	
	Corticoviridae	Nonenveloped, isometric	Circular dsDNA	
	Cystoviridae	Enveloped, spherical	Segmented dsRNA	
	Fuselloviridae	Nonenveloped, lemon-shaped	Circular dsDNA	

ICTV classification of prokaryotic (bacterial and archaeal) viruses[1]

Order	Family	Morphology	Nucleic acid	Examples
	Globuloviridae	Enveloped, isometric	Linear dsDNA	
	Guttaviridae	Nonenveloped, ovoid	Circular dsDNA	
	Inoviridae	Nonenveloped, filamentous	Circular ssDNA	M13
	Leviviridae	Nonenveloped, isometric	Linear ssRNA	MS2, Qβ
	Microviridae	Nonenveloped, isometric	Circular ssDNA	ΦX174
	Plasmaviridae	Enveloped, pleomorphic	Circular dsDNA	
	Tectiviridae	Nonenveloped, isometric	Linear dsDNA	

Table 1: Taxonomy of Bacteriophages

FAMILY	PROPERTIES	SHAPE
Myoviridae	Contractile tail	
Siphoviridae	Noncontractile long tail,	
Podoviridae	Short tail	
Microviridae	ssDNA (C), 27 nm, 12 knoblike capsomers	
Corticoviridae	dsDNA (C), complex capsid, lipids, 63 nm	
Tectiviridae	dsDNA (L), inner lipid vesicle, pseudo-tail, 60 nm	
Leviviridae	ssRNA (L), 23 nm, like poliovirus	
Cystoviridae	dsRNA (L), segmented, lipidic envelope, 70–80 nm	
Inoviridae	ssDNA (C), filaments or rods, 85–1950 x 7 nm	
Plasmaviridae	dsDNA (C), lipidic envelope, no capsid, 80 nm	

History

In 1896, Ernest Hanbury Hankin reported that something in the waters of the Ganges and Yamuna rivers in India had marked antibacterial action against cholera and could pass through a very fine porcelain filter In 1915, British bacteriologist Frederick Twort, superintendent of the Brown Institution of London, discovered a small agent that infected and killed bacteria. He believed the agent must be one of the following:

1. a stage in the life cycle of the bacteria;
2. an enzyme produced by the bacteria themselves; or
3. a virus that grew on and destroyed the bacteria.

Twort's work was interrupted by the onset of World War I and shortage of funding. Independently, French-Canadian microbiologist Félix d'Hérelle, working at the Pasteur Institute in Paris, announced on 3 September 1917, that he had discovered "an invisible, antagonistic microbe of the dysentery bacillus". For d'Hérelle, there was no question as to the nature of his discovery: "In a flash I had understood: what caused my clear spots was in fact an invisible microbe … a virus parasitic on bacteria." D'Hérelle called the virus a bacteriophage or bacteria-eater (from the Greek *phagein* meaning to eat). He also recorded a dramatic account

of a man suffering from dysentery who was restored to good health by the bacteriophages.[8] It was D'Herelle who conducted much research into bacteriophages and introduced the concept of phage therapy.[9]

In 1969, Max Delbrück, Alfred Hershey and Salvador Luria were awarded the Nobel Prize in Physiology and Medicine for their discoveries of the replication of viruses and their genetic structure.]

Structure

Bacteriophages come in different sizes and shapes but most of them have the same basic features: a head or capsid and a tail. A bacteriophage's head structure, regardless of its size or shape, is made up of one or more proteins which protectively coats the nucleic acid. Though there are some phages that don't have a tail, most of them do have one attached to its head structure. It is a hollow tube through which the nucleic acid passes through when the bacteriophage infects a host cell. Some of the more complex phages such as T4 have a tail with a base plate as well as one or more tail fibers that aid the phage in attaching itself to a bacterial cell

How Bacteriophages Work

In order to infect a host cell, the bacteriophage attaches itself to the bacteria's cell wall, specifically on a receptor found on the bacteria's surface. Once it becomes tightly bound to the cell, the bacterial virus injects its genetic material (its nucleic acid) into the host cell. Depending on the type of phage, one of two cycles will occur – the lytic or the lysogenic cycle. During a lytic cycle, the phage will make use of the host cell's chemical energy as well as its biosynthetic machinery in order to produce phage nucleic acids (phage DNA and phage mRNA) and phage proteins. Once the production phase is finished, the phage nucleic acids and structural proteins are then assembled. After a while, certain proteins produced within the cell will cause the cell wall to lyse, allowing the assembled phages within to be released and to infect other bacterial cells.

Viral reproduction can also occur through the lysogenic cycle. The main difference between the two types of cycles is that during lysogeny, the host cell is not destroyed or does not undergo lysis. Once the host cell is infected, the phage DNA integrates or combines with the bacterial chromosome, creating the prophage. When the bacterium reproduces, the prophage is replicated along with the host chromosomes. Thus, the daughter cells also contain the prophage which carries the potential of producing phages. The lysogenic cycle can continue indefinitely (daughter cells with prophage present within continuing to replicate) unless exposed to adverse conditions which can trigger the termination of the lysogenic state and cause the expression of the phage DNA and the start of the lytic cycle. These adverse conditions include exposure to UV or mutagenic chemicals and desiccation.

Applications

Bacteriophages have several applications. In some countries such as Russia and other Eastern European nations, phages are used therapeutically for the treatment of pathogenic bacterial infections that are resistant to antibiotics. Also known as phage therapy, this method involves the use of a phage to destroy the infective bacteria such as E. coli or salmonella. Bacteriophage is also used in identifying pathogenic bacteria (also called phage typing) in diagnostic laboratories. One other use for bacteriophages is for killing specific bacteria found

in food. For example, LISTEX by Micreos is made up of bacteriophages that can kill the L. monocytogenes bacteria in cheese.Viruses that attack bacteria were observed by Twort and d'Herelle in 1915 and 1917. They observed that broth cultures of certain intestinal bacteria could be dissolved by addition of a bacteria-free filtrate obtained from sewage. The lysis of the bacterial cells was said to be brought about by a virus which meant a "filterable poison" ("virus" is Latin for "poison").

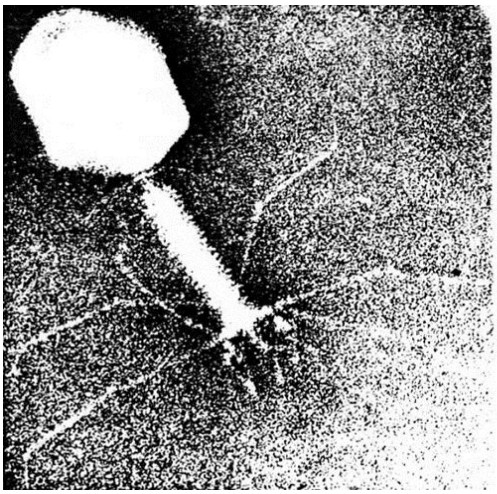
Probably every known bacterium is subject to infection by one or more viruses or "bacteriophages" as they are known ("phage" for short, from Gr. "phagein" meaning "to eat" or "to nibble"). Most research has been done on the phages that attack *E. coli*, especially the T-phages and phage lambda.

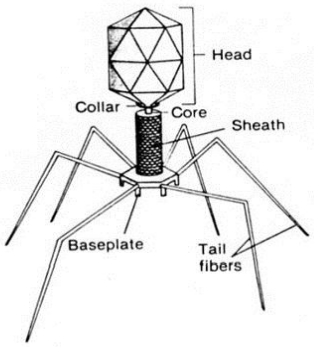
Like most viruses, bacteriophages typically carry only the genetic information needed for replication of their nucleic acid and synthesis of their protein coats. When phages infect their host cell, the order of business is to replicate their nucleic acid and to produce the protective protein coat. But they cannot do this alone. They require precursors, energy generation and ribosomes supplied by their bacterial host cell.

Bacterial cells can undergo one of two types of infections by viruses termed **lytic infections** and **lysogenic (temperate)** infections. In *E. coli*, lytic infections are caused by a group seven phages known as the T-phages, while lysogenic infections are caused by the phage lambda.

Lytic Infections

The T-phages, T1 through T7, are referred to as lytic phages because they always bring about the lysis and death of their host cell, the bacterium *E. coli*. T-phages contain double-stranded DNA as their genetic material. In addition to their protein coat or capsid (also referred to as the "head"), T-phages also possess a tail and some related structures. A diagram and electron micrograph of bacteriophage T4 is shown below. The tail includes a core, a tail sheath, base plate, tail pins, and tail fibers, all of which are composed of different proteins. The tail and related structures of bacteriophages are generally involved in attachment of the phage and securing the entry of the viral nucleic acid into the host cell.

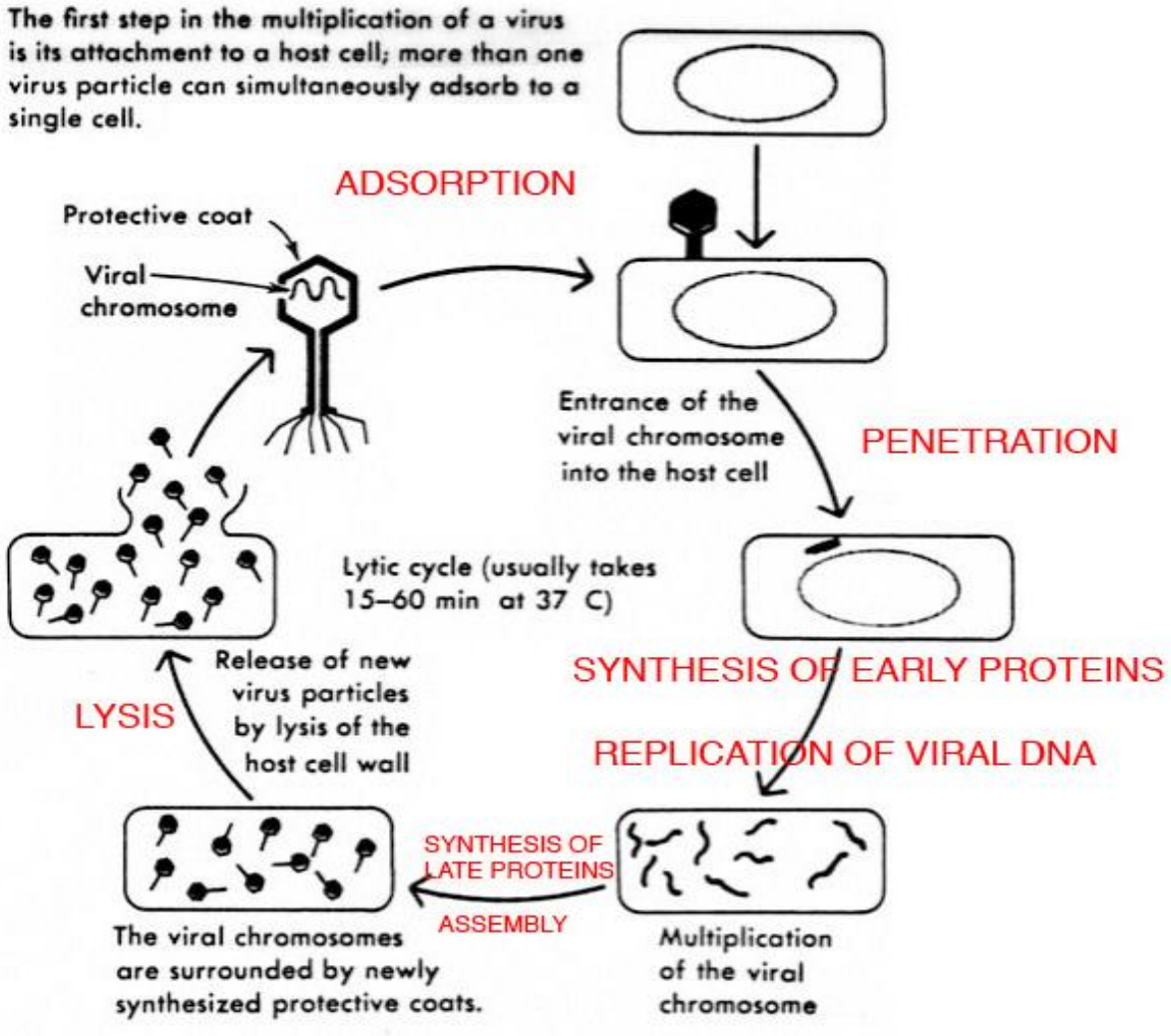




Left. Electron Micrograph of bacteriophage T4. Right. Model of phage T4. The phage possesses a genome of linear ds DNA contained within an icosahedral head. The tail consists of a hollow core through which the DNA is injected into the host cell. The tail fibers are involved with recognition of specific viral "receptors" on the bacterial cell

surface.

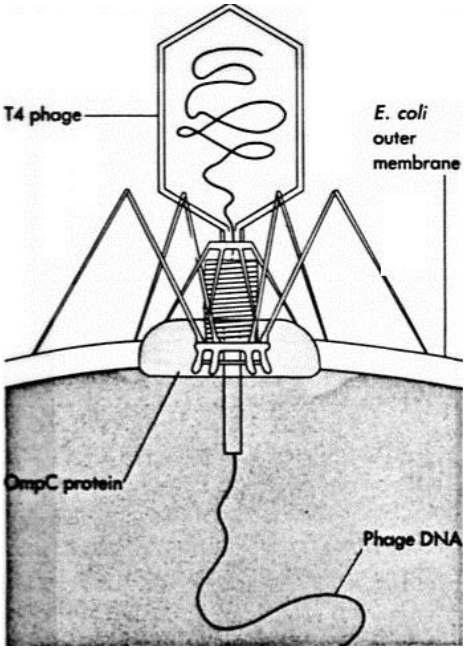
Before viral infection, the cell is involved in replication of its own DNA and transcription and translation of its own genetic information to carry out biosynthesis, growth and cell division. After infection, the viral DNA takes over the machinery of the host cell and uses it to produce the nucleic acids and proteins needed for production of new virus particles. Viral DNA replaces the host cell DNA as a template for both replication (to produce more viral DNA) and transcription (to produce viral mRNA). Viral mRNAs are then translated, using host cell ribosomes, tRNAs and amino acids, into viral proteins such as the coat or tail proteins. The process of DNA replication, synthesis of proteins, and viral assembly is a carefully coordinated and timed event. The overall process of lytic infection is diagrammed in the figure below. Discussion of the specific steps follows.



The lytic cycle of a bacterial virus, e.g. bacteriophage T4.

The first step in the replication of the phage in its host cell is called **adsorption**. The phage particle undergoes a chance collision at a chemically complementary site on the bacterial surface, then adheres to that site by means of its tail fibers.

Following adsorption, the phage injects its DNA into the bacterial cell. The tail sheath contracts and the core is driven through the wall to the membrane. This process is called penetration and it may be both mechanical and enzymatic. Phage T4 packages a bit of lysozyme in the base of its tail from a previous infection and then uses the lysozyme to degrade a portion of the bacterial cell wall for insertion of the tail core. The DNA is injected into the periplasm of the bacterium, and generally it is not known how the DNA penetrates the membrane. The adsorption and penetration processes are illustrated below.



Adsorption, penetration and injection of bacteriophage T4 DNA into an*E. coli* cell. T4 attaches to an outer membrane porin protein, ompC.

Immediately after injection of the viral DNA there is a process initiated called **synthesis of early proteins**. This refers to the transcription and translation of a section of the phage DNA to make a set of proteins that are needed to replicate the phage DNA. Among the early proteins produced are a repair enzyme to repair the hole in the bacterial cell wall, a DNAase enzyme that degrades the host DNA into precursors of phage DNA, and a virus specific DNA polymerase that will copy and replicate phage DNA. During this period the cell's energy-generating and protein-synthesizing abilities are maintained, but they have been subverted by the virus. The result is the **synthesis of several copies of the phage DNA**.

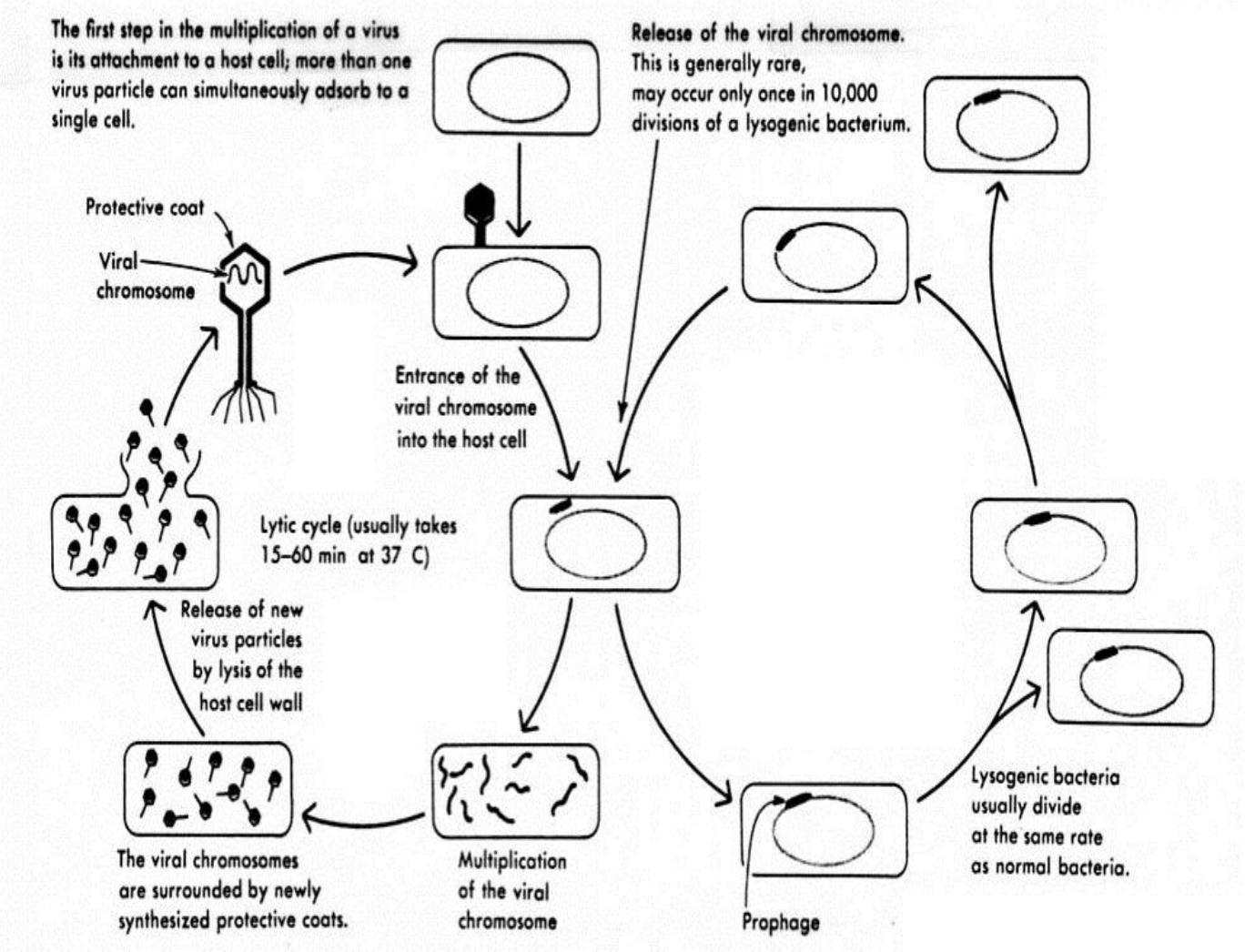
The next step is the synthesis of late proteins. Each of the several replicated copies of the phage DNA can now be used for transcription and translation of a second set of proteins called the **late proteins**. The late proteins are mainly structural proteins that make up the capsomeres and the various components of the tail assembly. Lysozyme is also a late protein that will be packaged in the tail of the phage and be used to escape from the host cell during the last step of the replication process.

Having replicated all of their parts, there follows an **assembly** process. The proteins that make up the capsomeres assemble themselves into the heads and "reel in" a copy of the phage DNA. The tail and accessory structures assemble and incorporate a bit of lysozyme in the tail plate. The viruses arrange their escape from the host cell during the assembly process.

While the viruses are assembling, lysozyme is being produced as a late viral protein. Part of this lysozome is used to escape from the host cell by lysing the cell wall peptiodglycan from the inside. This accomplishes the **lysis of the host cell** and the **release of the mature viruses**, which spread to nearby cells, infect them, and complete the cycle. The life cycle of a T-phage takes about 25-35 minutes to complete. Because the host cells are ultimately killed by lysis, this type of viral infection is referred to as lytic infection.

Lysogenic Infections

Lysogenic or temperate infection rarely results in lysis of the bacterial host cell. Lysogenic viruses, such as lambda which infects *E. coli*, have a different strategy than lytic viruses for their replication. After penetration, the virus DNA integrates into the bacterial chromosome and it becomes replicated every time the cell duplicates its chromosomal DNA during normal cell division. The life cycle of a lysogenic bacteriophage is illustrated below.



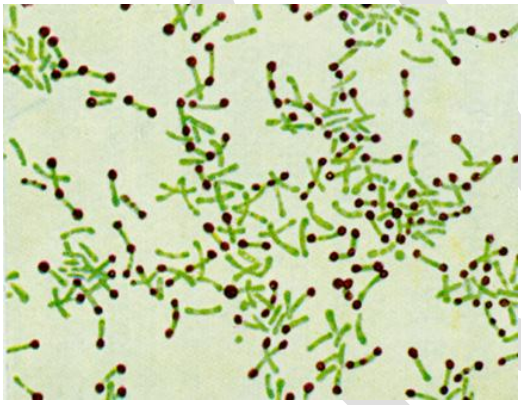
The lysogenic cycle of a temperate bacteriophage such as lambda.

Temperate viruses usually do not kill the host bacterial cells they infect. Their chromosome becomes

integrated into a specific section of the host cell chromosome. Such phage DNA is called **prophage** and the host bacteria are said to be **lysogenized**. In the prophage state all the phage genes except one are repressed. None of the usual early proteins or structural proteins are formed.

The phage gene that is expressed is an important one because it codes for the synthesis of a **repressor** molecule that prevents the synthesis of phage enzymes and proteins required for the lytic cycle. If the synthesis of the repressor molecule stops or if the repressor becomes inactivated, an enzyme encoded by the prophage is synthesized which excises the viral DNA from the bacterial chromosome. This excised DNA (the phage genome) can now behave like a lytic virus, that is to produce new viral particles and eventually lyse the host cell (see diagram above). This **spontaneous derepression** is a rare event occurring about one in 10,000 divisions of a lysogenic bacterium., but it assures that new phage are formed which can proceed to infect other cells.

Usually it is difficult to recognize lysogenic bacteria because lysogenic and nonlysogenic cells appear identical. But in a few situations, the prophage supplies genetic information such that the lysogenic bacteria exhibit a new characteristic (new phenotype), not displayed by the nonlysogenic cell, a phenomenon called **lysogenic conversion**. Lysogenic conversion has some interesting manifestations in pathogenic bacteria that only exert certain determinants of virulence when they are in a lysogenized state. Hence, *Corynebacterium diphtheriae* can only produce the toxin responsible for the disease if it carries a temperate virus called phage beta. Only lysogenized streptococci produce the erythrogenic toxin (pyrogenic exotoxin) which causes the skin rash of scarlet fever; and some botulinum toxins are synthesized only by lysogenized strains of *C. botulinum*.



***Corynebacterium diphtheriae* only produces diphtheria toxin when lysogenized by beta phage.*C. diphtheriae* strains that lack the prophage do not produce diphtheria toxin and do not cause the disease diphtheria. Surprisingly, the genetic information for production of the toxin is found to be on the phage chromosome, rather than the bacterial chromosome.**

A similar phenomenon to lysogenic conversion exists in the relationship between an animal tumor virus and its host cell. In both instances, viral DNA is incorporated into the host cell genome, and there is a coincidental change in the phenotype of the cell. Some human cancers may be caused by viruses which establish a state in human cells analogous to lysogeny in bacteria.

The **Double-Stranded DNA (dsDNA) tailed phages**, or Caudovirales, account for 95% of all the phages reported in the scientific literature, and possibly make up the majority of phages on the planet. Nineteen families that infect bacteria and archaeaare currently recognized; of these, 15 have double-stranded DNA genomes.
Under the Baltimore classification scheme, the Caudovirales are group I viruses as they have double-stranded DNA (dsDNA) genomes, which can be anywhere from 18,000 base pairs to 500,000 base pairs in length. The virus particles have a distinct shape; each virion has an icosohedral head that contains the viral genome, and is attached to a flexible tail by a connector protein. The order encompasses a wide range of viruses, many of which contain genes of similar nucleotide sequence and function. Some tailed bacteriophage genomes can vary quite significantly in nucleotide sequence, however, even among the same genus. Due to their characteristic structure and possession of potentially homologous genes, it is believed these bacteriophages possess a common origin. There are at least 350 recognized species in this order.
Upon encountering a host bacterium, the tail section of the virion binds to receptorson the cell surface and delivers the DNA into the cell by use of an injectisome-like mechanism (an *injectisome* is a nanomachine that evolved for the delivery of proteins by type III secretion). The tail section of the virus punches a hole through the bacterial cell wall and plasma membrane and the genome passes down the tail into the cell. Once inside, the genes are expressed from transcripts made by the host machinery, using host ribosomes. Typically, the genome is replicated by use of concatemers, in which overlapping segments of DNA are made, and then put together to form the whole genome.
Viral capsid proteins come together to form a precursor prohead, into which the genome enters. Once this has occurred, the prohead undergoes maturation by cleavage of capsid subunits to form an icosohedral phage head with 5-fold symmetry. After the head maturation, the tail is joined in one of two ways: either the tail is constructed separately and joined with the connector, or the tail is constructed directly onto the phage head. The tails consist of helix-based proteins with 6-fold symmetry. After maturation of virus particles, the cell is lysed by lysins, holins, or a combination of the two.
Because the lack of homology between the amino acid and DNA sequences of these viruses precludes these from being used as taxonomic markers (as is common for other organisms), the three families here are defined on the basis of morphology. This classification scheme was originated by Bradley in 1969 and has since been extended. All viruses in this order have icosahedral or oblate heads, but differ in the length and contractile abilities of their tails. The Myoviridae have long tails that are contractile, the Podoviridae have short noncontractile tails, and the Siphoviridae have long non-contractile tails. Siphoviridae constitute the majority of the known tailed viruses .

phage T4 is a bacteriophage that infects Escherichia coli bacteria. The T4 phage is a member of the T-even phages, a group including enterobacteriophages T2 and T6. T4 is capable of undergoing only a lytic lifecycle and not the lysogenic lifecycle.
Genome and structure
The T4 phage's double-stranded DNA genome is about 169 kbp long[1] and encodes 289 proteins. The T4 genome is terminally redundant and is first replicated as a unit, then several genomic units are recombined end-to-end to form a concatemer. When packaged, the concatemer is cut at unspecific positions of the same length, leading to several genomes that represent circular permutations of the original.[2] The T4 genome bears eukaryote-like intron sequences.
Translation
The Shine-Dalgarno sequence GAGG dominates in bacteriophage T4 early genes, whereas the sequence GGAG is a target for the T4 endonuclease RegB that initiates the early mRNA degradation.
Virus particle structure
T4 is a relatively large phage, at approximately 90 nm wide and 200 nm long (most phages range from 25 to 200 nm in length). The DNA genome is held in an icosahedral head, also known as a capsid. The T4's tail is hollow so that it can pass its nucleic acid into the cell it is infecting after attachment. The tail attaches to a host cell with the help of tail fibres. The tail fibres are also important in recognizing host cell surface receptors, so they determine if a bacterium is within the phage's host range.

The structure of the 6 megadalton T4 baseplate that comprises 127 polypeptide chains of 13 different proteins (gene products 5, 5.4, 6, 7, 8, 9, 10, 11, 12, 25, 27, 48 and 53) has recently been described in atomic detail. An atomic model of the proximal region of the tail tube formed by gp54 and the main tube protein gp19 have also been created. The tape measure protein gp29 is present in the baseplate-tail tube complexes, but it could not be modeled.

Infection process

The T4 phage initiates an Escherichia coli infection by binding OmpC porin proteins and Lipopolysaccharide (LPS) on the surface of E. coli cells with its long tail fibers (LTF) A recognition signal is sent through the LTFs to the baseplate. This unravels the short tail fibers (STF) that bind irreversibly to the E. coli cell surface. The baseplate changes conformation and the tail sheath contracts, causing GP5 at the end of the tail tube to puncture the outer membrane of the cell. The lysozyme domain of GP5 is activated and degrades the periplasmic peptidoglycan layer. The remaining part of the membrane is degraded and then DNA from the head of the phage can travel through the tail tube and enter the E. coli cell.

Reproduction

The lytic lifecycle (from entering a bacterium to its destruction) takes approximately 30 minutes (at 37 °C) and consists of:

Adsorption and penetration (starting immediately)
Arrest of host gene expression (starting immediately)
Enzyme synthesis (starting after 5 minutes)
DNA replication (starting after 10 minutes)
Formation of new virus particles (starting after 12 minutes)

After the life cycle is complete, the host cell bursts open and ejects the newly built viruses into the environment, destroying the host cell. T4 has a burst size of approximately 100-150 viral particles per infected host. Complementation, deletion, and recombination tests can be used to map out the rII gene locus by using T4. These bacteriophage infect a host cell with their information and then blow up the host cell, thereby propagating themselves.

Replication and packaging

The rate of DNA replication in a living cell was measured as the rate of phage T4 DNA elongation in phage-infected E. coli.[8] During the period of exponential DNA increase at 37 °C, the rate was 749 nucleotides per second. The mutation rate per base pair per replication during phage T4 DNA synthesis is 1.7 per 10−8,[9] a highly accurate DNA copying mechanism, with only 1 error in 300 copies. The phage also codes for unique DNA repair mechanisms. The T4 DNA packaging motor has been found to load DNA into phage capsids at a rate up to 2000 base pairs per second. The power involved, if scaled up in size, would be equivalent to that of an average automobile engine.

Multiplicity reactivation

Survival curves for phage T4 with DNA damaged by UV (top) or MMC (bottom) after single phage T4 infecting host cells (monocomplexes) or two or more phage T4 simultaneously infecting host cells (multicomplexes).

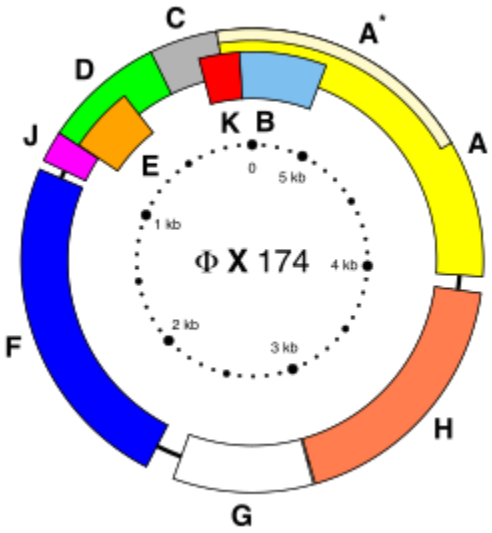
Multiplicity reactivation (MR) is the process by which two or more virus genomes, each containing inactivating genome damage, can interact within an infected cell to form a viable virus genome. Salvador Luria, while studying UV irradiated phage T4 in 1946, discovered MR and proposed that the observed reactivation of damaged phage occurs by a recombination mechanism. This preceded the confirmation of DNA as the genetic material in 1952 in related phage T2 by the Hershey–Chase experiment.[14]

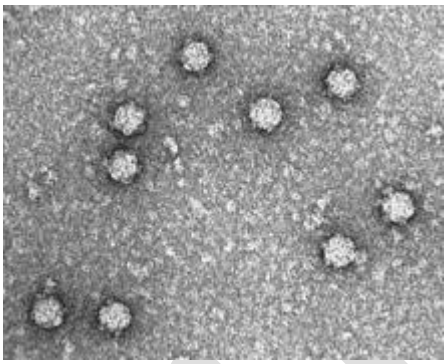
As remembered by Luria (1984) the discovery of reactivation of irradiated phage (referred to as "multiplicity reactivation") immediately started a flurry of activity in the study of repair of radiation damage within the early phage group . It turned out later that the repair of damaged phage by mutual help that Luria had discovered was only one special case of DNA repair. Cells of all types, not just, bacteria and their viruses, but all organisms studied, including humans, are now known to have complex biochemical processes for repairing DNA damages

(see DNA repair). DNA repair processes are also now recognized as playing critical roles in protecting against aging, cancer, and infertility.
MR is usually represented by "survival curves" where survival of plaque forming ability of multiply infected cells (multicomplexes) is plotted against dose of genome damaging agent. For comparison, the survival of phage plaque forming ability of singly infected cells (monocomplexes) is also plotted against dose of genome damaging agent. The top figure shows the survival curves for phage T4 multicomplexes and monocomplexes with increasing dose of UV light. Since survival is plotted on a log scale it is clear that survival of multicomplexes exceeds that of monocomplexes by very large factors (depending on dose). The UV inactivation curve for multicomplexes has an initial shoulder. Other phage T4 DNA damaging agents with shoulders in their multicomplex survival curves are X-rays and ethyl methane sulfonate (EMS). The presence of a shoulder has been interpreted to mean that two recombinational processes are used. The first one repairs DNA with high efficiency (in the "shoulder"), but is saturated in its ability as damage increases; the second pathway functions at all levels of damage. Surviving T4 phage released from multicomplexes show no increase in mutation, indicating that MR of UV irradiated phage is an accurate process.
The bottom figure shows the survival curves for inactivation of phage T4 by the DNA damaging agent mitomycin C (MMC). In this case the survival curve for multicomplexes has no initial shoulder, suggesting that only the second recombinational repair process described above is active. The efficiency of repair by this process is indicated by the observation that a dose of MMC that allows survival of only 1 in 1,000 monocomplexes allows survival of about 70% of multicomplexes. Similar multicomplex survival curves (without shoulders) were also obtained for the DNA damaging agents P32 decay, psoralen plus near-UV irradiation (PUVA), N-methyl-N'-nitro-N-nitrosoguanidine (MNNG), methyl methane sulfonate (MMS) and nitrous acid.
Several of the genes found to be necessary for MR in phage T4 proved to be orthologs for genes essential for recombination in prokaryotes, eukaryotes and archaea. This includes, for instance, T4 gene uvsX[20] which specifies a protein that has three-dimensional structural homology to RecA from Escherichia coli and the homologous protein RAD51 in eukaryotes and RadA in archaea. It has been suggested that the efficient and accurate recombinational repair of DNA damages during MR may be analogous to the recombinational repair process that occurs during meiosis in eukaryotes.

Bacteriophage phiX174

The **phi X 174** (or **ΦX174**) bacteriophage is a virus and was the first DNA-basedgenome to be sequenced. This work was completed by Fred Sanger and his team in 1977. In 1962, Walter Fiers and Robert Sinsheimer had already demonstrated the physical, covalently closed circularity of ΦX174 DNA. Nobel prize winner Arthur Kornberg used ΦX174 as a model to first prove that DNA synthesized in a test tube by purified enzymes could produce all the features of a natural virus, ushering in the age of synthetic biology. In 2003, it was reported by Craig Venter's group that the genome of ΦX174 was the first to be completely assembled *in vitro* from synthesized oligonucleotides. The ΦX174 virus particle has also been successfully assembled *in vitro*. Recently, it was shown how its highly overlapping genome can be fully decompressed and still remain functional.





This bacteriophage has a [+] circular single-stranded DNA genome of 5386nucleotides encoding 11 proteins. Of these 11 genes, only 8 are essential to viral morphogenesis. The GC-content is 44% and 95% of nucleotides belong to coding genes.

Protein	Copies	Function[
A	--	Nicks RF DNA to initiate rolling-circle replication; ligates ends of linear phage DNA to form single-stranded circular DNA
A*	--	Inhibits host cell DNA replication; blocks superinfecting phage; not essential
B	60 in procapsid	Internal scaffolding protein involved in procapsid assembly
C	--	DNA packaging
D	240 in procapsid	External scaffolding protein involved in procapsid assembly
E	--	Host cell lysis
F	60 in virion	Major capsid protein
G	60 in virion	Major spike protein
H	12 in virion	DNA pilot protein (or minor spike protein)
J	60 in virion	Binds to new single-stranded phage DNA; accompanies phage DNA into procapsid
K	--	Optimizes burst size; not essential

Infection begins when G protein binds to lipopolysaccharides on the bacterial host cell surface. H protein (or the DNA Pilot Protein) pilots the viral genome through the bacterial membrane of *E.coli* bacteria (Jazwinski et al. 1975) most likely via a predicted N-terminal transmembrane domain helix (Tusnady and Simon, 2001). However, it has become apparent that H protein is a multifunctional protein (Cherwa, Young and Fane, 2011). This is the only viral capsid protein of ΦX174 to lack a crystal structure for a couple of reasons. It has low aromatic content and high glycine content, making the protein structure very flexible and in addition, individual hydrogen atoms (the R group for glycines) are difficult to detect in protein crystallography. Additionally, H protein induces lysis of the bacterial host at high concentrations as the predicted N-terminal transmembrane helix easily pokes holes through the bacterial wall. By bioinformatics, this protein contains four predicted coiled-coil domains which has a significant homology to known transcription factors. Additionally, it was determined by Ruboyianes et al. (2009) that *de novo* H protein was required for optimal synthesis of other viral

proteins. Interestingly, mutations in H protein that prevent viral incorporation, can be overcome when excess amounts of Protein B, the internal scaffolding protein, are supplied.
The DNA is ejected through a hydrophilic channel at the 5-fold vertex (McKenna et al. 1992). It is understood that H protein resides in this area but experimental evidence has not verified its exact location. Once inside the host bacterium, replication of the [+] ssDNA genome proceeds via negative sense DNA intermediate. This is done as the phage genome supercoils and the secondary structure formed by such supercoiling attracts a primosome protein complex. This translocates once around the genome and synthesizes a [-]ssDNA from the positive original genome. [+]ssDNA genomes to package into viruses are created from this by a rolling circle mechanism. This is the mechanism by which the double stranded supercoiled genome is nicked on the negative strand by a virus-encoded A protein, also attracting a bacterial DNA Polymerase to the site of cleavage. DNAP will use the negative strand as a template to make positive sense DNA. As it translocates around the genome it displaces the outer strand of already-synthesised DNA, which is immediately coated by ssBP proteins. The A protein will cleave the complete genome every time it recognises the origin sequence.
As D protein is the most abundant gene transcript, it is the most protein in the viral procapsid. Similarly, gene transcripts for F, J, and G are more abundant than for H as the stoichiometry for these structural proteins is 5:5:5:1.The primosome are protein complexes which attach/bind the enzyme helicase on the template. primosomes gives RNA primers for DNA synthesis to strands.
Phi X is regularly used as a positive control in DNA sequencing due to its relatively small genome size in comparison to other organisms and the extensive work that has been done on it.

A Milestone at the PDB
The 10,000th entry in the Protein Data Bank, the bacteriophage phiX174, is a perfect example of how the science of protein structure has progressed in four decades. In 1960, the world got its first look at the structure of a protein. That first structure was the small protein myoglobin, composed of one protein chain and one heme group--about 1260 atoms in all. By contrast, the 10,000th entry in the PDB contains 420 protein chains and over half a million atoms. Enormous structures like this are not uncommon in the Protein Data Bank. The stakes have risen dramatically since the structure of myoglobin was first revealed.
Animal, Mineral, or Vegetable?
A bacteriophage is a virus that attacks bacteria. The phiX174 bacteriophage attacks the common human bacteria *Escherichia coli*, infecting the cell and forcing it to make new viruses. Do you think that viruses are living organisms? PhiX174 is composed of a single circle of DNA surrounded by a shell of proteins. That's all. It can inject its DNA into a bacterial cell, then force the cell to create many new viruses. These viruses then burst out of the cell, and go on to hijack more bacteria. By itself, it is like an inert rock. But given the proper bacterial host, it is a powerful reproducing machine. What do you think? Is it alive?
A Molecular Time Bomb
The capsid of phiX174 is designed to find bacterial cells, and then infect them with its DNA. Sixty copies of the capsid protein (colored red here) form a spherical shell around the DNA, and the spike proteins (colored orange here) form 12 pentagonal spikes on the surface. It is thought that the DNA is ejected through the middle of the spikes when the virus infects an *Escherichia coli* cell. The DNA itself encodes 11 genes. In order to fit into this tiny protein shell, however, the DNA is so short that the genes must actually overlap.
Assembling a Virus
As one can imagine, assembling 120 protein chains into a perfectly symmetrical shell is a difficult task. PhiX174 uses special scaffolding proteins to ensure that everything ends up in the right place. The capsid and spike proteins spontaneously form pentagonal units, with five copies of each chain. The scaffolding proteins (shown here in light blue and purple) then arrange these pentagon building-blocks into the whole icosahedron, complete with DNA inside. The scaffolding proteins are small, and bind to the inner and outer surfaces of the pentagons, aligning them one next to the other in the proper orientation.
The DNA Inside

PhiX174 has the distinction of being the first DNA genome sequence that was determined. The virus contains one piece of DNA, 5386 bases long, wrapped into a small circle. In the mature virus, this small circle of DNA is packaged inside the icosahedral protein shell, safe for delivery to an unfortunate bacterial target. The PDB entry 1cd3 includes atomic coordinates for the capsid proteins, but the DNA inside is not included. It does not conform to the beautiful icosahedral symmetry of the capsid, and thus cannot be resolved by x-ray crystallography. We must imagine it packed inside, trapped as the last pentamer closes the capsid.
Exploring the Structure
You can easily look at one of the subunits of this bacteriophage. There are seven separate chains in the PDB file 1cd3 . The spike protein, chain G, is small and compact and the capsid protein, chain F, is large. Both are very stable structures composed of two beta-sheets, forming a structure commonly called a "beta-sandwich." The ribbon diagram shows the chain of the spike protein. Notice how the beta-strands, each depicted with an arrow, arrange side-by-side to form the two sheets. Beta-sandwich structures like this are found in many different viruses.

Four copies of a small scaffolding protein (chains 1, 2, 3 and 4) are arranged in the angle between the capsid and spike proteins, ending up on the outside of the final virus capsid. Another small scaffold protein (chain B) is found on the inside of the capsid, where it assists in the capture of DNA. Compare this detailed atomic view of one subunit of the capsid to the picture of the whole capsid shown above, which contains 60 identical copies of each of these seven proteins.

The pictures were created with RasMol. You can create similar pictures by using one of the viewers on the page for PDB entry 1cd3 .
One step multiplication curve for bacteriophages.
The single-step growth experiment of Ellis and Delbruck demonstrates the cyclic replication of the phage. These authors devised a method to demonstrate only a single step of the many steps of phage replication. Essentially they drastically diluted the mixture after attachment of phage to bacteria, so when the infected cells lysed, no new host cells could be found for a second round of infection. A number of modifications have been introduced since the original experiment was reported. For instance, instead of diluting the initial bacterium:phage mixture, antibodies specific for the phage attachment apparatus may be added to the mixture to

'neutralize' and thus render all of the unadsorbed phage unable to adsorb to any bacterium.
How do you perform this experiment:
Bacteriophages are infected with a very large number of phage particles: The large number of phage ensures all bacteriem are rapidly infected. The high level of infection is called multiplicity of infection (MOI) and can be achieved with a phage to host ratio of 5 to 10 plaque forming units (PFU) per cell. Adsorption of virions to cells is allowed to proceed for suitable time : To replicate, a virus should induce its host to synthesize components that are necessary for the assembly of new virus particles. The virus accomplishes this process by first attaching to the host (adsorption) and then injecting its nucleic acid into the cell (injection or penetration). The viral DNA can stay free in the cell and be replicated as such, or it can be incorporated into the host chromosome and be replicated simultaneously with it. Viral proteins are next synthesized with the host's machinery under the direction of viral DNA and the new virus particles are assembled mechanically. These particles can find their way out of the cell or lyse the cell and be released into the medium, ready to infect new cells.
The phage/cell mixture is then diluted synchronizing the infection or adding antivirus antiserum. This stop the absorption of virions to cells that are infected and also prevent infection of new cells other than those that has been infected. Antivirus antiserum contains antibodies directed against the virus. It binds and occupy all the attachment sites of the viral particles so that no new bacteria cells can attach a virus. As the infection of bacteriophages is synchronised, the interaction of virus with the cell population can be seen as a single interaction between phage and the cell. In order to visualize the infection over time, samples are removed at specified intervals and plated to quantitate the phage present in the culture.

At the start of the experiment, the plaque count is relatively constant over a time period because each infected bacterium will yield only one plaque. A rise in plaque forming units (pfu) to a plateau level occurs as bacteria are lysed and the newly synthesized phage are released into the medium. These phage particles fail to meet susceptible bacteria (due to the dilution of the adsorption mixture) and thus remain free in the culture fluid. The average number of phage released per bacterium is called the **burst size** and this value may be calculated from the data. The burst size varies in accordance with the specific virus, and may range from 10 to 100 for the DNA transducing phages to approximately 20,000 pfu for the RNA viruses. Plaque assay for bacteriophages are performed by mixing the phage into a layer of bacteria which are spread out as an overlay on the surface of an agar plate. As the plate is incubated, the bacteria grow and they become visible as a turbid layer on the plate. When a phage infects bacteria cells, a zone of Lysis or growth inhibition can occur. This produces clear zone in the bacterial lawn known as **plaque.** Each plaque originates from a single phage particle. If the number of phage particles was monitored during growth, a growth curve could be drawn which would be similar to that of the bacterial growth curve except in the last stage.

The phage growth curve starts with a latent or eclipse period (similar to the bacterial lag phase). During this phase, the infection, adsorption, injection and syntheses of new viral DNA and protein coat occur. The next phase is called the maturation or release stage (similar to the log phase in bacteria) when new phage particles are assembled and released. The cycle can then start over with the infection of new cells. In this manner, the shape of the curve would look step-wise and that is why the process is called "one-step phage growth curve".

Stages of one step multiplication curve:

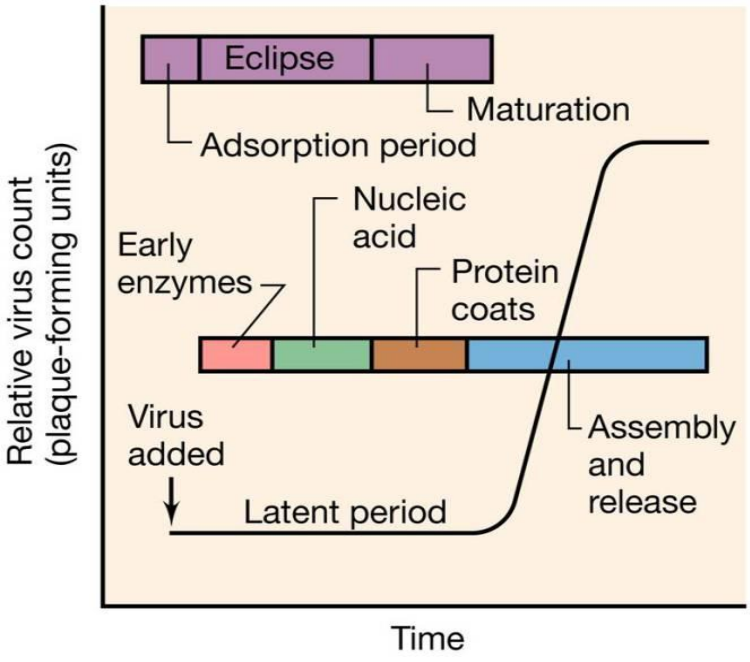
Eclipse: or initial period can be defined as the time period taken for the appearance of first intracellular phages. No phage particles can be detected during this period as the phages are being uncoated and phage DNA is being injected foe replication.

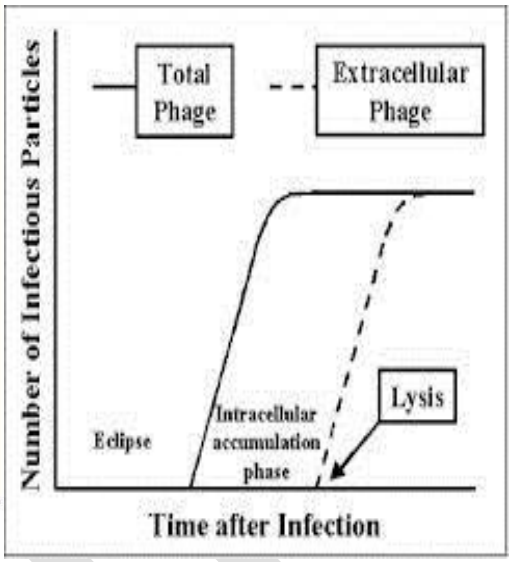
Synthetic period: During synthetic period intracellular particles are being produced. As in the Eclipse period, there are no phage particles released during the synthetic period.

Latent Period: The first two periods are combined in the third period known as latent period. The latent period is described as the time period prior to the release of infection particles or appearance of extra cellular phages. In the latent period, attachment, entry, replication, transcription, translation and assembly of progeny phages occur.

Rise Period: In this period lysis occurs and extracellular phages appear and they increase in number of concentration of bacteriophages rises.

- **Burst Size:** Average yield of infectious virus per cell is called burst size.
- Burst Size = Final titre of virus / Initial viral titre.
- There is much variation in bursts size between different kind of cells.
- In one study with phage, a burst size of 170 was obtained when growing bacteria cells and value of 20 was obtained with resting bacteria cells. This is because rapid growing cells means, its cell machinery are active and are metabolically active than the resting bacteria cell. So yield of infectious virus increases with growing cells than resting bacteria cell.
- Extra step, **lysis from without (LO):** LO described as an early lysis of bacteria induced by high-multiplicity virion adsorption and that occurs without phage production and leads to killing of bacteria. LO can be induced by adding chloroform, which break open the host cell and the intracellular phages are released. It is an artificial lysis.





Phage therapy

Phages were discovered to be antibacterial agents and were used in the former Soviet Republic of Georgia (pioneered there by Giorgi Eliava with help from the co-discoverer of bacteriophages, Felix d'Herelle) and the United States during the 1920s and 1930s for treating bacterial infections. They had widespread use, including treatment of soldiers in the Red Army. However, they were abandoned for general use in the West for several reasons:

- Medical trials were carried out, but a basic lack of understanding of phages made these invalid.[11]
- Antibiotics were discovered and marketed widely. They were easier to make, store and to prescribe.
- Former Soviet research continued, but publications were mainly in Russian or Georgian languages and were unavailable internationally for many years.
- Clinical trials evaluating the antibacterial efficacy of bacteriophage preparations were conducted without proper controls and were methodologically incomplete preventing the formulation of important conclusions.

Their use has continued since the end of the Cold War in Georgia and elsewhere in Central and Eastern Europe. Globalyz Biotech is an international joint venture that commercializes bacteriophage treatment and its various applications across the globe. The company has successfully used bacteriophages in administering Phage therapy to patients suffering from bacterial infections, including: Staphylococcus (including MRSA), Streptococcus, Pseudomonas, Salmonella, skin and soft tissue, gastrointestinal, respiratory, and orthopedic infections. In 1923, the Eliava Institute was opened in Tbilisi, Georgia, to research this new science and put it into practice.

The first regulated, randomized, double-blind clinical trial was reported in the Journal of Wound Care in June 2009, which evaluated the safety and efficacy of a bacteriophage cocktail to treat infected venous leg ulcers in human patients. The FDA approved the study as a Phase I clinical trial. The study's results demonstrated the safety of therapeutic application of bacteriophages but did not show efficacy. The authors explain that the use of

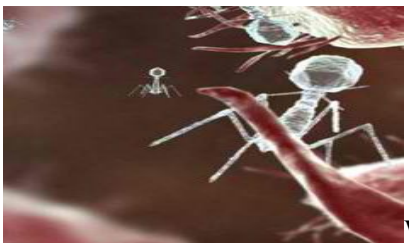
certain chemicals that are part of standard wound care (e.g. lactoferrin or silver) may have interfered with bacteriophage viability.[12] Another controlled clinical trial in Western Europe (treatment of ear infections caused by *Pseudomonas aeruginosa*) was reported shortly after in the journal Clinical Otolaryngology in August 2009.[13] The study concludes that bacteriophage preparations were safe and effective for treatment of chronic ear infections in humans. Additionally, there have been numerous animal and other experimental clinical trials evaluating the efficacy of bacteriophages for various diseases, such as infected burns and wounds, and cystic fibrosis associated lung infections, among others. Meanwhile, bacteriophage researchers are developing engineered viruses to overcome antibiotic resistance, and engineering the phage genes responsible for coding enzymes which degrade the biofilm matrix, phage structural proteins and also enzymes responsible for lysis of bacterial cell wall.

D'Herelle "quickly learned that bacteriophages are found wherever bacteria thrive: in sewers, in rivers that catch waste runoff from pipes, and in the stools of convalescent patients." This includes rivers traditionally thought to have healing powers, including India's Ganges River

Phage therapy is the therapeutic use of lytic bacteriophages to treat pathogenic bacterial infections. Phage therapy is an alternative to antibiotics being developed for clinical use by research groups in Eastern Europe and the U.S. After having been extensively used and developed mainly in former Soviet Union countries for about 90 years, phage therapies for a variety of bacterial and poly microbial infections are now becoming available on an experimental basis in other countries, including the U.S. The principles of phage therapy have potential applications not only in human medicine, but also in dentistry, veterinary science, food science and agriculture.

An important benefit of phage therapy is derived from the observation that bacteriophages are much more specific than most antibiotics that are in clinical use. Theoretically, phage therapy is harmless to the eucaryotic host undergoing therapy, and it should not affect the beneficial normal flora of the host. Phage therapy also has few, if any, side effects, as opposed to drugs, and does not stress the liver. Since phages are self-replicating in their target bacterial cell, a single, small dose is theoretically efficacious. On the other hand, this specificity may also be disadvantageous because a specific phage will only kill a bacterium if it is a match to the specific subspecies. Thus, phage mixtures may be applied to improve the chances of success, or clinical samples can be taken and an appropriate phage identified and grown.

Phages are currently being used therapeutically to treat bacterial infections that do not respond to conventional antibiotics, particularly in the country of Georgia. They are reported to be especially successful where bacteria have constructed a biofilm composed of a polysaccharide matrix that antibiotics cannot penetrate.



What comes to mind when you hear the word “bacteria”? Most people, if not all, will answer “disease,” “sickness,” or “bad for the health.” What not all people know is there are actually both good and bad bacteria and some bacterial species are probiotic – bacteria that are helpful to its host. In fact,

bacterial infections can be treated with bacteriophages: viruses that have the ability to infect and fight harmful bacteria, culminating in their destruction. Bacteriophage or phage therapy is therefore very useful in various fields like medicine, veterinary science, dentistry, and even agriculture.

History of Phage Therapy

Bacteriophages were discovered by two people: the English bacteriologist Frederick Twort in 1915 and the French-Canadian microbiologist Felix d'Herelle in 1917. Immediately after their discovery, the thought of using phages to fight bacterial infections was already apparent. D'Herelle began testing the therapeutic effects that phages may have on chickens and cows first and the tests were successful. Eventually, human tests were conducted and the development of phage therapy became more extensive especially with the foundation of the Eliavia Institute in 1923; the pharmaceutical company Eli Lilly began the commercialization of phage therapy in the US during the 1940s. During the Second World War, phages were used to treat bacterial diseases among soldiers of the Soviet Union, particularly gangrene and dysentery. The development of antibiotics in the 1950s led to a temporary setback on phage therapy as the use of antibiotics became more favourable. However, there has been a renewed interest in the development and employment of phage therapy in more applications.

Advantages over Antibiotics

Viruses and bacteria evolve over time and can develop a resistance to antibiotics. In theory, this resistance can also apply to phages, but it may be less difficult to overcome compared to antibiotics.

Because phages are target specific, meaning only a one or very few bacterial strains are targeted upon, it is easier to develop new phages than new antibiotics. A time period of only a few days or weeks is needed to acquire new phages for resistant strains of bacteria, whereas it can take years to obtain new antibiotics. When resisting bacteria evolve, the assigned phages also evolve, so when super bacterium appears, an equivalent super phage fights it as long as the phage is derived from the same environment.

Compared to antibiotics, phages go deeper into the infected area. Antibiotics, on the other hand, have concentration properties that quickly decrease as they go below the surface of the infection. The replication of phages is concentrated on the infected area where they are needed the most, while antibiotics are metabolized and removed from the body. In addition, secondary resistance does not happen among phages, but happens quite often among antibiotics. Secondary resistance is acquired and occurs when there aren't enough blood drug levels. Certain infections in people and experimentally infected animals have been proven to be more effectively treated with phage therapy than using antibiotics. Since 1966, the average success rate of studies that used phages in various ways (systematically, topically, intravenously, or orally) is from 80 to 95%, with minimal or no allergic and/or gastrointestinal side effects. The infections studied are from *E. coli*, *Acinetobacter*, *Psuedomonas*, and *Staphylococcus aureus*. Multiple side effects like allergies, intestinal disorders, and yeast infections have been observed when using antibiotics.

Applications

Fighting and destroying bacterial infections (both in humans and animals) are the primary applications of phage therapy, but it can also be employed for other uses. It can be the key to fighting the NDM-1, a gene that can be included in the DNA of bacteria, enabling them to resist antibiotics. Waste water from sewage systems are not really considered waste because it is a rich source of phage strains for various kinds of bacteria that lead to the most up-to-date medicines. Skin grafting for extensive wounds, trauma, burns, and skin cancer can also be improved by using phage therapy to lessen the *Psuedomonas aeruginosa* infection. Some experiments for cells in tissue culture have also discovered antitumor agents in phages. Bacteria cause food to spoil faster, and phages have been studied for their potential to increase the freshness of food and decrease the incidents of food spoilage.

Phage therapy has many other potential benefits and giving it ample support can pave the way to a healthier future.

Replication

Bacteriophages may have a lytic cycle or a lysogenic cycle, and a few viruses are capable of carrying out both. With *lytic phages* such as the T4 phage, bacterial cells are broken open (lysed) and destroyed after immediate replication of the virion. As soon as the cell is destroyed, the phage progeny can find new hosts to infect. Lytic phages are more suitable for phage therapy. Some lytic phages undergo a phenomenon known as lysis inhibition, where completed phage progeny will not immediately lyse out of the cell if extracellular phage concentrations are high. This mechanism is not identical to that of temperate phage going dormant and is usually temporary. In contrast, the *lysogenic cycle* does not result in immediate lysing of the host cell. Those phages able to undergo lysogeny are known as temperate phages. Their viral genome will integrate with host DNA and replicate along with it relatively harmlessly, or may even become established as a plasmid. The virus remains dormant until host conditions deteriorate, perhaps due to depletion of nutrients; then, the endogenous phages (known as prophages) become active. At this point they initiate the reproductive cycle, resulting in lysis of the host cell. As the lysogenic cycle allows the host cell to continue to survive and reproduce, the virus is replicated in all of the cell's offspring. An example of a bacteriophage known to follow the lysogenic cycle and the lytic cycle is the phage lambda of *E. coli*.[16]

Sometimes prophages may provide benefits to the host bacterium while they are dormant by adding new functions to the bacterial genome in a phenomenon called lysogenic conversion. Examples are the conversion of harmless strains of*Corynebacterium diphtheriae* or *Vibrio cholerae* by bacteriophages to highly virulent ones, which cause Diphtheria orcholera, respectively.[17][18] Strategies to combat certain bacterial infections by targeting these toxin-encoding prophages have been proposed.[19]

Attachment and penetration

To enter a host cell, bacteriophages attach to specific receptors on the surface of bacteria, including lipopolysaccharides, teichoic acids, proteins, or even flagella. This specificity means a bacteriophage can infect only certain bacteria bearing receptors to which they can bind, which in turn determines the phage's host range. Host growth conditions also influence the ability of the phage to attach and invade them.[20] As phage virions do not move independently, they must rely on random encounters with the right receptors when in solution (blood, lymphatic circulation, irrigation, soil water, etc.).

Myovirus bacteriophages use a hypodermic syringe-like motion to inject their genetic material into the cell. After making contact with the appropriate receptor, the tail fibers flex to bring the base plate closer to the surface of the

cell; this is known as reversible binding. Once attached completely, irreversible binding is initiated and the tail contracts, possibly with the help of ATP present in the tail,[3] injecting genetic material through the bacterial membrane. Podoviruses lack an elongated tail sheath similar to that of a myovirus, so they instead use their small, tooth-like tail fibers enzymatically to degrade a portion of the cell membrane before inserting their genetic material.

Synthesis of proteins and nucleic acid

Within minutes, bacterial ribosomes start translating viral mRNA into protein. For RNA-based phages, RNA replicase is synthesized early in the process. Proteins modify the bacterial RNA polymerase so it preferentially transcribes viral mRNA. The host's normal synthesis of proteins and nucleic acids is disrupted, and it is forced to manufacture viral products instead. These products go on to become part of new virions within the cell, helper proteins that help assemble the new virions, or proteins involved in cell lysis. Walter Fiers (University of Ghent, Belgium) was the first to establish the complete nucleotide sequence of a gene (1972) and of the viral genome of bacteriophage MS2 (1976).[21]

Virion assembly

In the case of the T4 phage, the construction of new virus particles involves the assistance of helper proteins. The base plates are assembled first, with the tails being built upon them afterward. The head capsids, constructed separately, will spontaneously assemble with the tails. The DNA is packed efficiently within the heads. The whole process takes about 15 minutes.

Release of virions

Phages may be released via cell lysis, by extrusion, or, in a few cases, by budding. Lysis, by tailed phages, is achieved by an enzyme calledendolysin, which attacks and breaks down the cell wall peptidoglycan. An altogether different phage type, the filamentous phages, make the host cell continually secrete new virus particles. Released virions are described as free, and, unless defective, are capable of infecting a new bacterium. Budding is associated with certain *Mycoplasma* phages. In contrast to virion release, phages displaying a lysogenic cycle do not kill the host but, rather, become long-term residents as prophage.

Genome structure

Given the millions of different phages in the environment, phages genomes come in a variety of forms and sizes. RNA phage such as MS2 have the smallest genomes of only a few kilobases. However, some DNA phages such as T4 may have large genomes with hundreds of genes.

Bacteriophage genomes can be highly mosaic, i.e. the genome of many phage species appear to be composed of numerous individual modules. These modules may be found in other phage species in different arrangements.Mycobacteriophages – bacteriophages with mycobacterial hosts – have provided excellent examples of this mosaicism. In these mycobacteriophages, genetic assortment may be the result of repeated instances of site-specific recombination and illegitimate recombination (the result of phage genome acquisition of bacterial host genetic sequences. It should be noted, however, that evolutionary mechanisms shaping the genomes of bacterial viruses vary between different families and depend on the type of the nucleic acid, characteristics of the virion structure, as well as the mode of the viral life cycle.

Systems biology

Phages often have dramatic effects on their hosts. As a consequence, the transcription pattern of the infected bacterium may change considerably. For instance, infection of *Pseudomonas aeruginosa* by the temperate phage PaP3 changed the expression of 38% (2160/5633) of its host's genes. Many of these effects are probably indirect, hence the challenge becomes to identify the direct interactions among bacteria and phage.[24]

Several attempts have been made to map Protein–protein interactions among phage and their host. For instance, bacteriophage lambda was found to interact with its host E. coli by 31 interactions. However, a large-scale study revealed 62 interactions, most of which were new. Again, the significance of many of these interactions remains unclear, but these studies suggest that there are most likely several key interactions and many indirect interactions whose role remains uncharacterized.

In the environment

Metagenomics has allowed the in-water detection of bacteriophages that was not possible previously.

Bacteriophages have also been used in hydrological tracing and modelling in river systems, especially where surface water and groundwater interactions occur. The use of phages is preferred to the more conventional dye marker because they are significantly less absorbed when passing through ground waters and they are readily detected at very low concentrations. Non-polluted water may contain ca. 2×10^8 bacteriophages per mL.

Other areas of use

Since 2006, the United States Food and Drug Administration (FDA) and United States Department of Agriculture (USDA) have approved several bacteriophage products. LMP-102 (Intralytix) was approved for treating ready-to-eat (RTE) poultry and meat products. In that same year, the FDA approved LISTEX (developed and produced by Micreos) using bacteriophages on cheese to kill *Listeria monocytogenes* bacteria, giving them generally recognized as safe (GRAS) status.[29] In July 2007, the same bacteriophage were approved for use on all food products.[30] In 2011 USDA confirmed that LISTEX is a clean label processing aid and is included in USDA.[31] Research in the field of food safety is continuing to see if lytic phages are a viable option to control other food-borne pathogens in various food products.

In 2011 the FDA cleared the first bacteriophage-based product for in vitro diagnostic use. The KeyPath MRSA/MSSA Blood Culture Test uses a cocktail of bacteriophage to detect *Staphylococcus aureus* in positive blood cultures and determine methicillin resistance or susceptibility. The test returns results in about 5 hours, compared to 2–3 days for standard microbial identification and susceptibility test methods. It was the first accelerated antibiotic susceptibility test approved by the FDA.

Government agencies in the West have for several years been looking to Georgia and the former Soviet Union for help with exploiting phages for counteracting bioweapons and toxins, such as anthrax and botulism. Developments are continuing among research groups in the US. Other uses include spray application in horticulture for protecting plants and vegetable produce from decay and the spread of bacterial disease. Other applications for bacteriophages are as biocides for environmental surfaces, e.g., in hospitals, and as preventative treatments for catheters and medical devices before use in clinical settings. The technology for phages to be applied to dry surfaces, e.g., uniforms, curtains, or even sutures for surgery now exists. Clinical trials reported in the *Lancet* show success in veterinary treatment of pet dogs with otitis.

Phage display is a different use of phages involving a library of phages with a variable peptide linked to a surface protein. Each phage's genome encodes the variant of the protein displayed on its surface (hence the name), providing a link between the peptide variant and its encoding gene. Variant phages from the library can be selected through their binding affinity to an immobilized molecule (e.g., botulism toxin) to neutralize it. The bound, selected phages can be multiplied by reinfecting a susceptible bacterial strain, thus allowing them to retrieve the peptides encoded in them for further study. The SEPTIC bacterium sensing and identification method uses the ion emission and its dynamics during phage infection and offers high specificity and speed for detection. Phage-ligand technology makes use of proteins, which are identified from bacteriophages, characterized and recombinantly expressed for various applications such as binding of bacteria and bacterial components (e.g. endotoxin) and lysis of bacteria

Bacteriophages are also important model organisms for studying principles of evolution and ecology.

Model bacteriophages

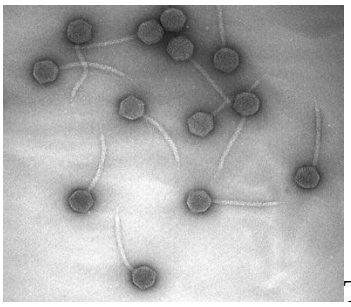
The following bacteriophages are extensively studied:

- λ phage
- T2 phage
- T4 phage (169 kbp genome, 200nm long T7 phage
- T12 phage
- R17 phage
- M13 phage
- MS2 phage (23–25 nm in size
- G4 phage
- P1 phage
- Enterobacteria phage P2
- P4 phage
- Phi X 174 phage
- N4 phage
- Pseudomonas phage Φ6
- Φ29 phage
- 186 phage

Cultural References

- In 1925 in the Pulitzer Prize-winning novel *Arrowsmith*, Sinclair Lewis fictionalized the discovery and application of bacteriophages as a therapeutic agent.
- The 1999 Greg Bear novel *Darwin's Radio* deals with an epidemic in the form of long-dormant sections of human DNA, introduced in prehistoric times by lysogenic bacteriophages, which begin to express themselves. The sequel, *Darwin's Children*, takes place in the post-epidemic world.
- The *Stargate Atlantis Legacy* novel *The Third Path* deals with a virulent bacteriophage that has turned a bacterial solvent into a deadly plastic-consuming threat with the potential to be fatal to humans. Part of the novel focuses on the characters efforts to stop the bacteriophage, ultimately creating another one to destroy the first.

Lambda Phages

 The lambda phage, also called *Enterobacteria phage λ* and coliphage λ, is a type of temperate bacteriophage or bacterial virus that infects the *Escherichia coli* (E. coli) species of bacteria. The virus may be housed in the genome of its host via lysogeny.

History of Lambda Phage

In 1950, Esther Lederberg, an American microbiologist, was performing experiments on E. coli mixtures. She happened to observe streaks of mixtures of two types of E. coli strains that seemed to have been nibbled on and had viral plaque. One E. coli strain had been treated with ultraviolet light, so the damage was more apparent. It was later determined that this was caused by bacterial viruses, which replicated and spread resulting in cell destruction. The discovery led to the employment of Lambda phage as a model organism in microbial genetics as well as in molecular genetics.

Structure

A lambda phage has a head measuring around 50-60 nanometers in diameter and a flexible tail that is around 150 nanometers long and may contain tail fibers.The head consists of various proteins and over a thousand protein molecules including X1, X2, B, B*, E, D, and W. The head functions as a capsid that contains its genome, which contains 48,490 base pairs of double-stranded linear DNA. This number also includes 12-base single-stranded parts at its 5’ ends. The single-stranded parts are known as sticky sites and are also called the cos site, which encircles the DNA in the host cytoplasm. Hence, when in circular form, the phage genome is comprised of 48,502 pairs in length. The weight of the genome is estimated to be 32×10^6 Da, which is around half of the weight of the phage. The tail has a 135 nanometer-tube that is hollow and contains a conical cap which is around 15 nanometers. The tail’s inner diameter is around 3 nanometers, while on the outside, it is around 9-18 nanometers depending on the knob-like structures that give the tail a rough appearance.

Life cycle

When E. coli is infected with a lambda phage, two cycles may happen: lytic or lysogenic. The lytic cycle happens when progeny phage particles are produced. The lytic cycle is the more common life cycle that comes after most infections. The first step of this cycle is the attachment of the phage to the host cell, injecting its DNA into the cell. Nucleic acid from the phage is replicated, and the phage’s genes are expressed, allowing the production of phage proteins. The phage proteins are assembled into phage particles, which are released when the host cell undergoes lysis (it breaks down). The lysis is mediated by lysis genes *S, R, Rz,* and *Rz1* which, upon expression, yield proteins that work together to break down the host bacterium’s cell wall.This mode of lambda replication typically yields many phage particles.

The lysogenic cycle, in contrast, does not produce a huge number of progeny phage or break down the host cell. Instead, the λ DNA recombines with its host's genome to produce a prophage. This typically is the favoured pathway when unfavourable environmental conditions prevent intense replication of the bacterial cells. Like the lytic cycle, the first step of the lysogenic cycle is also the attachment of the phage and the injection of its DNA into the host cell. The phage DNA then integrates with the host chromosome, producing an integrated DNA combination called the prophage DNA. Host cells that carry this DNA are said to be in the lysogenic state. The prophage DNA is replicated along each time the host bacterial cell replicates itself, producing more cells, each with a copy of the prophage DNA. When these cells are exposed to certain chemicals or to ultraviolet light, phage induction happens; the prophage DNA is then cut out of the host genome and proceeds to the lytic cycle.

Applications

The lambda phage has different applications, most of which are related to DNA cloning. This is because lambda phage can be used as a vector for generating recombinant DNA, which are combined DNA sequences that result from using laboratory techniques like molecular cloning to assemble genetic material from several sources. The site-specific recombinase of lambda phage can be used for shuffling cloned DNAs via the gateway cloning system, a molecular biology technique that ensures the effective transfer of DNA fragments between plasmids.

The lambda phage's ability to mediate genetic recombincation is due to its red operon, which is a functioning unit of genomic DNA that has a cluster of genes controlled by a promoter or a single regulatory signal. This red operon can be expressed to yield the proteins red alpha (or exo), beta, and gamma, which can be used in recombination-mediated genetic engineering, a method commonly employed in bacterial genetics, generation of target vectors, and DNA modification. Undoubtedly, the lambda phage is a powerful genetic tool that can be used in many important studies.

Temperate Phages

A bacteriophage is a kind of virus that can infect and replicate itself inside bacterial cells. The virus has a protein-encapsulated DNA or RNA genome and can have simple or complex anatomies. There are many types of bacteriophages including M13, T phage, lambda phage, MS2, G4, and Phix174.

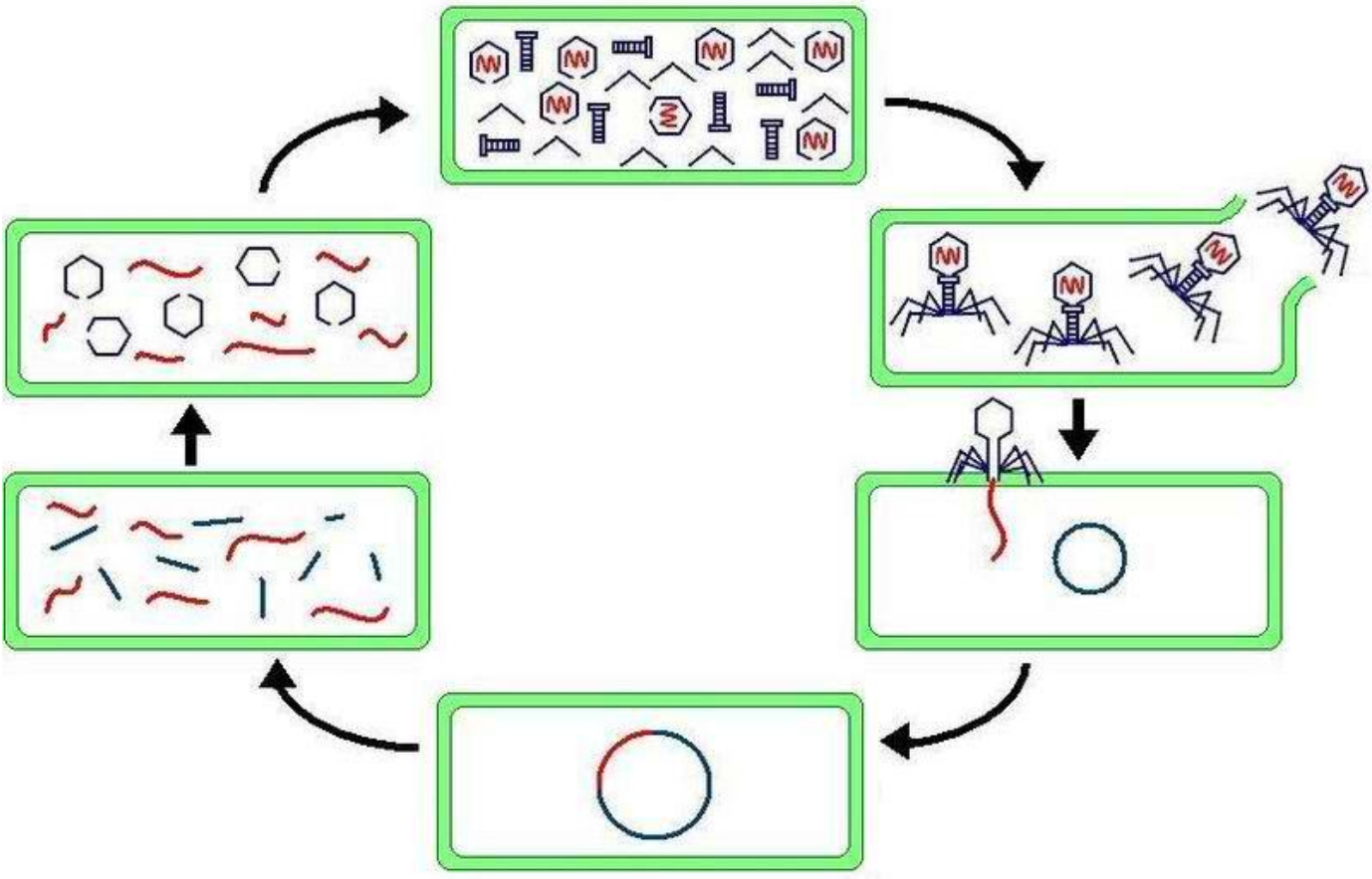
One of the characteristics of bacteriophages is their temperateness. Temperateness refers to the ability of some bacteriophages, particularly lambda phage, to choose between two cycles: lysogenic or lytic. "Temperance" generally refers to the moderation of actions, and in the case of phages, moderation is seen through the ability to not express anti-bacterial virulence.

Viral reproduction

Viruses cannot multiply through the division of cells because they are acellular (they do not have cells). Instead, they seek a host cell in which they replicate and assemble themselves using the metabolism and machinery of the host cell. Different species of viral populations undergo different viral life cycles, but for temperate phages, as previously mentioned, they must pick between two.

The lytic-lysogeny decision

Decision making isn't just done by people; it is also done by temperate phages as they need to choose between two different life cycles, productive (lytic) or reductive (lysogenic). There is a predominance of lytic among temperate phages, as induction can cause lysogenic to convert to lytic. However, in most cases, temperate phages reel toward the lysogenic cycle especially when phage absorption in the infected bacteria is apparent. It is inferred that other local bacteria are undergoing the same phage infection, making the bacteria decrease in density. Because of this "crisis," the go-to cycle is lysogenic. On the other hand, when there is an abundance of uninfected bacteria, undergoing the lytic cycle is preferable because to increase the number of healthy bacteria, phages that have productive infections are needed.



Lysogenic cycle

In the lysogenic cycle, the genomes of temperate phages are not expressed. However, they are integrated into the genome of the bacteria and produce prophages, which are created without disrupting the bacterial cell. Moreover, because of this integration, passive replication of the bacteriophage occurs when daughter bacterial cells are produced. These prophage-containing bacteria cells are called lysogens – phages that can exist as dormant DNA within its host cell. These lysogens have the ability to stay in the lysogenic cycle for a very long time, but through induction, they can be directed to the lytic cycle at any point in time. When induction occurs, prophage DNA is cut off from the bacterial genome and coat proteins are produced via transcription and translation of the prophage DNA for the regulation of lytic growth.

Lytic cycle

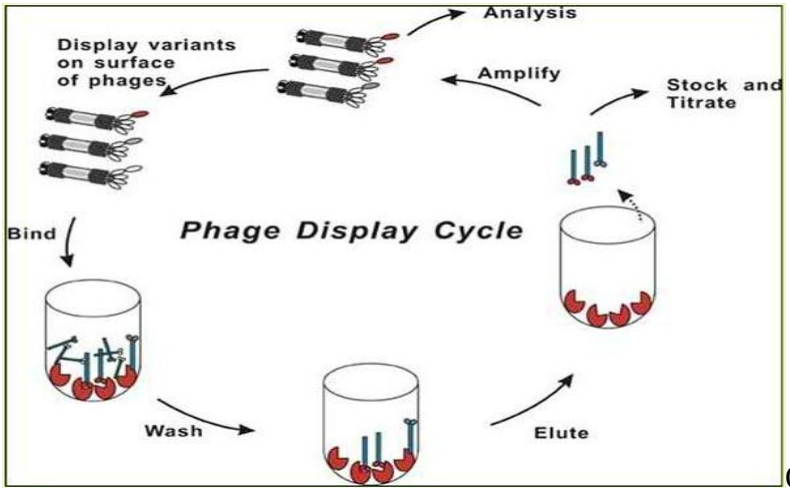
The lytic cycle is similar to the lysogenic cycle in that the host DNA machinery is used to replicate the phage, but the phage is considered a separate molecule from the host DNA. When a temperate phage undergoes the lytic cycle, it becomes a virulent phage. The infected cell and its membrane disintegrate as the viral DNA, which is considered a separate molecule from the bacterial cell, replicates separately from the DNA of the host bacteria, eventually overwhelming it.

The lytic cycle is divided into different stages. The first stage is the penetration in which the virus enters the host cell and injects its nucleic acids into it, releasing genetic material (either DNA or RNA) and infecting the cell. Viral components are then produced using the machinery of the host cell, culminating in the biosynthesis of mRNA and protein production. The host cell begins to copy the viral nucleic acids, which combine with viral proteins to produce phage particles within the cell. When there are already too many viral particles within the host, its membrane splits and the released viruses begin infecting other cells.

Applications

Temperate phages have various biological and molecular applications. They can be used to genetically manipulate eukaryotic cells, especially species that have large genomes like plants and mammals. Gene therapy, manipulation of cell lines, and construction of transgenic organisms can also employ phage enzymes. The temperate phage Mu-1 has a DNA-modifying function, which is of great importance especially in virology. Various food and biotechnology products and chemicals also employ the bacterial fermentation of phages. In most laboratories, temperate phages are considered more of lytic phages because most lytic-lysogenic decisions result in the former. However, whether phages are lytic or lysogenic, it is apparent that even they are capable of making a decision, particularly for replication.

Phage Display



One of the laboratory techniques employed in studying different protein interactions is Phage Display. With this in vitro screening method, protein ligands and macromolecules can be easily identified and interactions between protein and protein, peptide and protein, & DNA and protein can be studied further.

History of Phage Display

The first described instance of Phage Display occurred in 1985, when George P. Smith fused a peptide with a gene III from a filamentous phage. He filed a patent detailing the process of generating phage display libraries in the same year. Eventually, further development of Phage Display technology led by different groups from the MRC Laboratory of Molecular Biology, as well as from The Scripps Research Institute, led to the possibility of displaying proteins for the purpose of therapeutic protein engineering. The technique has been continuously improved to screen and amplify huge collections of proteins showing the connection of phenotype and genotype better.

Structure

A filamentous phage has a diameter of around 6.5 nanometers, with a length that depends on the size of its genome. It comes from a huge family of bacterial viruses that also infect other forms of bacteria. It contains a small genome with an intergenic region containing the necessary sequences for the replication and encapsidation of DNA. A phage particle consists of five coat proteins. The particle has a hollow tube that houses so many copies of the primary coat protein. There are also binding interactions between the adjacent subunits' hydrophobic midsections. One end of the particle is blunt, and the other is sharp. The blunt end contains plenty of copies of the two tiniest ribosomally translated proteins, while the sharp end contains around only 5 copies of the pIII and pVI genes, which are necessary for the detachment of the phage from the cell membrane.

How it works

Phage Display is a method wherein a library of phage particles that express very diverse peptides is generated. The objective is to choose those that will bind a desired target; the target can be a protein, a peptide, or a piece of DNA.

The most often used vector to build a random peptide display is the filamentous phage M13. In this display, the DNA which encodes the peptide or protein of interest is integrated into the pIII or pVI gene. To make sure that the fragments are completely inserted into the three possible reading frames, multiple cloning sites are sometimes employed, allowing the proper translation of the cDNA in its correct frame. The DNA hybrid and the phage gene are then put inside E. coli bacterial cells. Examples of these bacterial cells include XL1-Blue E. coli, SS320, TG1, and ER2738. The peptide or protein of interest is eventually expressed in either the minor or major coat protein. If another kind of vector is used, for example, a phagemid vector or simplified display construct vector, a helper phage must infect the E. coli cells; otherwise, the phage particles will not be separated from the E. coli cells. A helper phage activates the packaging of the phage DNA and assembles the mature virions with their corresponding protein fragments, which are included in the outer coating of the minor or major protein coat. The generated phage library is then screened by addition into a microtiter plate containing immobilised target proteins or DNA sequences. Phages displaying a protein that bind to one target will remain, while the other phages can be discarded through washing. The remaining phage particles can be used to multiply the phage by infecting them into bacteria, thus increasing the diversity of the peptide display library.

Applications

The fast isolation of particular ligands through phage display has a wide variety of applications like epitope mapping, analyzing different protein interactions, vaccine development, drug design, and therapeutic target validation. Phage display is also used to pick inhibitors for the active and allosteric sites of G-protein binding modulatory peptides, enzymes, and receptor antagonists and agonists.

Determining the proper protein partners can be useful to identify the functions of various proteins. For drug discovery and design, Phage Display is employed in protein engineering or in vitro protein evolution. Therapeutic targeting with phage display is also primarily used to diagnose and determine tumour antigens, which is useful for cancer research. Antibody Phage Display significantly improved the discovery and development of antibody drugs. Phage display for antibody libraries paved the way for rapid vaccine design and therapy. These libraries are used to learn more about the human immune system and to create human antibodies in vitro with the use of diverse synthetic substances.Phage Display can be used in conjunction with other techniques, and with enough support and studies, more applications for it can be discovered.

****

Plant viruses – RNA viruses – TMV, Cowpea mosaic virus, Bromomosaic viruses, Satellite viruses – Double stranded DNA viruses - CaMV – Single stranded DNA viruses – Gemini virus. Structure and replication of Bacteriophage (T4) – Filamentous phage (ΦX174).

MORPHOLOGY AND GENERAL PROPERTIES OF VIRUSES

INTRODUCTION

Viruses occupy the twilight zone that separates the 'living' from the 'non-living'. They do not have a cellular organization and contain only one type of nucleic acid, either DNA or RNA but never both. The medical importance of viruses lies in their ability to cause a very large number of human diseases. Viral diseases range from minor ailments like common cold to terrifying diseases like rabies and AIDS.

In this chapter, we shall be discussing the morphology and general properties of viruses.

After reading this lesson you will be able to:

- explain the concept of viruses, in relation to other microorganisms
- describe the morphological features of viruses
- explain the multiplication of viruses (replication)
- describe the methods of cultivation of viruses
- explain the classification and naming (nomenclature) of viruses

Concept of Viruses in relation to other Organisms

Viruses occupy the twilight zone that separates the 'living' from the 'non-living'. They do not have a cellular organization and contain only one type of nucleic acid, either DNA or RNA but never both. Viruses are obligate intracellular parasites. They lack the enzymes necessary for protein and nucleic acid synthesis. They are dependent for replication on the synthetic machinery of host cells. They multiply by a complex process and not by binary fission. They are unaffected by antibacterial antibiotics. Viruses cause a wide range of human diseases. They cause infections like common cold, chicken pox, measles, viral encephalitis, rabies and AIDS.

Properties Bacteria Viruses

Cellular organization Present Absent Growth on inanimate media Yes No Binary fission Yes No DNA and RNA Both are present Either DNA or RNA Ribosomes Present Absent Sensitivity to antibacterial Yes No antibiotics

Morphology of Viruses

Size: The extracellular infectious virus particle is called virion. Viruses are much smaller than bacteria. They are too small to be seen under the light microscope. Some large viruses like the poxviruses can be seen under the light microscope when suitably stained. The viruses range in size from 20 nm to 300 nm. Poxviruses are one of the largest viruses and parvoviruses are one of the smallest viruses. The earliest method of estimating the size of virus particles was by passing them through collodion membrane filters of graded porosity. The average pore diameter of the finest filter that permitted passage of the virion gave an estimate of its size. With the development of the ultracentrifuge, a second method became available. From the rate of sedimentation of the virus in the ultracentrifuge, the particle size could be calculated using Stoke's law. The third and the most direct method of

measuring virus size is electron microscopy. By this method, both the shape and size of virions can be studied.

Structure, shape and symmetry: The virion consists essentially of a nucleic acid surrounded by a protein coat, the **capsid**. The capsid with the enclosed nucleic acid is called the **nucleocapsid**. The capsid protects the nucleic acid from harmful agents in the environment. It is composed of a large number of capsomers which form its morphological units. The chemical units of the Capsid are polypeptide molecules which are arranged symmetrically. They form a shell around the nucleic acid. The capsid shows two kinds of symmetry – icosahedral (cubical) and helical. An icosahedron is a polygon with 12 vertices and 20 facets or

sides. Each facet is in the shape of an equilateral triangle. Two types of capsomers are present in the icosahedral capsid. They are the pentagonal capsomers at the vertices (pentons) and the hexagonal capsomers making up the facets (hexons). There are always 12 pentons but the number of hexons varies with the virus group. Examples of viruses with icosahedral symmetry of the capsid are Adenovirus and Herpes Simplex Virus. In the nucleocapsids with helical symmetry, the capsomers and nucleic acid are wound together to form a helical or spiral tube, for example tobacco mosaic virus. All viruses do not show the typical icosahedral or helical symmetry. Some, like the poxviruses, show a complex symmetry. Virions may be enveloped or nonenveloped. The envelope of viruses is derived from the host cell membrane. This occurs when the virus is released from the host cell by budding. Protein subunits may be present as projecting spikes on the surface of the envelope. They are called **peplomers**. The influenza virus carries two kinds of peplomers: haemagglutinin and neuraminidase. Haemagglutinin is a triangular spike and neuraminidase is mushroom-shaped. Envelope is sensitive to the action of lipid solvents. Envelopes confer chemical, antigenic and biological properties on viruses.

The overall shape of the virus particle varies in different groups of viruses. Most animal viruses are roughly spherical. The rabies virus is bullet shaped. Poxviruses are brick-shaped.

Chemical properties: Viruses contain only one type of nucleic acid, either DNA or RNA. Viruses are unique because they carry genetic information on RNA. This property is not seen in any other organism in nature. Viruses also contain protein which makes up the capsid. Enveloped viruses contain lipids derived from the host cell membrane. Most viruses do not have enzymes for the synthesis of viral components or for energy production. Some viruses have enzymes, for example the influenza virus has neuraminidase.

Resistance: Viruses are destroyed by heat except a few. They are stable at low temperatures. For long term storage, they are kept at -70°C. A better method for prolonged storage is lyophilisation or freeze-drying. Viruses are inactivated by sunlight, UV rays and ionising radiation. They are, in general, more resistant than bacteria to chemical disinfectants. Phenolic disinfectants have a weak action on viruses.

Multiplication of Viruses

Multiplication of viruses is called viral **replication**. Viruses contain the genetic information for their replication but they lack the enzymes. They depend on host cell machinery for replication. The viral replication cycle can be divided into six phases – adsorption, penetration, uncoating, biosynthesis, maturation and release.

Adsorption: In this phase, the virus gets attached to the host cell. The host cell should have specific receptors on its surface. These receptors recognize viral surface components. This cell-virus interaction helps the virus to attach to the host cell surface.

Penetration: In this phase, the virus enters into the host cell. Bacteria have rigid cell wall. So, viruses which infect bacteria cannot penetrate into the bacterial cell. Only the nucleic acid of the virus enters the bacterial cell. Animal and human cells do not have cell walls. Therefore, whole virus enters the cell. Virus particle may be engulfed by a process called **viropexis**. In case of enveloped viruses, the viral envelope may fuse with the cell membrane of the host cell. Then the nucleocapsid is released into the cytoplasm.

Uncoating: This is the process in which the outer layers and capsid of the virus are removed. This mostly occurs by the action of lysosomal enzymes of the host cell. This can also occur by a viral uncoating enzyme. Finally, the viral nucleic acid is released into the cell.

Biosynthesis: In this phase, the viral nucleic acid and capsid are synthesised. The enzymes necessary in the various stages of viral synthesis, assembly and release are also synthesised. Certain 'regulator proteins' are synthesised. They shut down the normal metabolism of the host cell. They direct the production of viral components. In general, most DNA viruses synthesise their nucleic acid in the host cell nucleus. Exceptions are the poxviruses. They are DNA viruses, but they synthesise all their components in the host cell cytoplasm. Most RNA

viruses synthesise all their components in the cytoplasm. Orthomyxoviruses and some paramyxoviruses are exceptions. They synthesise some components in the host cell nucleus. Biosynthesis consists essentially of the following steps:

1. Transcription of messenger RNA (mRNA) from the viral nucleic acid
2. Translation of mRNA into "early proteins" or "non-structural proteins".

They are enzymes responsible for the synthesis of viral components.

3. Replication of viral nucleic acid
4. Synthesis of "late proteins" or "structural proteins". They are the components

of daughter virion capsids.

Maturation: This is the assembly of daughter virions following the synthesis of viral nucleic acid and proteins. It can take place in the host cell nucleus or cytoplasm. Herpesviruses and adenoviruses are assembled in the nucleus.

Picornaviruses and poxviruses are assembled in the nucleus.

Release: Viruses which infect bacteria (bacteriophages) are released by lysis of the infected bacterium. Animal viruses are usually released without cell lysis. Myxoviruses are released by budding from the cell membrane. The host cell is unaffected. Daughter virions are released into the surrounding medium and may infect other cells. In some viruses (for eg. varicella), transmission occurs directly from cell to cell. In this case, there is very little free virus in the medium. The poliovirus causes cell damage and may be released by cell lysis. From the stage of penetration till the appearance of mature daughter virions, the virus cannot be demonstrated inside the host cell. During this period, the virus seems to disappear. This is called the "eclipse phase". The time taken for a single cycle of replication is about 15-30 minutes for bacteriophages. It is about 15- 30 hours for animal viruses. A single infected cell may release a large number of progeny virions.

Methods of cultivation of viruses

Viruses are obligate intracellular parasites. They donot grow on culture media used for bacteria. The methods used for cultivation of viruses are:

1. **Animal inoculation:** Monkeys were used for the isolation of the poliovirus by Landsteiner and Popper in 1909. Infant mice are used for the isolation of coxsackievirus and arboviruses (dengue, chikungunya). Mice may be

inoculated by several routes – intracerebral, subcutaneous, intraperitoneal or subcutaneous. Other animals like guinea pigs, rabbits and ferrets are usedin some situations.

2. **Embryonated eggs:** The embryonated hen's egg was first used for the cultivation of viruses by Good pasture in 1931. This method was further developed by Burnet. Different parts of the egg are used for the cultivation of different viruses. Herpes simplex virus, when inoculated into the chorioallantoic membrane, produces visible lesions called pocks. Inoculation into the amniotic sac is done for the isolation of influenza virus. Yolk sac inoculation is done for the isolation of rabies virus.

3. **Cell culture:** Probably, the first application of tissue culture in virology was by Steinhardt and colleagues in 1913. They maintained the vaccinia virus in fragments of rabbit cornea. The turning point was the cultivation of poliovirus which was demonstrated by Enders, Weller and Robbins in 1949. They showed that poliovirus, till then considered a strictly neurotropic virus,could be grown in tissue culture of non-neural origin.

The various types of tissue cultures are described as follows:

(i) **Organ culture:** Small bits of organs can be maintained in vitro for days and weeks, preserving their original architecture and function. Organ culture is useful for viruses which are highly specialised parasites of certain organs. For example, tracheal ring organ culture is used for the isolation of coronavirus, a respiratory pathogen.

(ii) **Explant culture:** Fragments of minced tissue can be grown as 'explants' embedded in plasma clots. They may also be cultivated in suspension. Adenoid tissue explant cultures were used for the isolation of adenovirus.

(iii) **Cell culture:** This is routinely used for growing viruses. Tissues are dissociated into the component cells by the action of enzymes andmechanical shaking. The cells are washed, counted and suspended in a growth medium. The growth medium consists of essential amino acids, glucose, vitamins, salts and a buffer. Antibiotics are added to prevent bacterial contamination. The cell suspension is put into bottles, tubes and petridishes. The cells adhere to the glass or plastic surface, divide and form a confluent monolayer sheet within a week. Cell culture is further classified on the basis of origin, chromosomal characters and the number of generations through which they can be maintained. It is of three types – primary cell culture, diploid cell strain and continuous cell lines. Primary cell cultures are normal cells freshly taken from the body and cultured. They are capable of only limited growth in culture. They cannot be maintained in serial culture. Examples are monkey kidney, human embryonic kidney and chick embryo cell cultures. Diploid cell strains are cells of a single type that retain the original diploid chromosome number and karyotype during serial subcultivation for a limited number of times. After about fifty serial passages, they undergo 'senescence'. Diploid strains developed from human fibroblasts are a good example. Continuous cell lines are cells of a single type, usually derived from cancer cells. They are capable of continuous serial cultivation

indefinitely. Hela cell lines are derived from carcinoma of the cervix. Cell culture is used for the isolation of viruses and their cultivation for vaccine production. Viruses in cell cultures can be detected by various methods like cytopathic effect, special staining techniques and detection of viral nucleic acid by molecular techniques like polymerase chain reaction (PCR). Cytopathic effect is the morphological change in the cultured cells which is produced by the virus growing in those cells. These changes can be seen by microscopic examination of the cell cultures. The cytopathic effects (CPE) produced by different groups of viruses are characteristic and help in the presumptive identification of virus isolates. For example, measles virus produces syncytium formation; adenovirus produces large granular clumps resembling bunches of grapes; enteroviruses produce crenation of cells and degeneration of the entire cell sheet.

53.2.5 Classification and naming of viruses

Till about 1950 little was known of the basic properties of viruses. They were named haphazardly, based on the diseases they caused or on the place of their isolation. They were grouped according to affinity to different systems or organs of the body (tropism). So, human viruses were classified as dermotropic, that is those producing skin lesions (smallpox, chickenpox, measles), neurotropic, that is those affecting the nervous system (poliomyelitis, rabies), pneumotropic, that is those affecting the respiratory tract (influenza, common cold) and viscerotropic, that is those affecting visceral organs (hepatitis). Bawden (1941) made the pioneering suggestion that viral nomenclature and classification should be based on the properties of viruses and not upon host responses. From the early 1950s, viruses began to be classified into groups based on their physiochemical and structural features. Nomenclature and classification are now the official responsibility of the International Committee on Taxonomy o of Viruses (ICTV). Viruses are classified into two main divisions based on the type of nucleic acid they possess: riboviruses contain RNA and deoxyriboviruses contain DNA. Further classification is based on other properties like strandedness of nucleic acid, symmetry of nucleic acid, presence of envelope, size and shape of virion and number of capsomeres.

DNA viruses: A few medically important families of DNA viruses are - Herpesviridae, Adenoviridae, Hepadnaviridae, Parvoviridae and Papillomaviridae. The Herpesviridae family consists of enveloped double-stranded DNA viruses having an icosahedral capsid. Examples of this family are herpes simplex virus and varicella zoster virus. Herpes simplex virus causes skin lesions like herpes labialis. It can also cause viral encephalitis. Parvoviridae consists of nonenveloped single-stranded DNA viruses, for example Parvovirus B19. The Hepadnaviridae family includes Hepatitis B virus which is a partially doublestranded DNA virus. Papillomaviridae family includes human papilloma virus which is responsible for causing skin warts.

RNA viruses: Some medically important families of RNA viruses are – Picornaviridae, Orthomyxoviridae and Paramyxoviridae, Flaviviridae, Rhabdoviridae and Retroviridae. Members of the family Picornaviridae are small (20-30 nm), non-enveloped, icosahedral viruses with single-stranded RNA genome. Examples include poliovirus and coxsackievirus. The viruses included in Orthomyxoviridae are enveloped viruses carrying haemagglutinin and neuraminidase peplomers on the envelope. The genome consists of singlestranded RNA in several (eight) pieces. Thus, they have a segmented genome. An example of this family is influenza virus. Flaviviridae consists of enveloped single-stranded RNA viruses. Examples include yellow fever virus, Japanese encephalitis virus and dengue virus. The members of Retroviridae family are enveloped RNA viruses which have a special enzyme called ‘reverse transcriptase’.

This enzyme is an RNA dependent DNA polymerase. It is required in the synthesis of DNA from RNA. An example of the Retroviridae family is Human Immunodeficiency Virus (HIV) which causes AIDS (acquired immunodeficiency syndrome).

Based on the mechanism of replication, Baltimore (1970) categorised viruses into seven categories. This is called the Baltimore classification.

1. The genetic material in viruses is:

A. DNA only B. RNA only

C. Either DNA or RNA D. Both DNA and RNA

2. Protein subunits presenting as projecting spikes on the surface of the envelope are called:

A. Capsomeres B. Capsid

C. Nuceocapsid D. Peplomers

3. Which of the following is the correct sequence of viral replication?

A. Penetration, uncoating, adsorption, biosynthesis, maturation and release

B. Adsorption, penetration, uncoating, biosynthesis, maturation and release

C. Biosynthesis, penetration, uncoating, adsorption, maturation and release
D. Adsorption, biosynthesis, maturation, uncoating, penetration and release
4. Methods used for viral cultivation are:
A. Cell culture B. Animal inoculation
C. Embryonated eggs D. All of the above
5. Baltimore classified viruses on the basis of:
A. Diseases caused by them B. Structure
C. Replication mechanism D. Physiochemical properties

Structure and Classification of Viruses

General Concepts

Structure and Function

Viruses are small obligate intracellular parasites, which by definition contain either a RNA or DNA genome surrounded by a protective, virus-coded protein coat. Viruses may be viewed as mobile genetic elements, most probably of cellular origin and characterized by a long co-evolution of virus and host. For propagation, viruses depend on specialized host cells supplying the complex metabolic and biosynthetic machinery of eukaryotic or prokaryotic cells. A complete virus particle is called a virion. The main function of the virion is to deliver its DNA or RNA genome into the host cell so that the genome can be expressed (transcribed and translated) by the host cell. The viral genome, often with associated basic proteins, is packaged inside a symmetric protein capsid. The nucleic acid-associated protein, called nucleoprotein, together with the genome, forms the nucleocapsid. In enveloped viruses, the nucleocapsid is surrounded by a lipid bilayer derived from the modified host cell membrane and studded with an outer layer of virus envelope glycoproteins.

Classification of Viruses

Morphology: Viruses are grouped on the basis of size and shape, chemical composition and structure of the genome and mode of replication. Helical morphology is seen in nucleocapsids of many filamentous and pleomorphic viruses. Helical nucleocapsids consist of a helical array of capsid proteins (protomers) wrapped around a helical filament of nucleic acid. Icosahedral morphology is characteristic of the nucleocapsids of many "spherical" viruses. The number and arrangement of the capsomeres (morphologic subunits of the icosahedron) are useful in identification and classification. Many viruses also have an outer envelope.

Chemical Composition and Mode of Replication: The genome of a virus may consist of DNA or RNA, which may be single stranded (ss) or double stranded (ds), linear or circular. The entire genome may occupy either one nucleic acid molecule (monopartite genome) or several nucleic acid segments (multipartite genome). The different types of genome necessitate different replication strategies.

Nomenclature

Aside from physical data, genome structure and mode of replication are criteria applied in the classification and nomenclature of viruses, including the chemical composition and configuration of the nucleic acid, whether the genome is monopartite or multipartite. The genomic RNA strand of single-stranded RNA viruses is called sense (positive sense, plus sense) in orientation if it can serve as mRNA, and antisense (negative sense, minus sense) if a complementary strand synthesized by a viral RNA transcriptase serves as mRNA. Also considered in viral classification is the site of capsid assembly and, in enveloped viruses, the site of envelopment.

Structure and Function

Viruses are inert outside the host cell. Small viruses, e.g., polio and tobacco mosaic virus, can even be crystallized. Viruses are unable to generate energy. As obligate intracellular parasites, during replication, they fully depend on the complicated biochemical machinery of eukaryotic or prokaryotic cells. The main purpose of a virus is to deliver its genome into the host cell to allow its expression (transcription and translation) by the host cell.

A fully assembled infectious virus is called a virion. The simplest virions consist of two basic components: nucleic acid (single- or double-stranded RNA or DNA) and a protein coat, the capsid, which functions as a shell to protect the viral genome from nucleases and which during infection attaches the virion to specific receptors exposed on the prospective host cell. Capsid proteins are coded for by the virus genome. Because of its limited size (Table 41-1) the genome codes for only a few structural proteins (besides non-structural regulatory proteins involved in virus replication). Capsids are formed as single or double protein shells and consist of only one or a few structural protein species. Therefore, multiple protein copies must self assemble

to form the continuous three-dimensional capsid structure. Self assembly of virus capsids follows two basic patterns: helical symmetry, in which the protein subunits and the nucleic acid are arranged in a helix, and icosahedral symmetry, in which the protein subunits assemble into a symmetric shell that covers the nucleic acid-containing core.

TABLE 41-1 Chemical and Morphologic Properties of Animal Virus Families Relevant to Human Disease

	Virion							
Family	Viral Genome: Type, Configuration[a] and Number of Bases per strand (x 10^3)	Shape[b]	Diameter (nm)	Enveloped[c]	Capsid Symmetry	Number of Capsomeres[d]	Site of Capsid Assembly	Enzymes, e.g. Transcriptase present in Virion
Circoviridae	ssDNA, circular; 0.6-1.2	s	17-22	0	Icosahedral	32?	Nucleus	None
Parvoviridae	ssDNA, linear, sense or antisense; 4-6	s	18-26	0	Icosahedral	32	Nucleus	None
Papovaviridae	dsDNA, circular; 5.1 / 7.9	s	45 / 55	0	Icosahedral	72	Nucleus	None
Adenoviridae	dsDNA, linear; 35-40	s	75-80	0	Icosahedral	252	Nucleus	None
Herpesviridae	dsDNA, linear; 124-235	s	120-200	+	Icosahedral	162	Nucleus	Thymidine kinase
Iridoviridae	dsDNA, linear; 170-200	s	125-300	+	Icosahedral	ca. 1,500	Cytoplasm	DNA-dependent RNA polymerase
Poxviridae	dsDNA, linear, covalently closed; 130-370	x	240x300	+	Complex	–	Cytoplasm	DNA-dependent RNA polymerase Protein kinase
Hepadnaviridae	dsDNA, circular, 1 ss-region; 3.0-3.3/2.0	s	40-48	+	Icosahedral	180	Nucleus	DNA-dependent DNA polymerase

TABLE 41-1 Chemical and Morphologic Properties of Animal Virus Families Relevant to Human Disease (continued)

Family	Viral Genome: Type, Configuration[a] and Number of Bases per strand (X 10^3)	Shape[b]	Diameter (nm)	Enveloped[c]	Capsid Symmetry	Number of Capsomeres[d]	Site of Capsid Assembly	Enzymes, e.g. Transcriptase present in Virion
Picornaviridae	ssRNA, linear; 7-8.5	s	22-30	0	Icosahedral	32	Cytoplasm	None
Astroviridae	ssRNA, linear, sense 6.8-7.9	s	28-30	0	Icosahedral	32?	Cytoplasm	None
Caliciviridae	dsRNA, linear, sense 7.4-7.7	s	35-39	0	Icosahedral	90	Cytoplasm	None
Togaviridae	dsRNA, linear, sense 9.7-11.8	s	70	+	Icosahedral	?	Cytoplasm	None
Flaviviridae	dsRNA, linear, sense 10-12	s	45-50	+	Icosahedral	unknown	Cytoplasm	None
Reoviridae	dsRNA, linear, 10-12 segments; 18-23	s	60-80	0	Icosahedral	32 or 92	Cytoplasm	RNA-dependent RNA polymerase
Orthomyxoviridae	dsRNA, linear, 8 molecules, antisense; 10-13.6	s-pleom[e]	80-120	+	Helical	–	Cytoplasm	RNA-dependent RNA polymerase
Paramyxoviridae	dsRNA, linear, antisense; 15	s-pleom	150-300	+	Helical	–	Cytoplasm	RNA-dependent RNA polymerase
Rhabdoviridae	ssRNA, linear, antisense;11-15	u	60x180	+	Helical	–	Cytoplasm	RNA-dependent RNA polymerase
Bunyaviridae	ssRNA, linear, 3 molecules, antisense; 11-20	s-pleom	90-120	+	Helical	–	Cytoplasm	RNA dependent RNA polymerase
Coronaviridae	ssRNA, linear, sense; 30	s-pleom	120-160	+	Helical	–	Cytoplasm	None
Arenaviridae	ssRNA, linear, 2 species + ribosomal RNA; 3.4	s-pleom	110-130	+	Helical	–	Cytoplasm	RNA-dependent RNA polymerase
Retroviridae	ssRNA, linear, inverted dimer of sense strand; 7-11	s-pleom	90-120	+	Icosahedral (type C)	–	Cytoplasm	RNA-dependent DNA polymerase Protease, Integrase
Filoviridae	ssRNA, linear, antisense; 19.1	Bacilli-form[e]	80x800-2,500	+	Helical	–	Cytoplasm	RNA-transcrip-tase/poly merase

[a]ss = single stranded; ds = double stranded. [b]S = spherical; X = brickshaped or ovoid; U = elongated with parallel sides and a round end; pleom = pleomorphic. [c]Most enveloped viruses are sensitive to lipid solvents. [d]Applicable to viruses with icosahedral symmetry. [e]Filamentous forms also occur.

Table 41-1 Chemical and Morphologic Properties of Animal Virus Families Relevant to Human Disease.
Some virus families have an additional covering, called the envelope, which is usually derived in part from modified host cell membranes. Viral envelopes consist of a lipid bilayer that closely surrounds a shell of virus-encoded membrane-associated proteins. The exterior of the bilayer is studded with virus-coded, glycosylated (trans-) membrane proteins. Therefore, enveloped viruses often exhibit a fringe of glycoprotein spikes or knobs, also called peplomers. In viruses that acquire their envelope by budding through the plasma or another intracellular cell membrane, the lipid composition of the viral envelope closely reflects that of the particular host membrane. The outer capsid and the envelope proteins of viruses are glycosylated and important in determining the host range and antigenic composition of the virion. In addition to virus-specified envelope proteins, budding viruses carry also certain host cell proteins as integral constituents of the viral envelope. Virus envelopes can be considered an additional protective coat. Larger viruses often have a complex architecture consisting of both helical and isometric symmetries confined to different structural components. Small viruses, e.g., hepatitis B virus or the members of the picornavirus or

parvovirus family are orders of magnitude more resistant than are the larger complex viruses, e.g. members of the herpes or retrovirus families.

Classification of Viruses

Viruses are classified on the basis of morphology, chemical composition, and mode of replication. The viruses that infect humans are currently grouped into 21 families, reflecting only a small part of the spectrum of the multitude of different viruses whose host ranges extend from vertebrates to protozoa and from plants and fungi to bacteria.

Morphology

Helical Symmetry

In the replication of viruses with helical symmetry, identical protein subunits (protomers) self-assemble into a helical array surrounding the nucleic acid, which follows a similar spiral path. Such nucleocapsids form rigid, highly elongated rods or flexible filaments; in either case, details of the capsid structure are often discernible by electron microscopy. In addition to classification as flexible or rigid and as naked or enveloped, helical nucleocapsids are characterized by length, width, pitch of the helix, and number of protomers per helical turn. The most extensively studied helical virus is tobacco mosaic virus (Fig. 41-1). Many important structural features of this plant virus have been detected by x-ray diffraction studies. Figure 41-2 shows Sendai virus, an enveloped virus with helical nucleocapsid symmetry, a member of the paramyxovirus family.

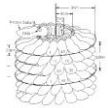


Figure 41-1

The helical structure of the rigid tobacco mosaic virus rod. About 5 percent of the length of the virion is depicted. Individual 17,400-Da protein subunits (protomers) assemble in a helix with an axial repeat of 6.9 nm (49 subunits per three turns). About 5 percent of the length of the virion is depicted. Individual 17,400-Da protein subunits (protomers) assemble in a helix with an axial repeat of 6.9 nm (49 subunits per three turns). Each turn contains a nonintegral number of subunits (16-1/3), producing a pitch of 2.3 nm. The RNA ($2\times1O^6$ Da) is sandwiched internally between adjacent turns of capsid protein, forming a RNA helix of the same pitch, 8 nm in diameter, that extends the length of virus, with three nucleotide bases in contact with each subunit. Some 2,130 protomers per virion cover and protect the RNA. The complete virus is 300 nm long and 18 nm in diameter with a hollow cylindrical core 4 nm in diameter.

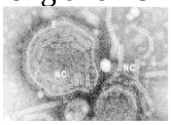


Figure 41-2

Fragments of flexible helical nucleocapsids (NC) of Sendai virus, a paramyxovirus, are seen either within the protective envelope (E) or free, after rupture of the envelope. The intact nucleocapsid is about 1,000 nm long and 17 nm in diameter; its pitch

Icosahedral Symmetry

An icosahedron is a polyhedron having 20 equilateral triangular faces and 12 vertices (Fig. 41-3). Lines through opposite vertices define axes of fivefold rotational symmetry: all structural features of the polyhedron repeat five times within each 360° of rotation about any of the fivefold axes. Lines through the centers of opposite triangular faces form axes of threefold rotational symmetry; twofold rotational symmetry axes are formed by lines through midpoints of opposite edges. An icosaheron (polyhedral or spherical) with fivefold, threefold, and twofold axes of rotational symmetry (Fig. 41-3) is defined as having 532 symmetry (read as 5,3,2).

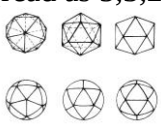


Figure 41-3

Icosahedral models seen, left to right, on fivefold, threefold, and twofold axes of rotational symmetry. These axes are perpendicular to the plane of the page and pass through the centers of each figure. Both polyhedral (upper) and spherical (lower) forms.

Viruses were first found to have 532 symmetry by x-ray diffraction studies and subsequently by electron microscopy with negative-staining techniques. In most icosahedral viruses, the protomers, i.e. the structural polypeptide chains, are arranged in oligomeric clusters called capsomeres, which are readily delineated by negative staining electron microscopy and form the closed capsid shell (Fig. 41-4 a/b). The arrangement of capsomeres into an icosahedral shell (compare Fig. 41-4 with the upper right model in Fig. 41-3) permits the classification of such viruses by capsomere number and pattern. This requires the identification of the nearest pair of vertex capsomeres (called penton: those through which the fivefold symmetry axes pass) and the distribution of capsomeres between them.

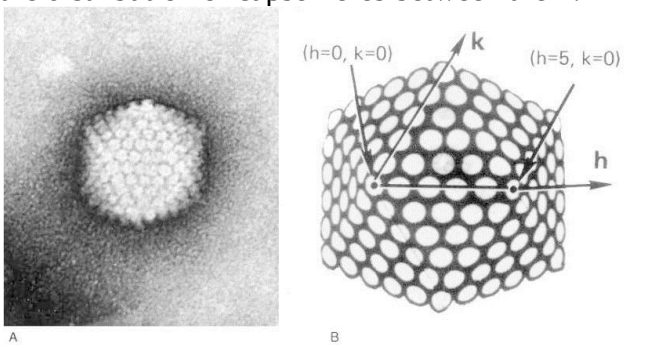


Figure 41-4

Adenovirus after negative stain electron microscopy. (A) The capsid reveals the typical isometric shell made up from 20 equilateral triangular faces. The 252 capsomeres, 12 pentons and the 240 hollow hexon capsomeres are arranged in a T = 25 symmetry

In the adenovirus model in Figure 41-4, one of the penton capsomeres is arbitrarily assigned the indices h = 0, k = 0 (origin), where h and k are the indicated axes of the inclined (60°) net of capsomeres. The net axes are formed by lines of the closest-packed neighboring capsomeres. In adenoviruses, the h and k axes also coincide with the edges of the triangular faces. Any second neighboring vertex capsomere has indices h = 5, k = 0 (or h = 0, k = 5). The capsomere number (C) can be determined to be 252 from the h and k indices and the equation: $C = 10(h^2 + hk + k^2) + 2$. This symmetry and number of capsomeres is typical of all members of the adenovirus family.

Virus Core Structure

Except in helical nucleocapsids, little is known about the packaging or organization of the viral genome within the core. Small virions are simple nucleocapsids containing 1 to 2 protein species. The larger viruses contain in a core the nucleic acid genome complexed with basic protein(s) and protected by a single- or double layered capsid (consisting of more than one species of protein) or by an envelope (Fig. 41-5).

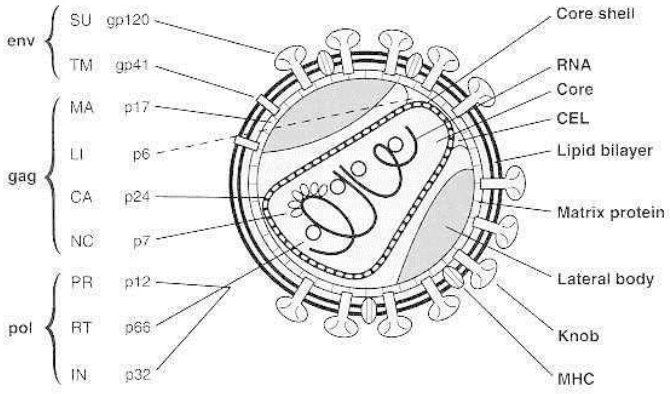


Figure 41-5

Two-dimensional diagram of HIV-1 correlating (immuno-) electron microscopic findings with the recent nomenclature for the structural components in a 2-letter code and with the molecular weights of the virus structural (glyco-) proteins. SU stands for outer surface glycoprotein, TM for transmembrane gp, MA for membrane associated or matrix protein, LI for core-envelope-link, CA for major capsid, NC for nucleocapsid protein, respectively. PR, RT and IN represent the virus-coded enzymes protease, reverse transcriptase and integrase that are functional during the life cycle of a retrovirus

Chemical Composition and Mode of Replication

RNA Virus Genomes

RNA viruses, comprising 70% of all viruses, vary remarkably in genome structure (Fig. 41-6). Because of the error rate of the enzymes involved in RNA replication, these viruses usually show much higher mutation rates than do the DNA viruses. Mutation rates of 10^{-4} lead to the continuous generation of virus variants which show great adaptability to new hosts. The viral RNA may be single-stranded (ss) or double-stranded (ds), and the genome may occupy a single RNA segment or be distributed on two or more separate segments (segmented genomes). In addition, the RNA strand of a single-stranded genome may be either a sense strand (plus strand), which can function as messenger RNA (mRNA), or an antisense strand (minus strand), which is complementary to the sense strand and cannot function as mRNA protein translation. Sense viral RNA alone can replicate if injected into cells, since it can function as mRNA and initiate translation of virus-encoded proteins. Antisense RNA, on the other hand, has no translational function and cannot per se produce viral components.

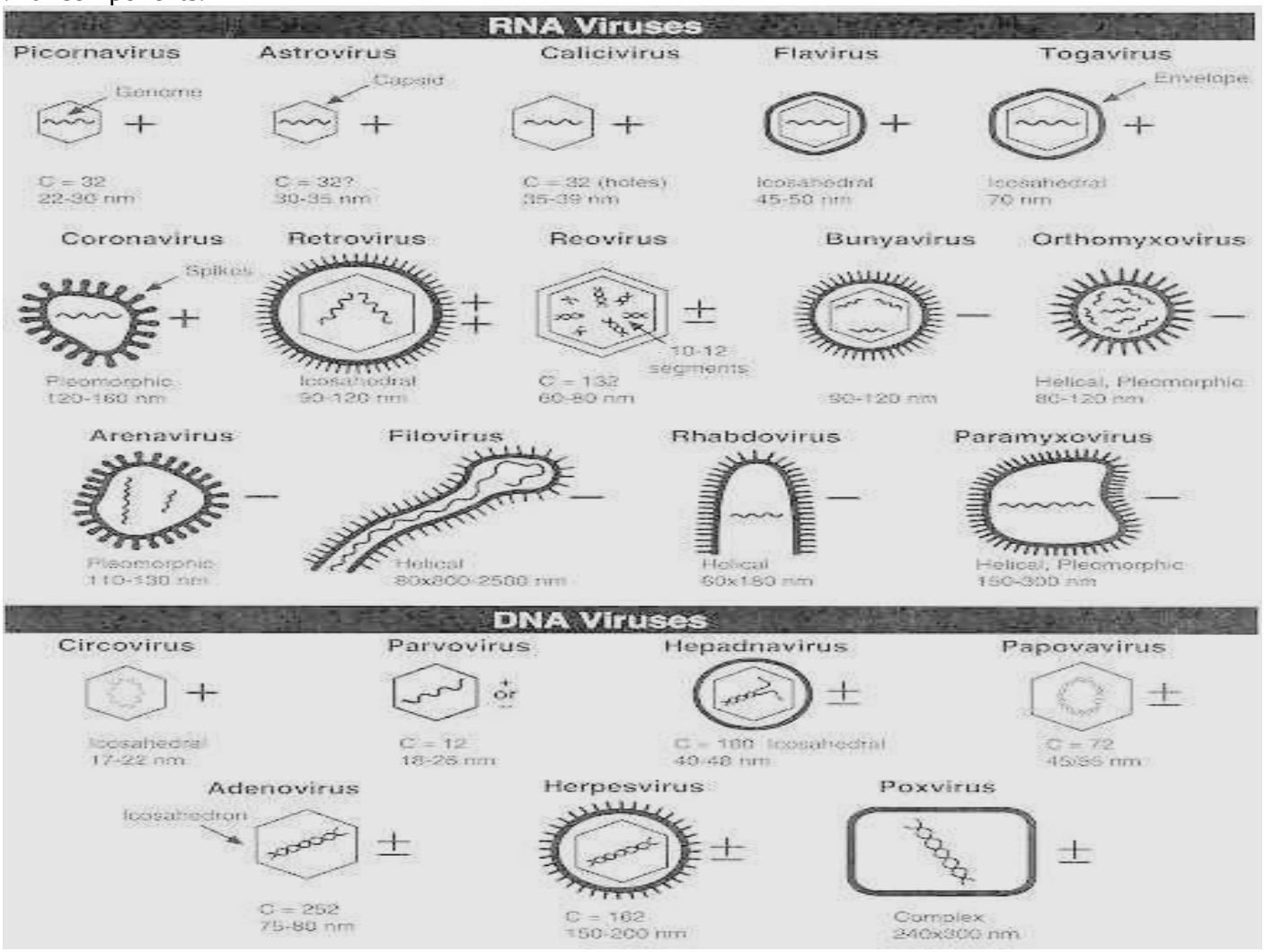


Figure 41-6

Schemes of 21 virus families infecting humans showing a number of distinctive criteria: presence of an envelope or (double-) capsid and internal nucleic acid genome. +, Sense strand; -, antisense strand; ±, dsRNA or DNA; 0, circular DNA; C, number +, Sense strand; -, antisense strand; ±, dsRNA or DNA; 0, circular DNA; C, number of capsomeres or holes, where known; nm, dimensions of capsid, or envelope when present; the hexagon designates the presence of an isometric or icosahedral outline.

DsRNA viruses, e.g., members of the reovirus family, contain 10, 11 or 12 separate genome segments coding for 3 enzymes involved in RNA replication, 3 major capsid proteins and a number of smaller structural proteins. Each segment consists of a complementary sense and antisense strand that is hydrogen bonded into a linear ds molecule. The replication of these viruses is complex; only the sense RNA strands are released from the infecting virion to initiate replication. The retrovirus genome comprises two identical, plus-sense ssRNA molecules, each monomer 7–11 kb in size, that are noncovalently linked over a short terminal region. Retroviruses contain 2 envelope proteins encoded by the env-gene, 4–6 nonglycosylated core proteins and 3 non-structural functional proteins (reverse transcriptase, integrase, protease: RT, IN, PR)

specified by the gag-gene (Fig. 41-5). The RT transcribes the viral ssRNA into double-stranded, circular proviral DNA. This DNA, mediated by the viral integrase, becomes covalently bonded into the DNA of the host cell to make possible the subsequent transcription of the sense strands that eventually give rise to retrovirus progeny. After assembly and budding, retroviruses show structural and functional maturation. In immature virions the structural proteins of the core are present as a large precursor protein shell. After proteolytic processing by the viral protease the proteins of the mature virion are rearranged and form the dense isometric or cone-shaped core typical of the mature virion, and the particle becomes infectious.

DNA Virus Genomes

Most DNA viruses (Fig. 41-6) contain a single genome of linear dsDNA. The papovaviruses, comprising the polyoma- and papillomaviruses, however, have circular DNA genomes, about 5.1 and 7.8 kb pairs in size. DsDNA serves as a template both for mRNA and for self-transcription. Three or 2 structural proteins make up the papovavirus capsid: in addition, 5-6 nonstructural proteins are encoded that are functional in virus transcription, DNA replication and cell transformation.

Single-stranded linear DNA, 4–6 kb in size, is found with the members of the Parvovirus family that comprises the parvo-, the erythro- and the dependoviruses. The virion contains 2–4 structural protein species which are differently derived from the same gene product. The adeno-associated virus (AAV, a dependovirus) is incapable of producing progeny virions except in the presence of helper viruses (adenovirus or herpesvirus). It is therefore said to be replication defective. Circular single-stranded DNA of only 1.7 to 2.3 kb is found in members of the Circovirus family which comprise the smallest autonomously propagated viruses. The isometric capsid measures 17 nm and is composed of 2 protein species only.

Virus Classification

On the basis of shared properties viruses are grouped at different hierarchical levels of order, family, subfamily, genus and species. More than 30,000 different virus isolates are known today and grouped in more than 3,600 species, in 164 genera and 71 families. Viral morphology provides the basis for grouping viruses into families. A virus family may consist of members that replicate only in vertebrates, only in invertebrates, only in plants, or only in bacteria. Certain families contain viruses that replicate in more than one of these hosts. This section concerns only the 21 families and genera of medical importance.

Besides physical properties, several factors pertaining to the mode of replication play a role in classification: the configuration of the nucleic acid (ss or ds, linear or circular), whether the genome consists of one molecule of nucleic acid or is segmented, and whether the strand of ss RNA is sense or antisense. Also considered in classification is the site of viral capsid assembly and, in enveloped viruses, the site of nucleocapsid envelopment. Table 41-1 lists the major chemical and morphologic properties of the families of viruses that cause disease in humans. The use of Latinized names ending in -viridae for virus families and ending in -virus for viral genera has gained wide acceptance. The names of subfamilies end in -virinae. Vernacular names continue to be used to describe the viruses within a genus. In this text, Latinized endings for families and subfamilies usually are not used. Table 41-2 shows the current classification of medically significant viruses.

TABLE 41-2 Current Classification of Major Groups of Viruses of Medical Significance

Family	Genera (or Subfamilies)	Vernacular Name of Type Species or Typical Member	Viruses Shown to Produce Infection in Humans
DNA Viruses			
Parvoviridae	*Erythrovirus*	B19 virus	B19 virus associated with erythema infectiosum and aplastic crisis of sickle cell anemia
	Depeondovirus	Adeno-associated virus (AAV) 2	Defective viruses (infect humans in presence of a helper adenovirus)
Papovaviridae	*Papillomavirus*	Human papilloma virus (HPV) 1	More than 60 HPV types
	Polyomavirus	Polyomavirus (simian, human, mouse)	JC and BK viruses, simian virus 40 (SV40)
Adenoviridae	*Mastadenovirus*	Human adenovirus 2	Human adenovirus serotypes 1-47
Herpesviridae	Alphaherpesvirinae	Human herpesvirus 1 Human herpesvirus 2	Herpes simplex virus 1 Herpes simplex virus 2
	Varicellovirus	Human herpesvirus 3	Varicella-zoster virus
	Gammaherpesvirinae	Human herpesvirus 4	Epstein-Barr virus
	Betaherpesvirinae	Human herpesvirus 5	Human cytomegalovirus
	Roseolovirus	Human herpesvirus 6	HHV-6; Roseola infantum
	Unclassified	Human herpesvirus 7	?
Poxviridae	Orthopoxvirus	Vaccinia virus	Vaccinia, Variola (eradicated), cowpox, monkeypox viruses
	Parapoxvirus	Orf virus	Orf, bovine pustular stomatitis, milker's node viruses
	Molluscipoxvirus	Molluscum contagiosum virus	Molluscum contagiosum
Hepadnaviridae	*Orthohepadna viruses*	Hepatitis B virus	Hepatitis B virus

TABLE 41-2 continued

Family	Genera (or Subfamilies)	Vernacular Name of Type Species or Typical Member	Viruses Shown to Produce Infection in Humans
Rhabdoviridae	*Vesiculovirus*	Vesicular stomatitis virus (VSV)	VSV and Chandipura virus
	Lyssavirus	Rabies virus	Rabies, Mokola, European bat, Duvenhage viruses
Bunyaviridae	*Bunyavirus*	Bunyamwera virus; La Crosse virus	various arthropod-transmitted viruses
	Hantavirus	Hantaan, Puumula, Seoul virus	Hemorrhagic fever with renal syndrome
	Nairovirus	Crimean-Congo hemorrhagic fever virus	Crimean-Congo hemorrhagic fever virus, Sakhalin virus group
	Phlebovirus	Sandfly fever (Sicilian) virus, Rift Valley fever virus, Uukuniemi virus	Sandfly fever virus, Rift Valley fever virus, Uukuniemi virus
Coronaviridae	*Coronavirus*	Avian infectious bronchitis virus	Human coronaviruses; several types
Arenaviridae	*Arenavirus*	Lymphocytic choriomeningitis virus	Lymphocytic choriomeningitis virus, Lassa viruses; Viruses of the Tacaribe complex
Retroviridae	BLV-HTLV-Retroviruses	Human T-lymphotropic virus I	Human T-cell leukemia viruses, Tropical spastic paresis
	Lentivirinae	Human immunodeficiency virus 1	HIV 1, HIV 2; acquired immunodeficiency syndrome
	Spomavirinae	Human spuma retrovirus	Human foamy virus; (in search of a disease)
Filoviridae	*Filovirus*	Marburg virus	Marburg, Ebola virus; hemorrhagic fever

Current Classification of Major Groups Of viruses of Medical Significance.

In the early days of virology, viruses were named according to common pathogenic properties, e.g. organ tropism and/or modes of transmission, and often also after their discoverers. From the early 1950s until the mid-1960s, when many new viruses were being discovered, it was popular to compose virus names by using sigla (abbreviations derived from a few or initial letters). Thus the name Picornaviridae is derived from pico (small) and RNA; the name Reoviridae is derived from respiratory, enteric, and orphan viruses because the agents were found in both respiratory and enteric specimens and were not related to other classified viruses; Papovaviridae is from papilloma, polyoma, and vacuolating agent (simian virus 40 [SV40]); retrovirus is from reverse transcriptase; Hepadnaviridae is from the replication of the virus in hepatocytes and their DNA genomes, as seen in hepatitis B virus. Hepatitis A virus is classified now in the family Picornaviridae, genus Hepatovirus. Although the current rules for nomenclature do not prohibit the introduction of new sigla, they require that the siglum be meaningful to workers in the field and be recognized by international study groups. The names of the other families that contain viruses pathogenic for humans are derived as follows: Adenoviridae (adeno, "gland"; refers to the adenoid tissue from which the viruses were first isolated); Astroviridae (astron means star); Arenaviridae (arena "sand") describes the

sandy appearance of the virion. Bunyaviridae (from Bunyamwera, the place in Africa where the type strain was isolated); Calicivirus (calix, "cup" or "goblet" from the cup-shaped depressions on the viral surfaces); Coronaviridae (corona, "crown") describes the appearance of the peplomers protruding from the viral surface; Filoviridae (from the Latin filum, "thread" or "filament") describes the morphology of these viruses. Herpesviridae (herpes, "creeping") describes the nature of the lesions; Orthomyxoviridae (ortho, "true," plus myxo "mucus," a substance for which the viruses have an affinity; Paramyxoviridae derived from para, "closely resembling" and myxo; Parvoviridae (parvus means, "small"); Poxviridae (pock means, "pustule"); Rhabdoviridae (rhabdo, "rod" describes the shape of the viruses and Togaviridae (toga, "cloak") refers to the tight viral envelope.

Several viruses of medical importance still remain unclassified. Some are difficult or impossible to propagate in standard laboratory host systems and thus cannot be obtained in sufficient quantity to permit more precise characterization. Hepatitis E virus, the Norwalk virus and similar agents that cause nonbacterial gastroenteritis in humans are now assigned to the calicivirus family. The fatal transmissible dementias in humans and other animals (scrapie in sheep and goat; bovine spongiform encephalopathy in cattle, transmissible mink encephalopathy; Kuru, Creutzfeldt-Jakob disease, and Gerstmann-Straussler-Scheinker syndrome in humans are caused by the accumulation of non-soluble amyloid fibrils in the central nervous systems. The agents causing transmissible subacute spongiform encephalopathies have been linked to viroids or virions (i.e. plant pathogens consisting of naked, but very stable circular RNA molecules of about 3-400 bases in size, or infectious genomes enwrapped into a host cell coat) because of their resistance to chemical and physical agents. According to an alternative theory, the term "prion" has been coined to point to an essential nonviral infectious cause for these fatal encephalopathies—prion standing for self-replicating proteinaceous agent devoid of demonstrable nucleic acid. Some of the transmissible amyloidoses show a familiar pattern and can be explained by defined mutations which render a primary soluble glycoprotein insoluble, which in turn leads to the pathogenomonic accumulation of amyloid fibers and plaques. The pathogenesis of the sporadic amyloidoses, however, is still a matter of highly ambitious research.

CLASS: I MSc MB **COURSE NAME: VIROLOGY**

COURSE CODE: 17MBP201 **UNIT: V**

Nosocomial infections, viral syndromes. Viral vaccines-interferons - Antiviral drugs - strategies to develop AIDS vaccines - Rabies vaccines preparation (animal and cell culture) and their immunization.

PLANT DISEASES CAUSED BY VIRUSES

- Plant viruses consist of a nucleoprotein that multiplies only in the living cells of a host. The presence of viruses in host cells often results in disease.
- 400 or more viruses are known to attack plants (2000 viruses are described for plants, animals, bacteria, etc.). viruses are generally specific, what infects a plant does not cause disease in an animal, and vice versa.
- The first record of a disease that was later found to be caused by a plant virus was on tulips in the 17th century in the Netherlands.
- First experimental demonstration of the infectious nature of viral disease was recorded by Lawrence, who described the transmission of a disease of jasmine by grafting.
- Adolf Mayer (1886) described a disease of tobacco called mosaikkranheit (tobacco mosaic). Disease could be transmitted to healthy plants with sap from diseased plants.
- Dmitrii Iwanowski (1892) demonstrated that the agent in tobacco mosaic was filterable. He demonstrated that the causal agent of tobacco mosaic could pass through a filter that retains bacteria.
- 1898 Martinus Beijerinck - demonstrated that the causal agent was not a microorganism but a *contagium vivum fluidum* (contagious living fluid). He was the first to use the term *virus*, which is the Latin word for poison. He concluded that this was not a toxin, because repeated inoculations of diluted infected sap yielded similar amounts of disease as it was passed from one plant to another. If it had been a toxin, it would eventually be diluted away.
- Loefler and Frosch (1898) described the first filterable infectious agent in animals - the foot-and-mouth disease virus and Walter Reed (1900) - described the first human virus, yellow fever virus.
- In 1929, F. O. Holmes provided a tool by which the virus could be measured by showing that the amount of virus present in a plant sample preparation is proportional to the number of local lesions produced on appropriate host plant leaves rubbed with the contaminated sap.
- 1935 W. M. Stanley isolated and purified some tiny white crystals from leaves of mosaic-infected tobacco plants. He treated healthy plants with TMV, which had been precipitated out of infected tobacco juice with the help of ammonium sulfate and a technique he had developed. The healthy plants contracted tobacco mosaic disease. Due to the high protein content of the purified virus particles, he concluded that the virus was an autocatalytic protein that could multiply within living cells. Although his conclusions were later proved incorrect, Stanley's work merited him receiving the Nobel Prize. He won the Nobel Prize in chemistry in 1946 for this work.
- 1937 - Bawden and Pirie demonstrated that virus consists of protein and nucleic acid (RNA).
- 1939 - Kausche - saw virus particles for the first time with the electron microscope.
- 1955-1960's Much was learned by various workers, regarding the infectivity of viral (TMV) RNA and the structure and arrangement of viral (TMV) coat protein.
- 1971 - T. O. Diener discovered viroids, which only consist of nucleic acids. Smaller than viruses, caused potato spindle tuber disease (250-400 bases long of single-stranded circular molecule of infectious RNA). About a dozen other viroids that cause disease in a variety of plants have been isolated. No viroids have ever been found in animals.

- 1980- Cauliflower mosaic virus, whose genome is a circular double-stranded DNA chromosome, was the first plant virus for which the exact sequence of all its 8,000 base pairs was determined. In 1982, the complete sequence of the bases in the single-stranded tobacco mosaic virus RNA was determined, as were those of smaller viral RNA and of viroids.
- 1986 - Use of transgenic plants to obtain resistance against viruses (TMV).

VIRUS DISEASES OF PLANTS ARE USUALLY DESCRIPTIVE OF THE TYPE OF SYMPTOMS THAT THESE CAUSE IN THE HOST

- For example, the symptoms of specific plant diseases form the basis for the following disease names: tobacco mosaic, turnip crinkle, barley yellow dwarf, ring spot of watermelon, cucumber mosaic, spotted wilt of tomato.
- Some viruses have a broader host range than the name of disease or virus may imply. For example, tobacco mosaic virus (TMV) infects tomato, eggplant, peppers, in addition to tobacco.

PROPERTIES AND MORPHOLOGY OF PLANT VIRUSES

- noncellular, ultramicroscopic particles, that multiply only in living cells. very, very small! (size measured in nanometers).
- most plant viruses consist of protein shells surrounded by a core of positive-stranded nucleic acid (normally ssRNA - nucleotides (guanine, uracil, cytosine, adenine) + 5 carbon sugar called ribose + a phosphate group), but sometimes these viruses contain dsRNA or dsDNA (2 strands of nucleotides with thymine substituted for uracil and deoxyribose instead of ribose).
- 5-40% of virus is nucleic acid 60-95% is protein
- Protein coats or shells can be different shapes, but are normally rod, filamentous, isometric, quasi-isometric/bacilliform or variants of these structures. For example, Tobacco Mosaic and Barley Stripe Mosaic viruses are rods, while broad bean wilt and maize chlorotic dwarf viruses are isometric or more spherical in shape.

VIRUS GENOME

Minimum number of genes in a plant RNA virus could be two: a coat protein and an RNA replicase gene (as is the case with RNA phages). Evidence indicates there are usually 3-5 gene products.
Plant positive-stranded RNA viruses frequently possess divided genomes. In addition, viral genomes are separately encapsulated. Viral genomes consisting of two or three different nucleic acid components, all required for infection are called bipartite, tripartite, or multipartite viruses. More than a single species of genomic RNA.
Multipartite viruses are potentially at an evolutionary disadvantage. Infectivity dilution curve for Alfalfa mosaic virus (requiring B, M, Tb particles for infectivity) is steeper than for tobacco necrosis virus (single particle). Partition of genome could potentially hinder transmission or infection by a virus.

SATELLITE VIRUSES AND RNAs

Kasinis in 1962, described the first satellite viruses. These viruses are serologically unrelated to their helpers and the two genomes exhibit little if any sequence similarity. Satellite viruses are dependent for its replication on the presence of a second, independently replicating virus.
Satellite RNAs have no coat protein of their own and are encapsulated with the help of other viral RNAs.

TRANSMISSION

- Mechanical transmission through sap by plants touching one another, through root grafts, and manhandling.
- Vegetative propagation and grafting.
- Seed, pollen, mites, nematodes, dodder, fungi (carried by zoospores and mycelium) and insects (aphids, leafhoppers, scale insects, thrips, grasshoppers, beetles, whiteflies). For example, cucumber mosaic virus and barley yellow dwarf virus moved by aphids.

DETECTION OF PLANT VIRUSES

Due to the inability to observe plant viruses visually by observing them directly through the light microscope, virologists must resort to the following methods of detecting their presence and in diagnoses.

1. Ability to transmit disease via plant sap by rubbing plant, grafting, dodder or insect transmission.
2. Indexing - indicator plants - sensitive to specific virus and will react a certain way if exposed..
3. Visual inspection with EM.
4. By eliminating possibility that symptoms are not due to other sources (e.g., herbicide, nutritional deficiencies.
5. Serological Tests (ELISA - enzyme-linked immuno sorbent assay).

Indirect (virus + Ab virus + Enzyme conjugated Ab) and direct (double-antibody sandwich technique) (Ab virus + virus + Enzyme-conjugated Ab).

1. Virus or Ab virus added to well and these become attached to walls.
2. Antibody or virus added to well and these attach to their counterpart (i.e., antigen to antibody).
3. Second antibody with enzyme conjugate attaches to first antibody/virus complex.
4. Substrate is catalyzed by enzyme and this causes a color change.

ELISA tests are extremely sensitive (small amounts of antisera are needed) results are quantitative, large samples can be run at same time (96 well plates), results can be gathered in a few hours instead of days. ELISAs along with serial dilutions of plant sap and applications of this to the leaves of susceptible hosts (by counting the number of lesions) can be used to quantify the amount of virus present.

MANAGEMENT

- Milk inactivates many viruses - use milk to wash tools/hands. "Milk does a plant body good!" Soap and water work well too!
- Removing diseased plants, killing and removing potential virus vectors (primarily weeds and insects).
- disease-resistant cultivars.
- disease or virus free seed, roots or tubers.
- cross protection (inoculation with a less-virulent strain of a virus protects the plant from a more virulent strain later when exposed to it).
- heat (some viruses are killed at temperatures that will not kill host). For example, dormant propagative organs dipped in hot water (35 C) for few minutes or hours, or by growing plants in greenhouse at 35-40 C for several days, weeks or months may inactivate virus.

TOBACCO MOSAIC

- Caused by Tobacco Mosaic Virus (TMV) worldwide distribution primarily infects tobacco and tomato, but more than 350 species are susceptible.
- tobacco leaves become mottled with light and dark green areas; leave become distorted, puckering or blistering, especially areas of new growth.
- stunting of plant growth. in tomato, mottling of leaves occurs and leaflets become long and pointed.
- TMV is a rod-shaped particle which are 300 nm long by 15-18 nm in diameter. It possesses ssRNA and a protein coat.
- difficult to inactivate, and can survive for 5 years in dead, dried tissues and many months in living plant tissues.
- many strains, that vary in virulence from severe to mild symptoms. virus is spread from plant to plant through injuries caused by crop worker, contaminated equipment and chewing insects.
- virus overwinters in dead plant tissues and debris, on contaminated equipment, in contaminated soil, greenhouse containers, bedding, tools, and in living hosts, including weeds like horsenettle, *Solanum carolinense*, and other crop plants (tomato, pepper, and eggplant).

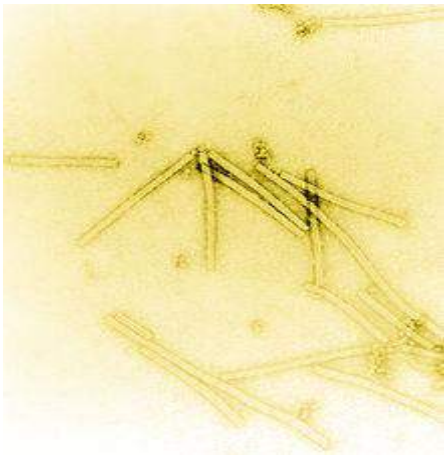
Management of Tobacco Mosaic Disease

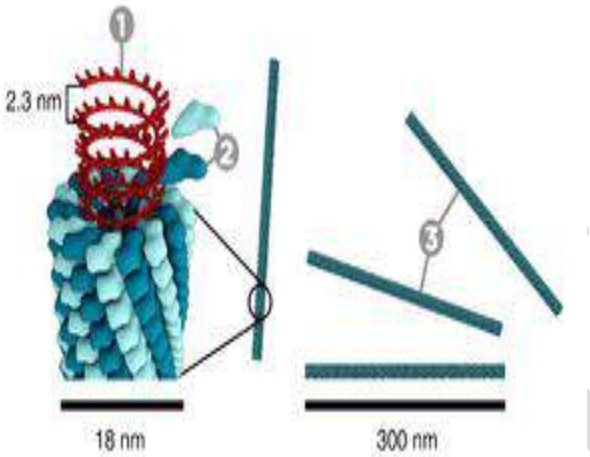
- use virus-free seed (tomato seed can by treated with acid or bleach)
- transplant in noninfested soil
- fumigatation with methyl bromide or heated.

- no chewing of tobacco or smoking around seedbeds or in greenhouses.
- to eliminate spreading of virus wash hand with soap and water or milk.
- spraying plants with milk (whole or skim) seems to help reduce
- infections. crop rotation with nonhost crops (corn, rice, other cereal grains).
- resistant cultivars

Tobacco Mosaic Virus: The Prototype Plant Virus

The stability of the TMV virus particle accounts for its having been the first virus to be identified, purified to homogeneity, and then biochemically and biologically characterized.





Small coat protein subunits (capsomeres) aggregate to form a helical protein coat or). The virus particle contains an axial channel that is 4 nm wide and the viral RNA lies within a groove in the surrounding protein helix. The nucleic acid core is not in the axial channel, but passes about halfway between the interior channel and the exterior surface of the rod. The overall particle is rod-shaped, narrow, and rigid. The pitch of the helix is 2.3 nm, and each turn contains 16 1/3 coat protein molecules. A full-length virion contains 130 helical turns.

TMV particle is resistant to nucleases and proteolytic enzymes. TMV particles will fall apart in both alkaline and acid solutions. Denaturation is often reversible, as long as temperature and pH are not too extreme. Removal of the denaturant allows the native structure of the viral protein to re-form and near its isoelectric point (pH 4 to 6), the TMV coat protein aggregates to form rod-shaped particles that look exactly like TMV virions.

When virus is subjected to neutral pH with either detergents (e.g., SDS) or 6 M urea or by extraction with phenol then RNA can be extracted in an intact form. When isolated TMV RNA are added to native TMV protein, these form stable "reconstituted" virus, which is more stable (stable from pH 3 to 9) then protein alone (unstable below pH of 4 and above pH 6).

Protein and RNA are more infectious than naked RNA alone (nearly 1000 times the amount of naked RNA is required to cause infection).

Proof that the viral RNA was the sole determinant of tobacco mosaic disease was obtained by a mixed reconstitution of RNA from Holmes ribgrass mosaic virus (RMV) with the protein subunits from TMV. Reconstituted virus caused localized lesions on plants instead of a systemic infection and formed new RMV virus (RMV RNA + protein coat containing histidine and methionine - not found in TMV). Refer to Figure 2.13 on page 50 in handout #2

Assembly of Helical Viruses

Aggregates of 33 protein molecules form the double disk. This combines with viral RNA. Attachment of the nucleic acid to the protein aggregate begins at the origin of assembly site (OAS) about 800 nucleotides from the 3' terminus of TMV common strain RNA. Rod growth toward the 5' terminus of the viral RNA is rapid, involving addition of double disks; encapsidation of the 3' terminus proceeds more slowly, through the addition of A protein monomers or small aggregates. Refer to Figure 6.6 in the textbook or to Figure 2.14 on

page 50 in handout #2). Cotranslation disassembly - the protein coat is displaced at the 5' end by ribosomes in host cell.

TMV RNA 3' TERMINUS

3' end of TMV RNA ends with the sequence -C-C-C-A and can be charged with an amino acid (histidine). This region is non-coding and be folded into a tRNA-like structure preceded by a series of four pseudoknots. Why? Four possibilities exist.

1. Donating an amino acid during some stage of protein synthesis.
2. Facilitating translation by disrupting base pairing between the 3' and 5' - terminal regions of the viral RNA
3. Acting as a recognition site for the viral replicase to initiate negative-strand synthesis
4. A molecular fossil from the original RNA world where tRNA-like structures tagged RNAs for replication and prevented the uncontrolled loss of nucleotides from third 3' terminus.

Subgenomic mRNAs and translational read-through in TMV replication.

Five open reading frames or ORFs are found in the genome of TMV. Subgenomic mRNAs and translational read-through are two strategies employed by TMV to regulate gene expression.

Plant positive-sense RNA viruses have developed several other mechanisms to facilitate and/or regulate the expression of individual genes. 5 strategies of regulating gene expression.

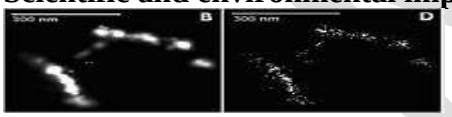
Environment

TMV is known as one of the most stable viruses. It has a very wide survival range. As long as the surrounding temperature remains below approximately 40 degrees Celsius, TMV can sustain its stable form. All it needs is a host to infect. If necessary, greenhouses and botanical gardens would provide the most favorable condition for TMV to spread out, due to the high population density of possible hosts and the constant temperature throughout the year.

Treatment and management

One of the common control methods for TMV is sanitation, which includes removing infected plants, and washing hands in between each planting. Crop rotation should also be employed to avoid infected soil/seed beds for at least two years. As for any plant disease, looking for resistant strains against TMV may also be advised. Furthermore, the cross protection method can be administered, where the stronger strain of TMV infection is inhibited by infecting the host plant with mild strain of TMV, similar to the effect of a vaccine. In the past ten years, the application of genetic engineering on a host plant genome has been developed to allow the host plant to produce the TMV coat protein within their cells. It was hypothesized that the TMV genome will be re-coated rapidly upon entering the host cell, thus it prevents the initiation of TMV replication. Later it was found that the mechanism that protects the host from viral genome insertion is through gene silencing.[20]

Scientific and environmental impact



TMV virus: super resolution light microscopy

The large amount of literature about TMV and its choice for many pioneering investigations in structural biology (including X-ray diffraction), virus assembly and disassembly, and so on, are fundamentally due to the large quantities that can be obtained, plus the fact that it does not infect animals. After growing a few infected tobacco plants in a greenhouse and a few simple laboratory procedures, a scientist can easily produce several grams of the virus. As a result of this, TMV can be treated almost as an organic chemical, rather than an infective agent.

James D. Watson, in his memoir *The Double Helix*, cites his x-ray investigation of TMV's helical structure as an important step in deducing the nature of the DNA molecule.[21]

Investigational uses

Due to its cylindrical shape, high aspect-ratio, self-assembling nature, and ability to incorporate metal coatings (nickel and cobalt) into its shell, TMV is an ideal candidate to be incorporated into battery

electrodes. Addition of TMV to a battery electrode increases the reactive surface area by an order of magnitude, resulting in an increase in the battery's capacity by up to six times compared to a planar electrode geometry.[

Cauliflower mosaic virus

Cauliflower mosaic virus (**CaMV**) is a member of the genus *Caulimovirus*, one of the six genera in the *Caulimoviridae* family, which are pararetroviruses that infect plants.[1] Pararetroviruses replicate through reverse transcription just like retroviruses, but the viral particles contain DNA instead of RNA.[2]

Definition

Aphid species *Myzus persicae*

Cauliflower mosaic virus (CaMV) is the type species of the family *Caulimoviridae*. This family is grouped together with *Hepadnaviruses* into the *Pararetrovirus* group due to its mode of replication via reverse transcription of a pre-genomic RNA intermediate.

CaMV infects mostly plants of the *Brassicaceae* family (such as cauliflower and turnip) but some CaMV strains (D4 and W260) are also able to infect *Solanaceae* species of the genera *Datura* and *Nicotiana*. CaMV induces a variety of systemic symptoms such as mosaic, necrotic lesions on leaf surfaces, stunted growth, and deformation of the overall plant structure. The symptoms exhibited vary depending on the viral strain, host ecotype, and environmental conditions.[3]

CaMV is transmitted in a non-circulatory manner by aphid species such as *Myzus persicae*.[4] Once introduced within a plant host cell, virions migrate to the nuclear envelope of the plant cell.

Structure

The CaMV particle is an icosahedron with a diameter of 52 nm built from 420 capsid protein (CP) subunits arranged with a triangulation T = 7, which surrounds a solvent-filled central cavity.[5][6]

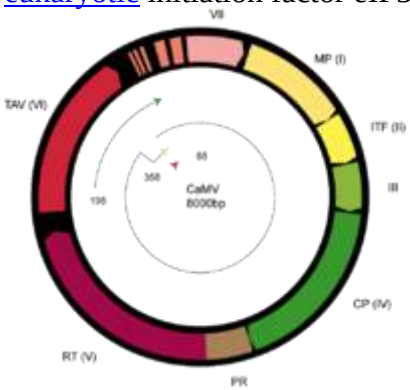
CaMV contains a circular double-stranded DNA molecule of about 8.0 kilobases, interrupted by nicks that result from the actions of RNAse H during reverse transcription. These nicks come from the Met-tRNA, and two RNA primers used in reverse transcription. After entering the host cell, these single stranded "nicks" in the viral DNA are repaired, forming a supercoiled molecule that binds to histones. This DNA is transcribed into a full length, terminally redundant[*clarification needed*], 35S RNA and a subgenomic 19S RNA.

Genome

The promoter of the 35S RNA is a very strong constitutive promoter responsible for the transcription of the whole CaMV genome. It is well known for its use in plant transformation. It causes high levels of gene expression in dicot plants. However, it is less effective in monocots, especially in cereals. The differences in behavior are probably due to differences in quality and/or quantity of regulatory factors. Interestingly, recent study has indicated that the CaMV 35S promoter is also functional in some animal cells, although the promoter elements used are different from those in plants. While this promoter had low activity compared to canonical animal promoters, levels of reporter products were significant. This observation suggests that the 35S promoter may have potential for use in animals.[7]

The promoter was named CaMV 35S promoter ("35S promoter") because the coefficient of sedimentation of the viral transcript, whose expression is naturally driven by this promoter, is 35S. It is one of the most

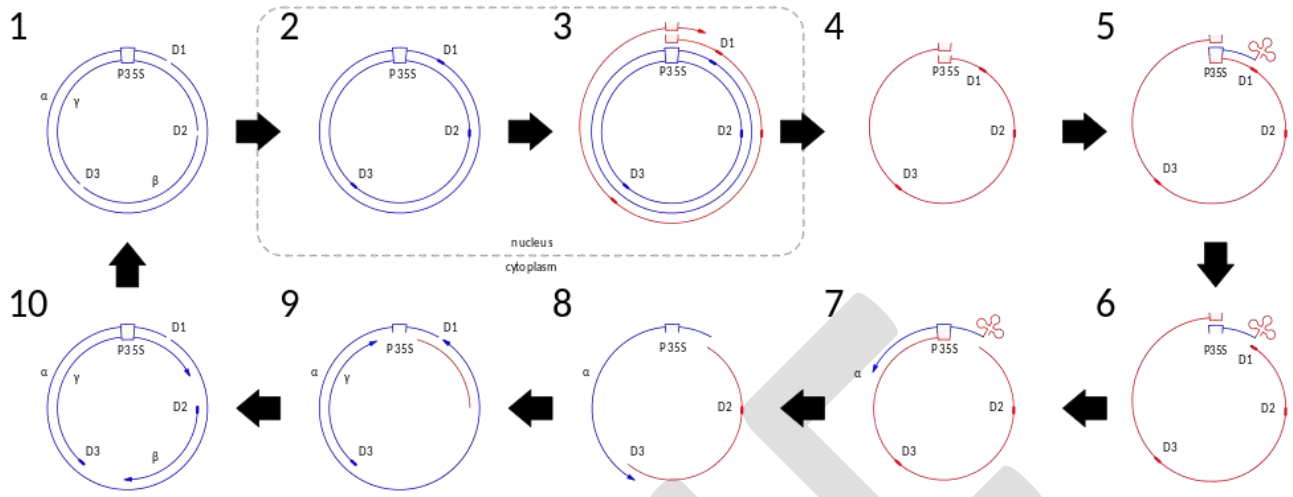
widely used, general-purpose constitutive promoters. It was discovered at the beginning of the 1980s, by Chua and collaborators at The Rockefeller University.
The 35S RNA is particularly complex, containing a highly structured 600 nucleotide long leader sequence with six to eight short open reading frames (ORFs).[8][9][10]
This leader is followed by seven tightly arranged, longer ORFs that encode all the viral proteins. The mechanism of expression of these proteins is unique, in that the ORF VI protein (encoded by the 19S RNA) controls translation reinitiation of major open reading frames on the polycistronic 35S RNA, a process that normally only happens on bacterial mRNAs. TAV function depends on its association with polysomes and eukaryotic initiation factor eIF3.[11]



- ORF I - Movement Protein
- ORF II - Insect Transmission Factor
- ORF III - Structural Protein, DNA-Binding Capabilities
- ORF IV - Capsid Protein
- ORF V - Protease, Reverse Transcriptase and RNaseH
- ORF VI - Translational Activator, Inclusion Body Formation/Trafficking; Possibly more functions (See Below)
- ORF VII - Unknown (Appears to not be required for infection)

Genomic map of CaMV
In addition to its functions regarding translational activation and formation of inclusion bodies, P6 has been shown to interact with a number of other CaMV proteins, such as P2 and P3, suggesting that it may also contribute in some degree to viral assembly and aphid-mediated transmission. In addition, P6 has been shown to bind to P7; investigating interactions between the two may help to elucidate the as yet unknown function of P7.[12]
Another interesting function of P6 involves modification of host NONEXPRESSOROFPATHOGENESISRELATED1 (NPR1) during the course of infection. NPR1 is an important regulator of salicylic acid (SA) and jasmonic acid (JA)-dependent signaling, and is most closely associated with crosstalk between the two. Modification of NPR1 serves to inhibit plant cells' defensive responses by preventing SA-dependent signaling; modified NPR1 can properly traffic to the nucleus and bind the PR-1 promoter, but is unable to initiate transcription. Because active NPR1 is required for accumulation of SA, this leads to a further depletion of SA. Whereas regulation of SA-dependent signaling by P6-modified NPR1 is localized to the nucleus, regulation of JA-dependent signaling is cytoplasmic in nature and involves the COI1 pathway. In contrast to that of SA, JA-dependent signaling is increased in the presence of modified NPR1.[13]

Replication



CaMV replicates by reverse transcription:

1. Viral particles enter a plant cell and are unencapsidated. At this stage the viral DNA consists of three fragments, one on the – strand (α) and two on the + strand (β and γ) which are imperfectly assembled into a circular genome with three gaps or discontinuities (D1, D2, and D3).
2. The viral DNA enters the nucleus where the discontinuities are filled in. At this point the viral DNA also associates with host histones, forming a minichromosome (not shown).
3. The host DNA-dependent RNA polymerase transcribes from the 35S promoter all the way around the viral genome, surpassing the 35S promoter. (This creates two copies of the 35S promoter in the resulting RNA.) Transcription also initiates at the 19S promoter (not shown).
4. The viral RNAs pass into the host cytoplasm where they are transcribed.
5. The 3′ end of a $tRNA^{fMet}$ anneals to a site corresponding to discontinuity 1 (D1) near the 5′ end of the 35S RNA.
6. The $tRNA^{fMet}$ primes synthesis, by the viral reverse transcriptase (encoded by ORF V), of a new α strand.
7. RNase H removes the RNA from the DNA–RNA duplex, leaving behind the DNA.
8. This new DNA binds the 35S promoter at the 3′ end of the RNA template and synthesis of the α strand of DNA continues and RNase H continues to degrade RNA complexed to DNA.
9. Synthesis of the α strand completes. RNase H activity exposes purine-rich regions at the position of discontinuity 3 (D3), which primes the synthesis of the γ DNA strand.
10. RNase H activity exposes purine-rich regions at the position of discontinuity 2 (D2), which primes the synthesis of the β DNA strand. When the new γ strand of DNA reaches the 5′ end of the new α strand it switches to the 5′ end of the new α strand, recreating discontinuity 1 (D1). When the new γ strand of DNA reaches the 5′ end of the new β strand, it displaces the primer and some of the newly synthesized β strand, resulting in the recreation of discontinuity 2 (D2). When the new β strand of DNA reaches the 5′ end of the new γ strand, it displaces the primer and some of the newly synthesized γ strand, resulting in the recreation of discontinuity 3 (D3).

At this point the new viral genome can either be packaged into capsids and released from the cell or they can be transported by movement proteins into an adjacent, uninfected cell.[14]

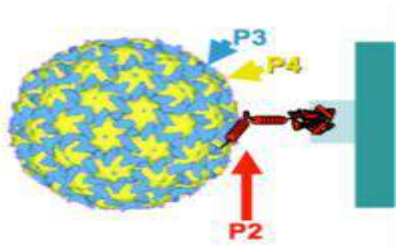
The Cauliflower mosaic virus promoter (CaMV 35S) is used in most transgenic crops to activate foreign genes which have been artificially inserted into the host plant. It is inserted into transgenic plants in a form which is different from that found when it is present in its natural *Brassica* plant hosts. This enables it to operate in a wide range of host-organism environments which would otherwise not be possible.

Prepared by – Dr. A. A. Arunkumar, Assistant Professor, Department of Microbiology, KAHE.

CaMV contains about 8 kb double-strand DNA genome and produces spherical particles. CaMV infections are systemic, and even its DNA is infectious when inoculated on abraded plant surfaces. The CaMV genome has 8 tightly packed genes, of which only two small genes, genes II and VII, are nonessential; as a result, only these two genes can be replaced/deleted without a loss of infectivity. In addition, modified CaMV genomes exceeding the natural genome size (8024 bp) by even a few hundred bp are not packaged into virions. These two factors seriously limit the size of DNA insert clonable in CaMV. The bacterial dihydrofolate reductase DHFR gene has been successfully cloned into the CaMV genome, in place of gene II, and has been successfully expressed in plants.

Molecular Mechanisms of Vector-Mediated CaMV Transmission

The virus is acquired from an infected host during feeding by the aphid vector. To occur, a transmissible complex is composed of virions and protein P2 located in the vector's stylets. The P2 N-terminal domain recognizes a protein receptor located at the tip of the stylet and the P2 C-terminal domain binds to the P3-decorated virions.[15]



Transmissible complex of CaMV

The mode of acquisition by the vector is controlled by the tissue and intracellular-specific localization of P2. This protein is only found in epidermis and parenchyma cells. Moreover, in these cells, P2 is localized in single viral electron-lucent inclusion bodies (ELIB).[16] In host cells, viral protein P2 and P3 are first produced in numerous viral factories (electron-dense inclusion bodies), and are later exported and co-localize with microtubules, before concentrating in ELIB. CaMV specifically uses the microtubules to form the transmissible body and thus enable vector transmission.[17] The complete molecular characterization and study of this virus was not carried further.

Evasion of Plant Defenses

Cauliflower mosaic virus possesses a number of mechanisms that allow it to counteract host plant cell defenses. While the pregenomic 35S RNA is responsible for genome replication by reverse transcriptase, it also contains a non-coding 600 base pair leader sequence that serves as an important mRNA for the production of factors involved in viral counter-defense. A number of hosts of CaMV possess small RNA-based viral silencing mechanisms that serve to limit viral infection. The products of the aforementioned 600-bp sequence are viral small RNAs (vsRNA) of 21, 22, and 24 nucleotides in length that serve as decoys, binding and inactivating effectors of host silencing machinery, such as Argonaute 1 (AGO1). As proof-of-principle, experimental overexpression of these vsRNAs allows for increased viral accumulation in infected plants.[18]

Concerns About Use of CaMV 35S Promoter in Transgenic Plants

Recently, some concerns have been raised about using the CaMV 35S promoter for expression in transgenic plants because sequence overlap exists between this promoter and the coding sequences of P6. Fifty four transgenic events certified for release in the USA contain up to 528 bp of ORF VI (encoding C-terminal domains of P6).[19] As P6 is a multifunctional protein whose full range of functions is unknown, there is some concern that expression of one or more of its domains may have unforeseen consequences in the transgenic organisms. Recent studies have attempted to determine what length of CaMV 35S promoter has

the least chance of inadvertently producing P6 domains, while still retaining full promoter activity. As one might expect, using shorter promoter lengths decreases the number of P6 domains included and also decreases the likelihood of unwanted effects.[19]

Geminiviridae

Geminiviridae is a family of plant viruses. There are currently 325 species in this family, divided among 7 genera. Diseases associated with this family include: bright yellow mosaic, yellow mosaic, yellow mottle, leaf curling, stunting, streaks, reduced yields.[1][2] They have single-stranded circular DNA genomes encoding genes that diverge in both directions from a virion strand origin of replication (i.e. geminivirus genomes are ambisense). According to the Baltimore classification they are considered class II viruses. It is the largest known family of single stranded DNA viruses.

Mastrevirus transmission is via various leafhopper species (e.g. maize streak virus and other African streak viruses are transmitted by *Cicadulina mbila*), curtoviruses and the only known topocuvirus species, *Tomato pseudo-curly top virus*, are transmitted by treehopper species (e.g.*Tomato pseudo-curly top virus* is transmitted by the treehopper *Micrutalis malleifera*), and begomoviruses are transmitted by the whitefly species, *Bemisia tabaci*.

These viruses are responsible for a significant amount of crop damage worldwide. Epidemics of geminivirus diseases have arisen due to a number of factors, including the recombination of different geminiviruses coinfecting a plant, which enables novel, possibly virulent viruses to be developed. Other contributing factors include the transport of infected plant material to new locations, expansion of agriculture into new growing areas, and the expansion and migration of vectors that can spread the virus from one plant to another.[3]

Virology

The genome can either be a single component between 2500–3100 nucleotides, or, in the case of some begomoviruses, two similar-sized components each between 2600 and 2800 nucleotides. They have elongated, geminate capsids with two incomplete T=1 icosahedra joined at the missing vertex. The capsids range in size from 18–20 nm in diameter with a length of about 30 nm. Begomoviruses with two component (i.e. bipartite) genomes have these components separated into two different particles both of which must usually be transmitted together to initiate a new infection within a suitable host cell.

Genus	Structure	Symmetry	Capsid	Genomic Arrangement	Genomic Segmentation
Eragrovirus	Twinned Icosahedral	Incomplete T=1	Non-Enveloped	Circular	Monopartite
Curtovirus	Twinned Icosahedral	Incomplete T=1	Non-Enveloped	Circular	Monopartite
Begomovirus	Twinned Icosahedral	Incomplete T=1	Non-Enveloped	Circular	Segmented
Becurtovirus	Twinned Icosahedral	Incomplete T=1	Non-Enveloped	Circular	Monopartite
Topocuvirus	Twinned Icosahedral	Incomplete T=1	Non-Enveloped	Circular	Monopartite
Turncurtovirus	Twinned Icosahedral	Incomplete T=1	Non-Enveloped	Circular	Monopartite

Genus	Structure	Symmetry	Capsid	Genomic Arrangement	Genomic Segmentation
Mastrevirus	Twinned Icosahedral	Incomplete T=1	Non-Enveloped	Circular	Monopartite

Taxonomy

Group: ssDNA

Order: Unassigned

Several additional genera have been proposed: Baminivirus, Nimivirus and Niminivirus.[4]

Some viruses have yet to be assigned a genus: one of these is the Grapevine Cabernet Franc-associated virus/Grapevine red blotch-associated virus/Grapevine redleaf-associated virus.[5]

Replication

Drawing of geminiviruses

Geminivirus genomes encode only a few proteins; thus, they are dependent on host cell factors for replication: these include factors such as DNA polymerase—and probably repair polymerases—in order to amplify their genomes, as well as transcription factors. Geminiviruses replicate via a rolling circle mechanism like bacteriophages such as M13, and many plasmids. Replication occurs within the nucleus of an infected plant cell. First the single-stranded circular DNA is converted to a double-stranded circular intermediate. This step involves the use of cellular DNA repair enzymes to produce a complementary negative-sense strand, using the viral genomic or plus-sense DNA strand as a template. The next step is the rolling circle phase, where the viral strand is cleaved at a specific site situated within the origin of replication by the viral Rep protein in order to initiate replication.[6] This process in a eukaryotic nucleus can give rise to concatemeric double-stranded forms of replicative intermediate genomes, although double-stranded unit circles can be isolated from infected plants and cells. New single-stranded DNA forms of the virus genome (plus-sense) are probably formed by interaction of the coat protein with replicating DNA intermediates, as genomes lacking a CP gene do not form ssDNA. The ssDNA is packaged into germinate particles in the nucleus. It is not clear if these particles can then leave the nucleus and be transmitted to surrounding cells as virions, or whether ssDNA associated with coat protein and a movement protein is the form of the genome that gets trafficked from cell to cell via the plasmodesmata.[7]

These viruses tend to be introduced into and initially infect differentiated plant cells, via the piercing mouthparts of the vector insect: however, these cells generally lack the host enzymes necessary for DNA replication, making it difficult for the virus to replicate. To overcome this block geminiviruses can induce plant cells to reenter the cell cycle from a quiescent state so that viral replication can occur.[8]

Genus	Host Details	Tissue Tropism	Entry Details	Release Details	Replication Site	Assembly Site	Transmission
Eragrovirus	Plants	None	Viral movement; mechanical inoculation	Budding	Nucleus	Nucleus	Treehopper; leafhopper
Curtovirus	Dicotyledonous plants	Phloem-limited	Viral movement;	Budding	Nucleus	Nucleus	Beet leefhopper

Genus	Host Details	Tissue Tropism	Entry Details	Release Details	Replication Site	Assembly Site	Transmission
			mechanical inoculation				
Begomovirus	Dicotyledonous plants	Phloem; sieve; phloem-limited	Viral movement; mechanical inoculation	Budding	Nucleus	Nucleus	Bemisia tabaci whiteflies
Becurtovirus	Spinach	Phloem; sieve; phloem-limited	Viral movement; mechanical inoculation	Budding	Nucleus	Nucleus	Viral movement; contact
Topocuvirus	Dicotyledonous plants	None	Cell receptor endocytosis	Budding	Nucleus	Nucleus	Leafhopper
Turncurtovirus	Turnip	None	Cell receptor endocytosis	Budding	Nucleus	Nucleus	Leafhopper
Mastrevirus	Monocots[9]	None	Viral movement; mechanical inoculation	Budding	Nucleus	Nucleus	Leafhopper

Evolution

These viruses may have evolved from a phytoplasma plasmid.[10] Geminiviruses are capable of horizontal gene transfer of genetic information to the plant host.[11]

HIV

"AIDS virus" redirects here. For the computer virus, see AIDS (computer virus).

The **human immunodeficiency virus** (**HIV**) is a lentivirus (a subgroup of retrovirus) that causes HIV infection and over time acquired immunodeficiency syndrome (AIDS).[1][2] AIDS is a condition in humans in which progressive failure of the immune system allows life-threatening opportunistic infections and cancers to thrive. Without treatment, average survival time after infection with HIV is estimated to be 9 to 11 years, depending on the HIV subtype.[3] Infection with HIV occurs by the transfer of blood, semen, vaginal fluid, pre-ejaculate, or breast milk. Within these bodily fluids, HIV is present as both free virus particles and virus within infected immune cells.

HIV infects vital cells in the human immune system such as helper T cells (specifically $CD4^+$ T cells), macrophages, and dendritic cells.[4] HIV infection leads to low levels of $CD4^+$ T cells through a number of mechanisms, including pyroptosis of abortively infected T cells,[5] apoptosis of uninfected bystander cells,[6] direct viral killing of infected cells, and killing of infected $CD4^+$ T cells by CD8 cytotoxic lymphocytes that recognize infected cells.[7] When $CD4^+$ T cell numbers decline below a critical level, cell-mediated immunity is lost, and the body becomes progressively more susceptible to opportunistic infections.

Virology

Classification

Comparison of HIV species

Species	Virulence	Infectivity	Prevalence	Inferred origin
HIV-1	High	High	Global	Common chimpanzee
HIV-2	Lower	Low	West Africa	Sooty mangabey

HIV is a member of the genus *Lentivirus*,[8] part of the family *Retroviridae*.[9] Lentiviruses have many morphologies and biological properties in common. Many species are infected by lentiviruses, which are characteristically responsible for long-duration illnesses with a long incubation period.[10] Lentiviruses are transmitted as single-stranded, positive-sense, enveloped RNA viruses. Upon entry into the target cell, the viral RNA genome is converted (reverse transcribed) into double-stranded DNA by a virally encoded reverse transcriptase that is transported along with the viral genome in the virus particle. The resulting viral DNA is then imported into the cell nucleus and integrated into the cellular DNA by a virally encoded integrase and host co-factors.[11] Once integrated, the virus may become latent, allowing the virus and its host cell to avoid detection by the immune system. Alternatively, the virus may be transcribed, producing new RNA genomes and viral proteins that are packaged and released from the cell as new virus particles that begin the replication cycle anew.

Two types of HIV have been characterized: HIV-1 and HIV-2. HIV-1 is the virus that was initially discovered and termed both LAV and HTLV-III. It is more virulent, more infective,[12] and is the cause of the majority of HIV infections globally. The lower infectivity of HIV-2 compared to HIV-1 implies that fewer of those exposed to HIV-2 will be infected per exposure. Because of its relatively poor capacity for transmission, HIV-2 is largely confined to West Africa.[13]

Structure and genome

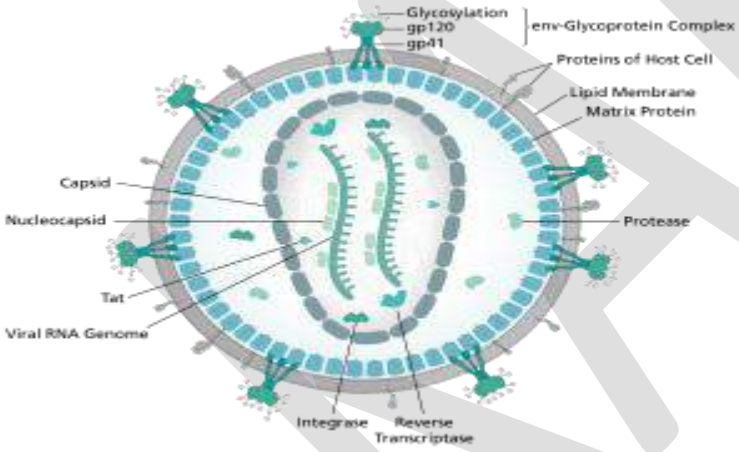
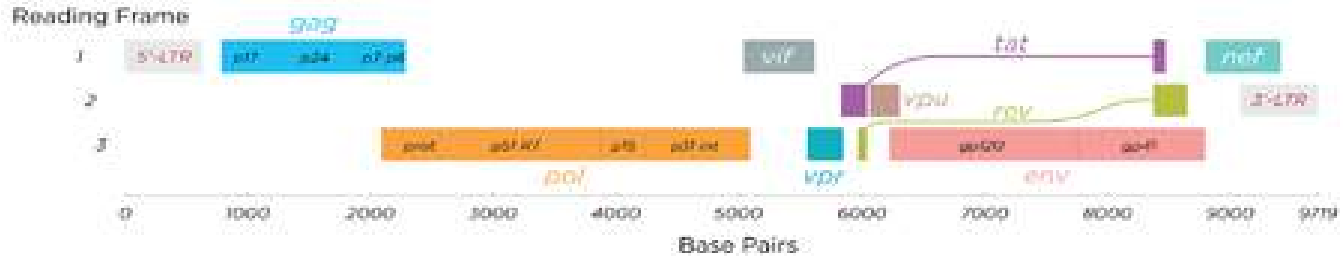


Diagram of HIV virion

HIV is different in structure from other retroviruses. It is roughly spherical[14] with a diameter of about 120 nm, around 60 times smaller than a red blood cell.[15] It is composed of two copies of positive single-stranded RNA that codes for the virus's nine genes enclosed by a conical capsid composed of 2,000 copies of the viral protein p24.[16] The single-stranded RNA is tightly bound to nucleocapsid proteins, p7, and enzymes needed for the development of the virion such as reverse transcriptase, proteases, ribonuclease and integrase. A matrix composed of the viral protein p17 surrounds the capsid ensuring the integrity of the virion particle.[16]

This is, in turn, surrounded by the viral envelope, that is composed of the lipid bilayer taken from the membrane of a human cell when the newly formed virus particle buds from the cell. The viral envelope contains proteins from the host cell and relatively few copies of the HIV Envelope protein,[16] which consists of a cap made of three molecules known as glycoprotein (gp) 120, and a stem consisting of three gp41 molecules which anchor the structure into the viral envelope.[17][18] The Envelope protein, encoded by the

HIV *env* gene, allows the virus to attach to target cells and fuse the viral envelope with the target cell membrane releasing the viral contents into the cell and initiating the infectious cycle.[17] As the sole viral protein on the surface of the virus, the Envelope protein is a major target for HIV vaccine efforts.[19] Over half of the mass of the trimeric envelope spike is N-linked glycans. The density is high as the glycans shield the underlying viral protein from neutralisation by antibodies. This is one of the most densely glycosylated molecules known and the density is sufficiently high to prevent the normal maturation process of glycans during biogenesis in the endoplasmic and Golgi apparatus.[20][21] The majority of the glycans are therefore stalled as immature 'high-mannose' glycans not normally present on secreted or cell surface human glycoproteins.[22] The unusual processing and high density means that almost all broadly neutralising antibodies that have so far been identified (from a subset of patients that have been infected for many months to years) bind to or, are adapted to cope with, these envelope glycans.[23]
The molecular structure of the viral spike has now been determined by X-ray crystallography[24] and cryo-electron microscopy.[25] These advances in structural biology were made possible due to the development of stable recombinant forms of the viral spike by the introduction of an intersubunit disulphide bond and an isoleucine to proline mutation in gp41.[26] The so-called SOSIP trimers not only reproduce the antigenic properties of the native viral spike but also display the same degree of immature glycans as presented on the native virus.[27] Recombinant trimeric viral spikes are promising vaccine candidates as they display less non-neutralising epitopes than recombinant monomeric gp120 which act to suppress the immune response to target epitopes.[28]



Structure of the RNA genome of HIV-1
The RNA genome consists of at least seven structural landmarks (LTR, TAR, RRE, PE, SLIP, CRS, and INS), and nine genes (*gag*, *pol*, and *env*, *tat*, *rev*, *nef*, *vif*, *vpr*, *vpu*, and sometimes a tenth *tev*, which is a fusion of tat env and rev), encoding 19 proteins. Three of these genes, *gag*, *pol*, and *env*, contain information needed to make the structural proteins for new virus particles.[16] For example, *env* codes for a protein called gp160 that is cut in two by a cellular protease to form gp120 and gp41. The six remaining genes, *tat*, *rev*, *nef*, *vif*, *vpr*, and *vpu* (or *vpx* in the case of HIV-2), are regulatory genes for proteins that control the ability of HIV to infect cells, produce new copies of virus (replicate), or cause disease.[16]
The two Tat proteins (p16 and p14) are transcriptional transactivators for the LTR promoter acting by binding the TAR RNA element. The TAR may also be processed into microRNAs that regulate the apoptosis genes ERCC1 and IER3.[29][30] The Rev protein (p19) is involved in shuttling RNAs from the nucleus and the cytoplasm by binding to the RRE RNA element. The Vif protein (p23) prevents the action of APOBEC3G (a cellular protein that deaminates Cytidine to Uridine in the single stranded viral DNA and/or interferes with reverse transcription[31]). The Vpr protein (p14) arrests cell division at G2/M. The Nef protein (p27) down-regulates CD4 (the major viral receptor), as well as the MHC class I and class II molecules.[32][33][34]
Nef also interacts with SH3 domains. The Vpu protein (p16) influences the release of new virus particles from infected cells.[16] The ends of each strand of HIV RNA contain an RNA sequence called the long terminal repeat (LTR). Regions in the LTR act as switches to control production of new viruses and can be triggered by proteins from either HIV or the host cell. The Psi element is involved in viral genome

packaging and recognized by Gag and Rev proteins. The SLIP element (TTTTTT) is involved in the frameshift in the Gag-Pol reading frame required to make functional Pol.[16]

Tropism

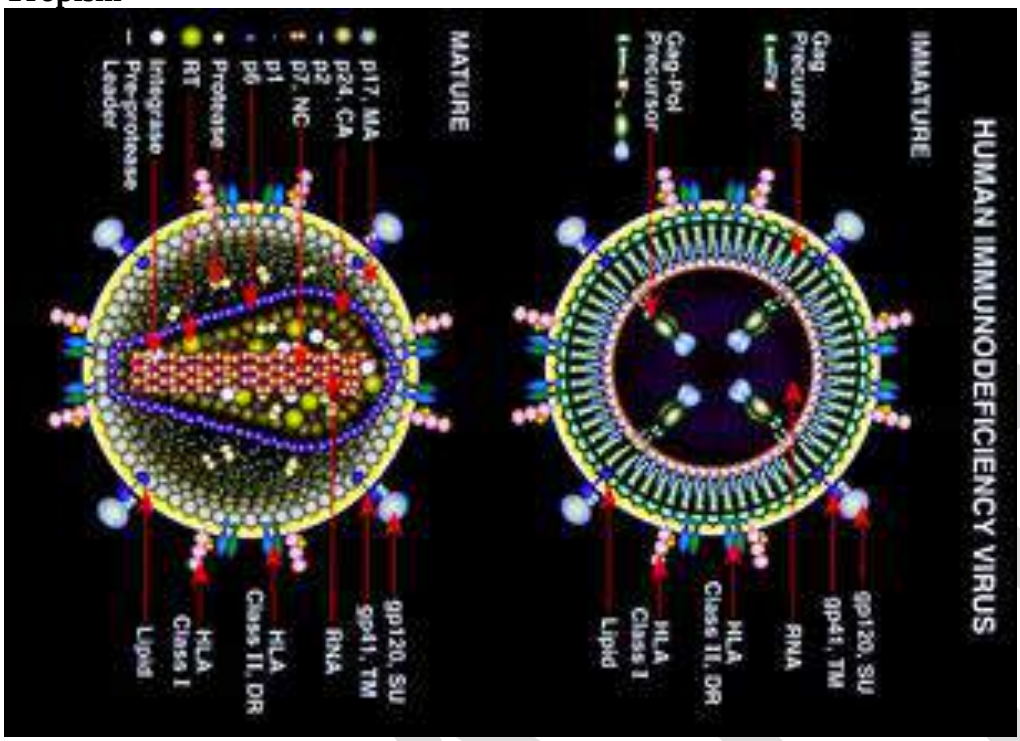


Diagram of the immature and mature forms of HIV

The term viral tropism refers to the cell types a virus infects. HIV can infect a variety of immune cells such as $CD4^+$ T cells, macrophages, and microglial cells. HIV-1 entry to macrophages and $CD4^+$ T cells is mediated through interaction of the virion envelope glycoproteins (gp120) with the CD4 molecule on the target cells and also with chemokine coreceptors.[17][35]

Macrophage (M-tropic) strains of HIV-1, or non-syncytia-inducing strains (NSI; now called R5 viruses[36]) use the β-chemokine receptor CCR5 for entry and are, thus, able to replicate in macrophages and $CD4^+$ T cells.[37] This CCR5 coreceptor is used by almost all primary HIV-1 isolates regardless of viral genetic subtype. Indeed, macrophages play a key role in several critical aspects of HIV infection. They appear to be the first cells infected by HIV and perhaps the source of HIV production when $CD4^+$ cells become depleted in the patient. Macrophages and microglial cells are the cells infected by HIV in the central nervous system. In tonsils and adenoids of HIV-infected patients, macrophages fuse into multinucleated giant cells that produce huge amounts of virus.

T-tropic isolates, or syncytia-inducing (SI; now called X4 viruses[36]) strains replicate in primary $CD4^+$ T cells as well as in macrophages and use the α-chemokine receptor, CXCR4, for entry.[37][38][39] Dual-tropic HIV-1 strains are thought to be transitional strains of HIV-1 and thus are able to use both CCR5 and CXCR4 as co-receptors for viral entry.

The α-chemokine SDF-1, a ligand for CXCR4, suppresses replication of T-tropic HIV-1 isolates. It does this by down-regulating the expression of CXCR4 on the surface of these cells. HIV that use only the CCR5 receptor are termed R5; those that use only CXCR4 are termed X4, and those that use both, X4R5. However, the use of coreceptor alone does not explain viral tropism, as not all R5 viruses are able to use CCR5 on macrophages for a productive infection[37] and HIV can also infect a subtype of myeloid dendritic cells,[40]

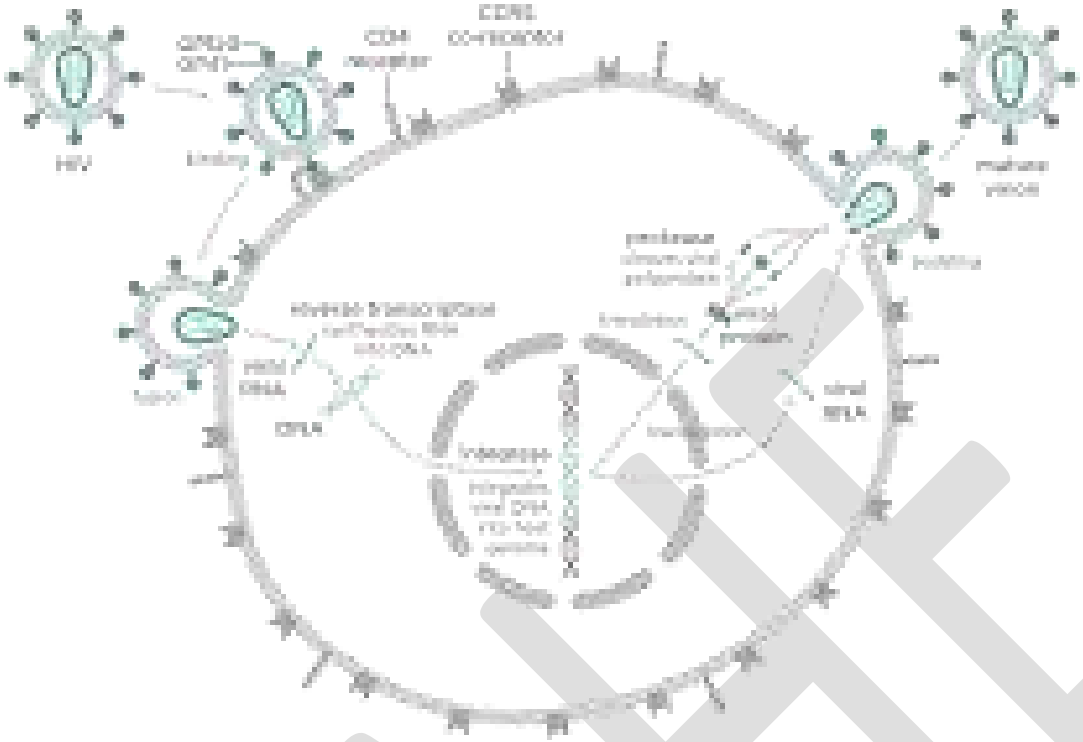
which probably constitute a reservoir that maintains infection when $CD4^+$ T cell numbers have declined to extremely low levels.

Some people are resistant to certain strains of HIV.[41] For example, people with the CCR5-Δ32 mutation are resistant to infection with R5 virus, as the mutation stops HIV from binding to this coreceptor, reducing its ability to infect target cells.

Sexual intercourse is the major mode of HIV transmission. Both X4 and R5 HIV are present in the seminal fluid, which is passed from a male to his sexual partner. The virions can then infect numerous cellular targets and disseminate into the whole organism. However, a selection process leads to a predominant transmission of the R5 virus through this pathway.[42][43][44] How this selective process works is still under investigation, but one model is that spermatozoa may selectively carry R5 HIV as they possess both CCR3 and CCR5 but not CXCR4 on their surface[45] and that genital epithelial cells preferentially sequester X4 virus.[46] In patients infected with subtype B HIV-1, there is often a co-receptor switch in late-stage disease and T-tropic variants appear that can infect a variety of T cells through CXCR4.[47] These variants then replicate more aggressively with heightened virulence that causes rapid T cell depletion, immune system collapse, and opportunistic infections that mark the advent of AIDS.[48] Thus, during the course of infection, viral adaptation to the use of CXCR4 instead of CCR5 may be a key step in the progression to AIDS. A number of studies with subtype B-infected individuals have determined that between 40 and 50 percent of AIDS patients can harbour viruses of the SI and, it is presumed, the X4 phenotypes.[49][50]

HIV-2 is much less pathogenic than HIV-1 and is restricted in its worldwide distribution. The adoption of "accessory genes" by HIV-2 and its more promiscuous pattern of coreceptor usage (including CD4-independence) may assist the virus in its adaptation to avoid innate restriction factors present in host cells. Adaptation to use normal cellular machinery to enable transmission and productive infection has also aided the establishment of HIV-2 replication in humans. A survival strategy for any infectious agent is not to kill its host but ultimately become a commensal organism. Having achieved a low pathogenicity, over time, variants more successful at transmission will be selected.[51]

Replication cycle



The HIV replication cycle

Entry to the cell

Mechanism of viral entry

1. Initial interaction between gp120 and CD4. **2.** Conformational change in gp120 allows for secondary interaction with CCR5. **3.** The distal tips of gp41 are inserted into the cellular membrane. **4.** gp41 undergoes significant conformational change; folding in half and forming coiled-coils. This process pulls the viral and cellular membranes together, fusing them.

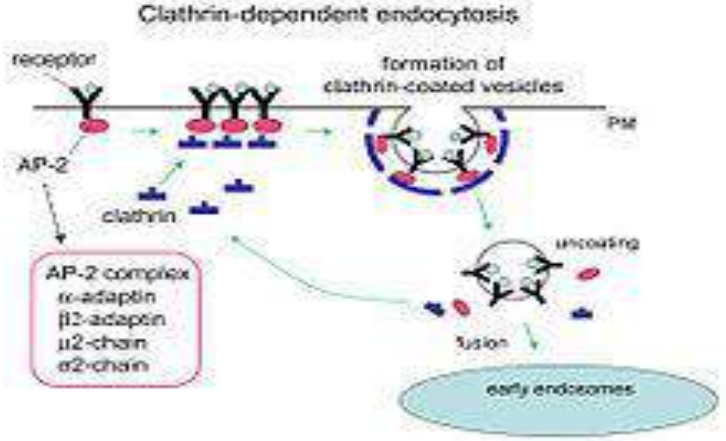
The HIV virion enters macrophages and $CD4^+$ T cells by the adsorption of glycoproteins on its surface to receptors on the target cell followed by fusion of the viral envelope with the cell membrane and the release of the HIV capsid into the cell.[52][53]

Entry to the cell begins through interaction of the trimeric envelope complex (gp160 spike) and both CD4 and a chemokine receptor (generally either CCR5 or CXCR4, but others are known to interact) on the cell surface.[52][53] gp120 binds to integrin $\alpha_4\beta_7$ activating LFA-1 the central integrin involved in the establishment of virological synapses, which facilitate efficient cell-to-cell spreading of HIV-1.[54] The gp160 spike contains binding domains for both CD4 and chemokine receptors.[52][53]

The first step in fusion involves the high-affinity attachment of the CD4 binding domains of gp120 to CD4. Once gp120 is bound with the CD4 protein, the envelope complex undergoes a structural change, exposing the chemokine binding domains of gp120 and allowing them to interact with the target chemokine receptor.[52][53] This allows for a more stable two-pronged attachment, which allows the N-terminal fusion peptide gp41 to penetrate the cell membrane.[52][53] Repeat sequences in gp41, HR1, and HR2 then interact, causing the collapse of the extracellular portion of gp41 into a hairpin. This loop structure brings the virus and cell membranes close together, allowing fusion of the membranes and subsequent entry of the viral capsid.[52][53]

After HIV has bound to the target cell, the HIV RNA and various enzymes, including reverse transcriptase, integrase, ribonuclease, and protease, are injected into the cell.[52][*not in citation given*] During the microtubule-based transport to the nucleus, the viral single-strand RNA genome is transcribed into double-strand DNA, which is then integrated into a host chromosome.

HIV can infect dendritic cells (DCs) by this CD4-CCR5 route, but another route using mannose-specific C-type lectin receptors such as DC-SIGN can also be used.[55] DCs are one of the first cells encountered by the virus during sexual transmission. They are currently thought to play an important role by transmitting HIV to T-cells when the virus is captured in the mucosa by DCs.[55] The presence of FEZ-1, which occurs naturally in neurons, is believed to prevent the infection of cells by HIV.[56]

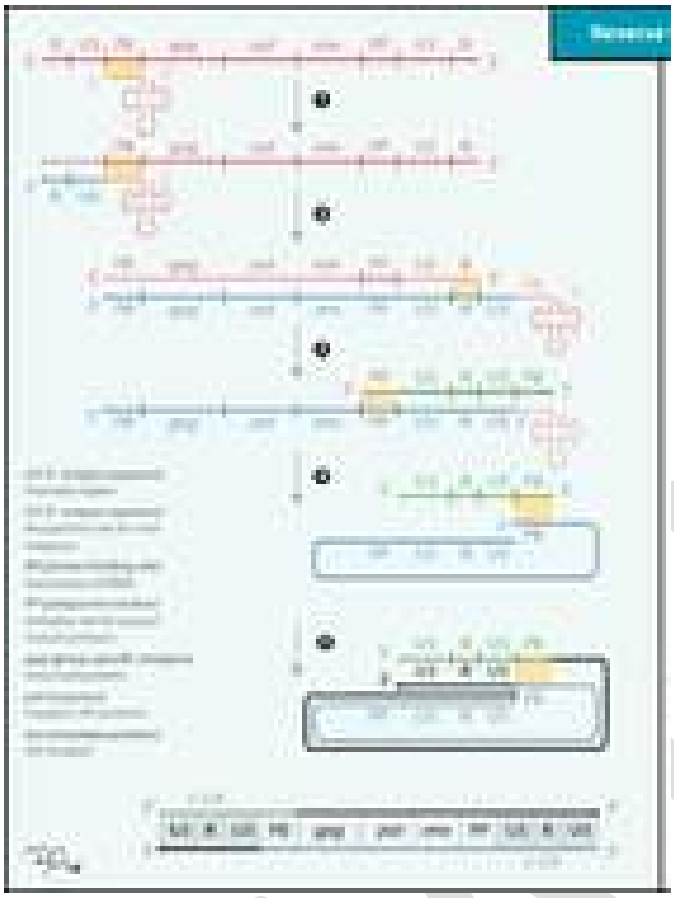


Clathrin-dependent endocytosis

HIV-1 entry, as well as entry of many other retroviruses, has long been believed to occur exclusively at the plasma membrane. More recently, however, productive infection by pH-independent, clathrin-dependent endocytosis of HIV-1 has also been reported and was recently suggested to constitute the only route of productive entry.[57][58][59][60][61]

Replication and transcription

Shortly after the viral capsid enters the cell, an enzyme called *reverse transcriptase* liberates the single-stranded (+)RNA genome from the attached viral proteins and copies it into a complementary DNA (cDNA) molecule.[62] The process of reverse transcription is extremely error-prone, and the resulting mutations may cause drug resistance or allow the virus to evade the body's immune system. The reverse transcriptase also has ribonuclease activity that degrades the viral RNA during the synthesis of cDNA, as well as DNA-dependent DNA polymerase activity that creates a sense DNA from the *antisense* cDNA.[63] Together, the cDNA and its complement form a double-stranded viral DNA that is then transported into the cell nucleus. The integration of the viral DNA into the host cell's genome is carried out by another viral enzyme called *integrase*.[62]



Reverse transcription of the HIV genome into double strand DNA

This integrated viral DNA may then lie dormant, in the latent stage of HIV infection.[62] To actively produce the virus, certain cellular transcription factors need to be present, the most important of which is NF-κB (NF kappa B), which is upregulated when T-cells become activated.[64] This means that those cells most likely to be killed by HIV are those currently fighting infection.

During viral replication, the integrated DNA provirus is transcribed into RNA, some of which then undergo RNA splicing to produce mature mRNAs. These mRNAs are exported from the nucleus into the cytoplasm, where they are translated into the regulatory proteins Tat (which encourages new virus production) and Rev. As the newly produced Rev protein accumulates in the nucleus, it binds to full-length, unspliced copies of virus RNAs and allows them to leave the nucleus.[65] Some of these full-length RNAs function as new copies of the virus genome, while others function as mRNAs that are translated to produce the structural proteins Gag and Env. Gag proteins bind to copies of the virus RNA genome to package them into new virus particles.[66]

HIV-1 and HIV-2 appear to package their RNA differently[*citation needed*]. HIV-1 will bind to any appropriate RNA[*citation needed*]. HIV-2 will preferentially bind to the mRNA that was used to create the Gag protein itself.[67]

Recombination

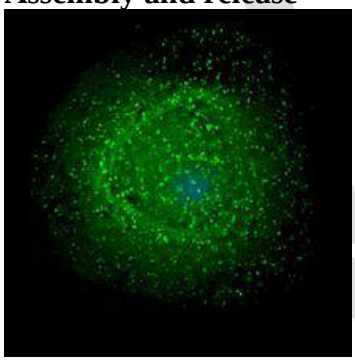
Two RNA genomes are encapsidated in each HIV-1 particle (see Structure and genome of HIV). Upon infection and replication catalyzed by reverse transcriptase, recombination between the two genomes can occur.[68][69] Recombination occurs as the single-strand (+)RNA genomes are reverse transcribed to form DNA. During reverse transcription the nascent DNA can switch multiple times between the two copies of

the viral RNA. This form of recombination is known as copy-choice. Recombination events may occur throughout the genome. From 2 to 20 events per genome may occur at each replication cycle, and these events can rapidly shuffle the genetic information that is transmitted from parental to progeny genomes.[69] Viral recombination produces genetic variation that likely contributes to the evolution of resistance to anti-retroviral therapy.[70] Recombination may also contribute, in principle, to overcoming the immune defenses of the host. Yet, for the adaptive advantages of genetic variation to be realized, the two viral genomes packaged in individual infecting virus particles need to have arisen from separate progenitor parental viruses of differing genetic constitution. It is unknown how often such mixed packaging occurs under natural conditions.[71]

Bonhoeffer et al.[72] suggested that template switching by the reverse transcriptase acts as a repair process to deal with breaks in the ssRNA genome. In addition, Hu and Temin[68] suggested that recombination is an adaptation for repair of damage in the RNA genomes. Strand switching (copy-choice recombination) by reverse transcriptase could generate an undamaged copy of genomic DNA from two damaged ssRNA genome copies. This view of the adaptive benefit of recombination in HIV could explain why each HIV particle contains two complete genomes, rather than one. Furthermore, the view that recombination is a repair process implies that the benefit of repair can occur at each replication cycle, and that this benefit can be realized whether or not the two genomes differ genetically. On the view that that recombination in HIV is a repair process, the generation of recombinational variation would be a consequence, but not the cause of, the evolution of template switching.[72]

HIV-1 infection causes chronic ongoing inflammation and production of reactive oxygen species.[73] Thus, the HIV genome may be vulnerable to oxidative damages, including breaks in the single-stranded RNA. For HIV, as well as for viruses generally, successful infection depends on overcoming host defensive strategies that often include production of genome-damaging reactive oxygen. Thus, Michod et al.[74] suggested that recombination by viruses is an adaptation for repair of genome damages, and that recombinational variation is a byproduct that may provide a separate benefit.

Assembly and release



HIV assembling on the surface of an infected macrophage.

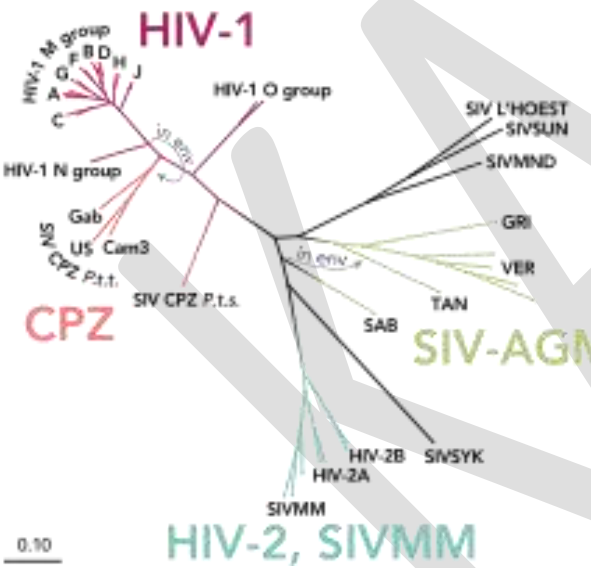
The final step of the viral cycle, assembly of new HIV-1 virions, begins at the plasma membrane of the host cell. The Env polyprotein (gp160) goes through the endoplasmic reticulum and is transported to the Golgi complex where it is cleaved by furin resulting in the two HIV envelope glycoproteins, gp41 and gp120.[75] These are transported to the plasma membrane of the host cell where gp41 anchors gp120 to the membrane of the infected cell. The Gag (p55) and Gag-Pol (p160) polyproteins also associate with the inner surface of the plasma membrane along with the HIV genomic RNA as the forming virion begins to bud from the host cell. The budded virion is still immature as the gag polyproteins still need to be cleaved into the actual matrix, capsid and nucleocapsid proteins. This cleavage is mediated by the also packaged viral protease and

can be inhibited by antiretroviral drugs of the protease inhibitor class. The various structural components then assemble to produce a mature HIV virion.[76] Only mature virions are then able to infect another cell.

Spread within the body

HIV is now known to spread between CD4+ T cells by two parallel routes: cell-free spread and cell-to-cell spread, i.e. it employs hybrid spreading mechanisms.[77] In the cell-free spread, virus particles bud from an infected T cell, enter the blood/extracellular fluid and then infect another T cell following a chance encounter.[77] HIV can also disseminate by direct transmission from one cell to another by a process of cell-to-cell spread. Two pathways of cell-to-cell transmission have been reported. Firstly, an infected T cell can transmit virus directly to a target T cell via a virological synapse.[54][78] Secondly, an antigen presenting cell (APC) can also transmit HIV to T cells by a process that either involves productive infection (in the case of macrophages) or capture and transfer of virions *in trans* (in the case of dendritic cells).[79] Whichever pathway is used, infection by cell-to-cell transfer is reported to be much more efficient than cell-free virus spread.[80] A number of factors contribute to this increased efficiency, including polarised virus budding towards the site of cell-to-cell contact, close apposition of cells which minimizes fluid-phase diffusion of virions, and clustering of HIV entry receptors on the target cell to the contact zone.[78] Cell-to-cell spread is thought to be particularly important in lymphoid tissues where CD4+ T lymphocytes are densely packed and likely to frequently interact.[77] Intravital imaging studies have supported the concept of the HIV virological synapse *in vivo*.[81] The hybrid spreading mechanisms of HIV contribute to the virus's ongoing replication against antiretroviral therapies.[77][82]

Genetic variability



The phylogenetic tree of the SIV and HIV

HIV differs from many viruses in that it has very high genetic variability. This diversity is a result of its fast replication cycle, with the generation of about 10^{10} virions every day, coupled with a high mutation rate of approximately 3×10^{-5} per nucleotide base per cycle of replication and recombinogenic properties of reverse transcriptase.[83][84][85]

This complex scenario leads to the generation of many variants of HIV in a single infected patient in the course of one day.[83] This variability is compounded when a single cell is simultaneously infected by two or more different strains of HIV. When simultaneous infection occurs, the genome of progeny virions may be composed of RNA strands from two different strains. This hybrid virion then infects a new cell where it

undergoes replication. As this happens, the reverse transcriptase, by jumping back and forth between the two different RNA templates, will generate a newly synthesized retroviral DNA sequence that is a recombinant between the two parental genomes.[83] This recombination is most obvious when it occurs between subtypes.[83]

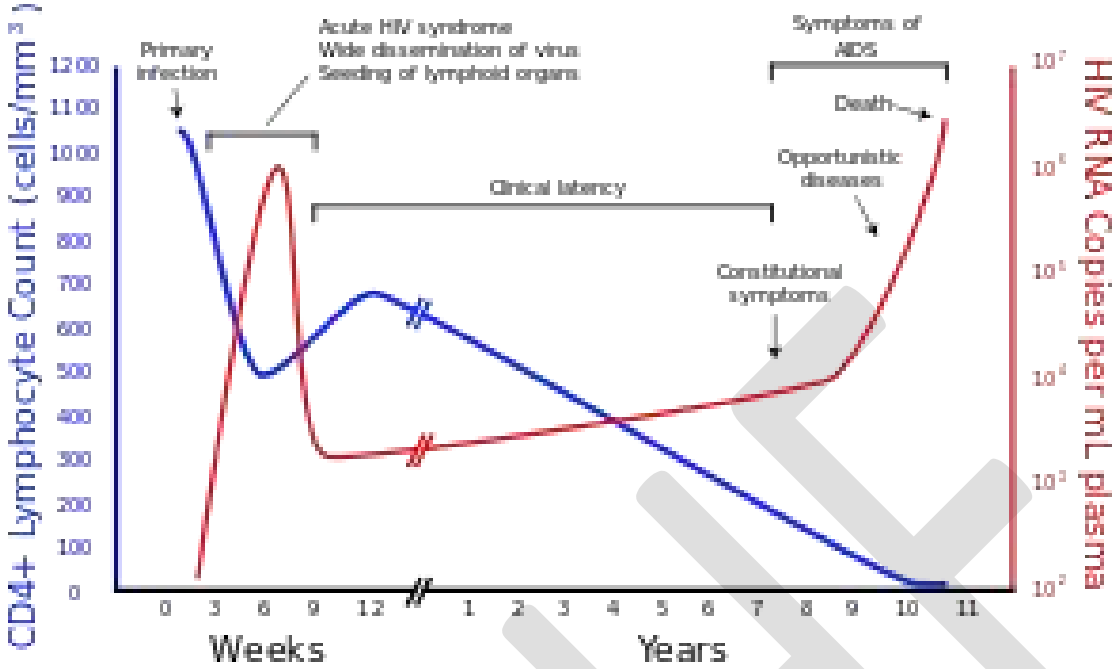
The closely related simian immunodeficiency virus (SIV) has evolved into many strains, classified by the natural host species. SIV strains of the African green monkey (SIVagm) and sooty mangabey (SIVsmm) are thought to have a long evolutionary history with their hosts. These hosts have adapted to the presence of the virus,[86] which is present at high levels in the host's blood but evokes only a mild immune response,[87] does not cause the development of simian AIDS,[88] and does not undergo the extensive mutation and recombination typical of HIV infection in humans.[89]

In contrast, when these strains infect species that have not adapted to SIV ("heterologous" hosts such as rhesus or cynomologus macaques), the animals develop AIDS and the virus generates genetic diversity similar to what is seen in human HIV infection.[90] Chimpanzee SIV (SIVcpz), the closest genetic relative of HIV-1, is associated with increased mortality and AIDS-like symptoms in its natural host.[91] SIVcpz appears to have been transmitted relatively recently to chimpanzee and human populations, so their hosts have not yet adapted to the virus.[86] This virus has also lost a function of the Nef gene that is present in most SIVs. For non-pathogenic SIV variants, Nef suppresses T-cell activation through the CD3 marker. Nef's function in non-pathogenic forms of SIV is to downregulate expression of inflammatory cytokines, MHC-1, and signals that affect T cell trafficking. In HIV-1 and SIVcpz, Nef does not inhibit T-cell activation and it has lost this function. Without this function, T cell depletion is more likely, leading to immunodeficiency.[91][92]

Three groups of HIV-1 have been identified on the basis of differences in the envelope (*env*) region: M, N, and O.[93] Group M is the most prevalent and is subdivided into eight subtypes (or clades), based on the whole genome, which are geographically distinct.[94] The most prevalent are subtypes B (found mainly in North America and Europe), A and D (found mainly in Africa), and C (found mainly in Africa and Asia); these subtypes form branches in the phylogenetic tree representing the lineage of the M group of HIV-1. Coinfection with distinct subtypes gives rise to circulating recombinant forms (CRFs). In 2000, the last year in which an analysis of global subtype prevalence was made, 47.2% of infections worldwide were of subtype C, 26.7% were of subtype A/CRF02_AG, 12.3% were of subtype B, 5.3% were of subtype D, 3.2% were of CRF_AE, and the remaining 5.3% were composed of other subtypes and CRFs.[95] Most HIV-1 research is focused on subtype B; few laboratories focus on the other subtypes.[96] The existence of a fourth group, "P", has been hypothesised based on a virus isolated in 2009.[97] The strain is apparently derived from gorilla SIV (SIVgor), first isolated from western lowland gorillas in 2006.[97]

HIV-2's closest relative is SIVsm, a strain of SIV found in sooty mangabees. Since HIV-1 is derived from SIVcpz, and HIV-2 from SIVsm, the genetic sequence of HIV-2 is only partially homologous to HIV-1 and more closely resembles that of SIVsm.[*citation needed*][98]

Diagnosis



A generalized graph of the relationship between HIV copies (viral load) and CD4 counts over the average course of untreated HIV infection; any particular individual's disease course may vary considerably.
CD4+ T cell count (cells per μL)
HIV RNA copies per mL of plasma

Many HIV-positive people are unaware that they are infected with the virus.[99] For example, in 2001 less than 1% of the sexually active urban population in Africa had been tested, and this proportion is even lower in rural populations.[99] Furthermore, in 2001 only 0.5% of pregnant women attending urban health facilities were counselled, tested or receive their test results.[99] Again, this proportion is even lower in rural health facilities.[99] Since donors may therefore be unaware of their infection, donor blood and blood products used in medicine and medical research are routinely screened for HIV.[100]

HIV-1 testing is initially by an enzyme-linked immunosorbent assay (ELISA) to detect antibodies to HIV-1. Specimens with a nonreactive result from the initial ELISA are considered HIV-negative unless new exposure to an infected partner or partner of unknown HIV status has occurred. Specimens with a reactive ELISA result are retested in duplicate.[101] If the result of either duplicate test is reactive, the specimen is reported as repeatedly reactive and undergoes confirmatory testing with a more specific supplemental test (e.g., western blot or, less commonly, an immunofluorescence assay (IFA)). Only specimens that are repeatedly reactive by ELISA and positive by IFA or reactive by western blot are considered HIV-positive and indicative of HIV infection. Specimens that are repeatedly ELISA-reactive occasionally provide an indeterminate western blot result, which may be either an incomplete antibody response to HIV in an infected person or nonspecific reactions in an uninfected person.[102]

HIV deaths in 2014.[103]

- Nigeria (15.76%)
- South Africa (12.51%)
- India (11.50%)
- Tanzania (4.169%)
- Mozambique (4.061%)
- Zimbabwe (3.49%)
- Cameroon (3.09%)
- Indonesia (3.04%)
- Kenya (2.98%)
- Uganda (2.97%)
- Malawi (2.94%)
- DR Congo (2.17%)

Ethiopia (2.11%) Other (29.21%)

Although IFA can be used to confirm infection in these ambiguous cases, this assay is not widely used. In general, a second specimen should be collected more than a month later and retested for persons with indeterminate western blot results. Although much less commonly available, nucleic acid testing (e.g., viral RNA or proviral DNA amplification method) can also help diagnosis in certain situations.[101] In addition, a few tested specimens might provide inconclusive results because of a low quantity specimen. In these situations, a second specimen is collected and tested for HIV infection.

Modern HIV testing is extremely accurate. A single screening test is correct more than 99% of the time.[104][*needs update*] The chance of a false-positive result in standard two-step testing protocol is estimated to be about 1 in 250,000 in a low risk population.[104] Testing post exposure is recommended initially and at six weeks, three months, and six months.[105]

The latest recommendations of the CDC show that HIV testing must start with an immunoassay combination test for HIV-1 and HIV-2 antibodies and p24 antigen. A negative result rules out HIV exposure, while a positive one must be followed by an HIV-1/2 antibody differentiation immunoassay to detect which is present. This gives rise to four possible scenarios:

- 1. HIV-1 (+) & HIV-2 (-): HIV-1 antibodies detected
- 2. HIV-1 (-) & HIV-2 (+): HIV-2 antibodies detected
- 3. HIV-1 (+) & HIV-2 (+): HIV antibodies detected
- 4. HIV-1 (-) or indeterminate & HIV-2 (-): Nucleic acid test must be carried out to detect the acute infection of HIV-1 or its absence.[106]

An updated algorithm published by the CDC in June 2014 recommends that diagnosis starts with the p24 antigen test. A negative result rules out infection, while a positive one must be followed by an HIV-1/2 antibody differentiation immunoassay. A positive differentiation test confirms diagnosis, while a negative or indeterminate result must be followed by nucleic acid test (NAT). A positive NAT result confirms HIV-1 infection whereas a negative result rules out infection (false positive p24).[107]

Research

HIV/AIDS research includes all medical research that attempts to prevent, treat, or cure HIV/AIDS, as well as fundamental research about the nature of HIV as an infectious agent and AIDS as the disease caused by HIV.

Many governments and research institutions participate in HIV/AIDS research. This research includes behavioral health interventions, such as research into sex education, and drug development, such as research into microbicides for sexually transmitted diseases, HIV vaccines, and antiretroviral drugs. Other medical research areas include the topics of pre-exposure prophylaxis, post-exposure prophylaxis, circumcision and HIV, and accelerated aging effects.

History

Discovery

AIDS was first clinically observed in 1981 in the United States.[108] The initial cases were a cluster of injection drug users and gay men with no known cause of impaired immunity who showed symptoms of *Pneumocystis carinii* pneumonia (PCP), a rare opportunistic infection that was known to occur in people with very compromised immune systems.[109] Soon thereafter, additional gay men developed a previously rare skin cancer called Kaposi's sarcoma (KS).[110][111] Many more cases of PCP and KS emerged, alerting U.S. Centers for Disease Control and Prevention (CDC) and a CDC task force was formed to monitor the outbreak.[112] The earliest retrospectively described case of AIDS is believed to have been in Norway beginning in 1966.[113]

In the beginning, the CDC did not have an official name for the disease, often referring to it by way of the diseases that were associated with it, for example, lymphadenopathy, the disease after which the discoverers of HIV originally named the virus.[114][115] They also used *Kaposi's Sarcoma and Opportunistic Infections*, the name by which a task force had been set up in 1981.[116] In the general press, the term *GRID*, which stood for gay-related immune deficiency, had been coined.[117] The CDC, in search of a name, and looking at the infected communities coined "the 4H disease," as it seemed to single out homosexuals, heroin users, hemophiliacs, and Haitians.[118][119] However, after determining that AIDS was not isolated to the gay community,[116] it was realized that the term GRID was misleading and *AIDS* was introduced at a meeting in July 1982.[120] By September 1982 the CDC started using the name AIDS.[121]

Robert Gallo, co-discoverer of HIV

In 1983, two separate research groups led by Robert Gallo and Luc Montagnier independently declared that a novel retrovirus may have been infecting AIDS patients, and published their findings in the same issue of the journal *Science*.[122][123] Gallo claimed that a virus his group had isolated from a person with AIDS was strikingly similar in shape to other human T-lymphotropic viruses (HTLVs) his group had been the first to isolate. Gallo's group called their newly isolated virus HTLV-III. At the same time, Montagnier's group isolated a virus from a patient presenting with swelling of the lymph nodes of the neck and physical weakness, two classic symptoms of AIDS. Contradicting the report from Gallo's group, Montagnier and his colleagues showed that core proteins of this virus were immunologically different from those of HTLV-I. Montagnier's group named their isolated virus lymphadenopathy-associated virus (LAV).[112] As these two viruses turned out to be the same, in 1986, LAV and HTLV-III were renamed HIV.[124]

Origins

Both HIV-1 and HIV-2 are believed to have originated in non-human primates in West-central Africa, and are believed to have transferred to humans (a process known as zoonosis) in the early 20th century.[125][126] HIV-1 appears to have originated in southern Cameroon through the evolution of SIV(cpz), a simian immunodeficiency virus (SIV) that infects wild chimpanzees (HIV-1 descends from the SIV(cpz) endemic in the chimpanzee subspecies *Pan troglodytes troglodytes*).[127][128] The closest relative of HIV-2 is SIV (smm), a virus of the sooty mangabey (*Cercocebus atys atys*), an Old World monkey living in litoral West Africa (from southern Senegal to western Côte d'Ivoire).[13] New World monkeys such as the owl monkey are resistant to HIV-1 infection, possibly because of a genomic fusion of two viral resistance genes.[129] HIV-1 is thought to have jumped the species barrier on at least three separate occasions, giving rise to the three groups of the virus, M, N, and O.[130]

Left to right: the African green monkey source of SIV, the sooty mangabey source of HIV-2, and the chimpanzee source of HIV-1

There is evidence that humans who participate in bushmeat activities, either as hunters or as bushmeat vendors, commonly acquire SIV.[131] However, SIV is a weak virus, and it is typically suppressed by the human immune system within weeks of infection. It is thought that several transmissions of the virus from individual to individual in quick succession are necessary to allow it enough time to mutate into HIV.[132] Furthermore, due to its relatively low person-to-person transmission rate, it can only spread throughout the population in the presence of one or more of high-risk transmission channels, which are thought to have been absent in Africa prior to the 20th century.

Specific proposed high-risk transmission channels, allowing the virus to adapt to humans and spread throughout the society, depend on the proposed timing of the animal-to-human crossing. Genetic studies of the virus suggest that the most recent common ancestor of the HIV-1 M group dates back to circa 1910.[133] Proponents of this dating link the HIV epidemic with the emergence of colonialism and growth of large colonial African cities, leading to social changes, including a higher degree of sexual promiscuity, the spread of prostitution, and the concomitant high frequency of genital ulcer diseases (such as syphilis) in nascent colonial cities.[134] While transmission rates of HIV during vaginal intercourse are typically low, they are increased many fold if one of the partners suffers from a sexually transmitted infection resulting in genital ulcers. Early 1900s colonial cities were notable due to their high prevalence of prostitution and genital ulcers to the degree that as of 1928 as many as 45% of female residents of eastern Leopoldville were thought to have been prostitutes and as of 1933 around 15% of all residents of the same city were infected by one of the forms of syphilis.[134]

An alternative view holds that unsafe medical practices in Africa during years following World War II, such as unsterile reuse of single use syringes during mass vaccination, antibiotic, and anti-malaria treatment campaigns, were the initial vector that allowed the virus to adapt to humans and spread.[132][135][136]

The earliest well documented case of HIV in a human dates back to 1959 in the Belgian Congo.[137] The virus may have been present in the United States as early as the mid-to-late 1950s, as a sixteen-year-old male presented with symptoms in 1966 died in 1969.

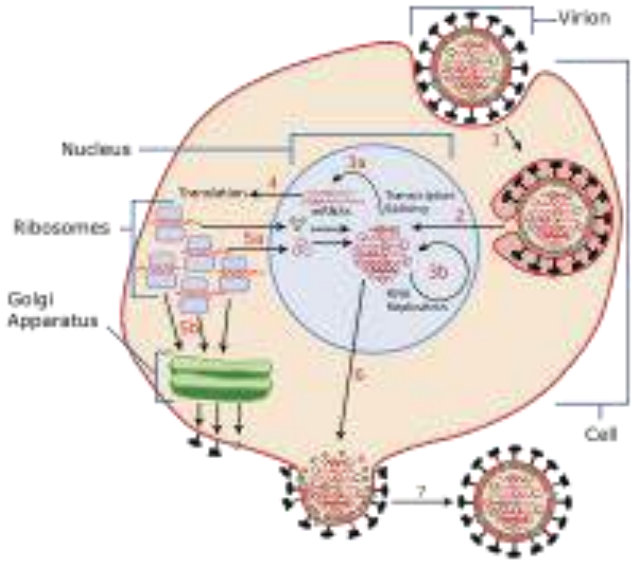
Rabies virus

Rabies virus is a neurotropic virus that causes rabies in humans and animals. Rabies transmission can occur through the saliva of animals and less commonly through contact with human saliva. Rabies virus, like many rhabdoviruses, has an extremely wide host range. In the wild it has been found infecting many mammalian species, while in the laboratory it has been found that birds can be infected, as well as cell cultures from mammals, birds, reptiles and insects.[1]

The rabies virus has a cylindrical morphology and is the type species of the *Lyssavirus* genus of the *Rhabdoviridae* family. These viruses are enveloped and have a single stranded RNA genome with negative-sense. The genetic information is packaged as a ribonucleoprotein complex in which RNA is tightly bound by the viral nucleoprotein. The RNA genome of the virus encodes five genes whose order is highly conserved. These genes code for nucleoprotein (N), phosphoprotein (P), matrix protein (M), glycoprotein (G) and the viral RNA polymerase (L).[2] The complete genome sequences range from 11,615 to 11,966 nt in length.[3]
All transcription and replication events take place in the cytoplasm inside a specialized "virus factory", the *Negri body* (named after Adelchi Negri[4]). These are 2–10 μm in diameter and are typical for a rabies infection and thus have been used as definite histological proof of such infection.[5]

Structure
Lyssaviruses have helical symmetry, so their infectious particles are approximately cylindrical in shape. They are characterized by an extremely broad host spectrum ranging from plants to insects and mammals; human-infecting viruses more commonly have icosahedral symmetry and take shapes approximating regular polyhedra.
The rabies genome encodes five proteins: nucleoprotein (N), phosphoprotein (P), matrix protein (M), glycoprotein (G) and polymerase (L). All rhabdoviruses have two major structural components: a helical ribonucleoprotein core (RNP) and a surrounding envelope. In the RNP, genomic RNA is tightly encased by the nucleoprotein. Two other viral proteins, the phosphoprotein and the large protein (L-protein or polymerase) are associated with the RNP. The glycoprotein forms approximately 400 trimeric spikes which are tightly arranged on the surface of the virus. The M protein is associated both with the envelope and the RNP and may be the central protein of rhabdovirus assembly.[6]
The rabies virus has a bullet like shape with a length of about 180 nm and a cross-sectional diameter of about 75 nm. One end is rounded or conical and the other end is planar or concave. The lipoprotein envelope carries knob-like spikes composed of Glycoprotein G. Spikes do not cover the planar end of the virion (virus particle). Beneath the envelope is the membrane or matrix (M) protein layer which may be invaginated at the planar end. The core of the virion consists of helically arranged ribonucleoprotein.
Genome Organization
The rhabdovirus virion is an enveloped, rod- or bullet-shaped structure containing five protein species.The nucleoprotein (N) coats the RNA at the rate of one monomer of protein to nine nucleotides, forming a nucleocapsid with helical symmetry. Associated with the nucleocapsid are copies of P (phosphoprotein) and L (large) protein. The L protein is well named, its gene taking up about half of the genome. Its large size is justified by the fact that it is a multifunctional protein. The M (matrix) protein forms a layer between the nucleocapsid and the envelope, and trimers of G (glycoprotein) form spikes that protrude from the envelope.The genomes of all rhabdoviruses encode these five proteins. Many rhabdoviruses encode one or more proteins in addition to these.[7]
Life cycle

Viral life cycle



- Entry
- Replication
- Latency
- Shedding

After receptor binding, rabies virus enters its host cells through the endosomal transport pathway. Inside the endosome, the low pH value induces the membrane fusion process, thus enabling the viral genome to reach the cytosol. Both processes, receptor binding and membrane fusion, are catalyzed by the glycoprotein G which plays a critical role in pathogenesis (mutant virus without G proteins cannot propagate).[2]
The next step after entry is the transcription of the viral genome by the P-L polymerase (P is an essential cofactor for the L polymerase) in order to make new viral protein. The viral polymerase can only recognize ribonucleoprotein and cannot use free RNA as template. Transcription is regulated by *cis*-acting sequences on the virus genome and by protein M which is not only essential for virus budding but also regulates the fraction of mRNA production to replication. Later in infection, the activity of the polymerase switches to replication in order to produce full-length positive-strand RNA copies. These complementary RNAs are used as templates to make new negative-strand RNA genomes. They are packaged together with protein N to form ribonucleoprotein which then can form new viruses.[5]

Infection

In September 1931, Joseph Lennox Pawan of Trinidad in the West Indies, a Government Bacteriologist, found Negri bodies in the brain of a bat with unusual habits. In 1932, Pawan first discovered that infected vampire bats could transmit rabies to humans and other animals.[8][9] For a brief history of some of the controversies surrounding the early discoveries relating to rabies in Trinidad, see the brief history by James Waterman.[10]
From the wound of entry, the rabies virus travels quickly along the neural pathways of the peripheral nervous system. The retrograde axonal transport of the rabies virus to the CNS (Central Nervous System) is the key step of pathogenesis during natural infection. The exact molecular mechanism of this transport is unknown although binding of the P protein from rabies virus to the dynein light chain protein DYNLL1 has been shown.[11] P also acts as an interferon antagonist, thus decreasing the immune response of the host. From the CNS, the virus further spreads to other organs. The salivary glands located in the tissues of the mouth and cheeks receive high concentrations of the virus, thus allowing it to be further transmitted due to

projectile salivation. Fatality can occur from two days to five years from the time of initial infection.[12] This however depends largely on the species of animal acting as a reservoir. Most infected mammals die within weeks, while strains of a species such as the African yellow mongoose (*Cynictis penicillata*) might survive an infection asymptomatically for years.[13]

Antigenicity

Upon viral entry into the body and also after vaccination, the body produces virus neutralizing antibodies which bind and inactivate the virus. Specific regions of the G protein have been shown to be most antigenic in leading to the production of virus neutralizing antibodies. These antigenic sites, or epitopes, are categorized into regions I-IV and minor site a. Previous work has demonstrated that antigenic sites II and III are most commonly targeted by natural neutralizing antibodies.[14] Additionally, a monoclonal antibody with neutralizing functionality has been demonstrated to target antigenic site I.[15] Other proteins, such as the nucleoprotein, have been shown to be unable to elicit production of virus neutralizing antibodies.[16] The epitopes which bind neutralizing antibodies are both linear and conformational.[17]

Evolution

All extant rabies viruses appear to have evolved within the last 1500 years.[18] There are seven genotypes of rabies virus. In Eurasia cases are due to three of these—genotype 1 (classical rabies) and to a lesser extent genotypes 5 and 6 (European bat lyssaviruses type-1 and -2).[19] Genotype 1 evolved in Europe in the 17th century and spread to Asia, Africa and the Americas as a result of European exploration and colonization. Bat rabies in North America appears to have been present since 1281 CE (95% confidence interval: 906–1577 CE).[20]

Application

Rabies virus is used in research for viral neuronal tracing to establish synaptic connections and directionality of synaptic transmission.[21]

Transmission

All warm-blooded species, including humans, may become infected with the rabies virus and develop symptoms. Birds were first artificially infected with rabies in 1884; however, infected birds are largely if not wholly asymptomatic, and recover.[24] Other bird species have been known to develop rabies antibodies, a sign of infection, after feeding on rabies-infected mammals.[25][26]

The virus has also adapted to grow in cells of poikilothermic ("cold-blooded") vertebrates.[27][28] Most animals can be infected by the virus and can transmit the disease to humans. Infected bats,[29][30] monkeys, raccoons, foxes, skunks, cattle, wolves, coyotes, dogs, mongooses (normally yellow mongoose)[31] and cats present the greatest risk to humans.

Rabies may also spread through exposure to infected bears, domestic farm animals, groundhogs, weasels, and other wild carnivorans. Lagomorphs, such as hares and rabbits, and small rodents such as chipmunks, gerbils, guinea pigs, hamsters, mice, rats, and squirrels, are almost never found to be infected with rabies and are not known to transmit rabies to humans.[32] Bites from mice, rats, or squirrels rarely require rabies prevention because these rodents are typically killed by any encounter with a larger, rabid animal, and would, therefore, not be carriers.[33] The Virginia opossum is resistant but not immune to rabies.[34]

The virus is usually present in the nerves and saliva of a symptomatic rabid animal.[35][36] The route of infection is usually, but not always, by a bite. In many cases, the infected animal is exceptionally aggressive, may attack without provocation, and exhibits otherwise uncharacteristic behavior.[37] This is an example of a viral pathogen modifying the behavior of its host to facilitate its transmission to other hosts.

Transmission between humans is extremely rare. A few cases have been recorded through transplant surgery.[38] The only well-documented cases of rabies caused by human-to-human transmission occurred among eight recipients of transplanted corneas and among three recipients of solid organs.[39] In addition to transmission from cornea and organ transplants, bite and non-bite exposures inflicted by infected humans

could theoretically transmit rabies, but no such cases have been documented, since infected humans are usually hospitalized and taken necessary precautions of. Casual contact, such as touching a person with rabies or contact with non-infectious fluid or tissue (urine, blood, feces) does not constitute an exposure and does not require post-exposure prophylaxis. Additionally, as the virus is present in sperm or vaginal secretions, spread through sex may be possible.[40]

After a typical human infection by bite, the virus enters the peripheral nervous system. It then travels along the afferent nerves toward the central nervous system.[41] During this phase, the virus cannot be easily detected within the host, and vaccination may still confer cell-mediated immunity to prevent symptomatic rabies. When the virus reaches the brain, it rapidly causes encephalitis, the prodromal phase, which is the beginning of the symptoms. Once the patient becomes symptomatic, treatment is almost never effective and mortality is over 99%. Rabies may also inflame the spinal cord, producing transverse myelitis.[42][43]

Diagnosis

Rabies can be difficult to diagnose, because, in the early stages, it is easily confused with other diseases or with aggressiveness.[44] The reference method for diagnosing rabies is the fluorescent antibody test (FAT), an immunohistochemistry procedure, which is recommended by the World Health Organization (WHO).[45] The FAT relies on the ability of a detector molecule (usually fluorescein isothiocyanate) coupled with a rabies-specific antibody, forming a conjugate, to bind to and allow the visualisation of rabies antigen using fluorescent microscopy techniques. Microscopic analysis of samples is the only direct method that allows for the identification of rabies virus-specific antigen in a short time and at a reduced cost, irrespective of geographical origin and status of the host. It has to be regarded as the first step in diagnostic procedures for all laboratories. Autolysed samples can, however, reduce the sensitivity and specificity of the FAT.[46] The RT PCR assays proved to be a sensitive and specific tool for routine diagnostic purposes,[47] particularly in decomposed samples[48] or archival specimens.[49] The diagnosis can be reliably made from brain samples taken after death. The diagnosis can also be made from saliva, urine, and cerebrospinal fluid samples, but this is not as sensitive and reliable as brain samples.[46] Cerebral inclusion bodies called Negri bodies are 100% diagnostic for rabies infection but are found in only about 80% of cases.[19] If possible, the animal from which the bite was received should also be examined for rabies.[50]

The differential diagnosis in a case of suspected human rabies may initially include any cause of encephalitis, in particular infection with viruses such as herpesviruses, enteroviruses, and arboviruses such as West Nile virus. The most important viruses to rule out are herpes simplex virus type one, varicella zoster virus, and (less commonly) enteroviruses, including coxsackieviruses, echoviruses, polioviruses, and human enteroviruses 68 to 71.[51]

New causes of viral encephalitis are also possible, as was evidenced by the 1999 outbreak in Malaysia of 300 cases of encephalitis with a mortality rate of 40% caused by Nipah virus, a newly recognized paramyxovirus.[52] Likewise, well-known viruses may be introduced into new locales, as is illustrated by the outbreak of encephalitis due to West Nile virus in the eastern United States.[53] Epidemiologic factors, such as season, geographic location, and the patient's age, travel history, and possible exposure to bites, rodents, and ticks, may help direct the diagnosis.

Cheaper rabies diagnosis will become possible for low-income settings: accurate rabies diagnosis can be done at a tenth of the cost of traditional testing using basic light microscopy techniques.[54] In Germany as of July 2016, rabies test strips, made by 6 companies, had high false-negative, suggesting that some manufacturers had poor quality control. The kits were made by companies in China, Germany, India, South Korea and the United States, and cost $3 to $11.[55]

Prevention

Almost all human cases of rabies were fatal until a vaccine was developed in 1885 by Louis Pasteur and Émile Roux. Their original vaccine was harvested from infected rabbits, from which the virus in the nerve

tissue was weakened by allowing it to dry for five to ten days.[56] Similar nerve tissue-derived vaccines are still used in some countries, as they are much cheaper than modern cell culture vaccines.[57]
The human diploid cell rabies vaccine was started in 1967. Less expensive purified chicken embryo cell vaccine and purified vero cell rabies vaccine are now available.[50] A recombinant vaccine called V-RG has been used in Belgium, France, Germany, and the United States to prevent outbreaks of rabies in undomesticated animals.[58] Immunization before exposure has been used in both human and nonhuman populations, where, as in many jurisdictions, domesticated animals are required to be vaccinated.[59]
The number of recorded human deaths from rabies in the United States has dropped from 100 or more annually in the early 20th century to one or two per year due to widespread vaccination of domestic dogs and cats and the development of human vaccines and immunoglobulin treatments. Most deaths now result from bat bites, which may go unnoticed by the victim and hence untreated.[60]
The Missouri Department of Health and Senior Services Communicable Disease Surveillance 2007 Annual Report states the following can help reduce the risk of contracting rabies:[61]

- Vaccinating dogs, cats, and ferrets against rabies
- Keeping pets under supervision
- Not handling wild animals or strays
- Contacting an animal control officer upon observing a wild animal or a stray, especially if the animal is acting strangely
- If bitten by an animal, washing the wound with soap and water for 10 to 15 minutes and contacting a healthcare provider to determine if post-exposure prophylaxis is required

September 28 is World Rabies Day, which promotes the information, prevention, and elimination of the disease.[62]

Treatment

Treatment after exposure can prevent the disease if administered promptly, generally within 10 days of infection.[19] Thoroughly washing the wound as soon as possible with soap and water for approximately five minutes is effective in reducing the number of viral particles.[63] Povidone-iodine or alcohol is then recommended to reduce the virus further.[64]
In the US, the Centers for Disease Control and Prevention recommends people receive one dose of human rabies immunoglobulin (HRIG) and four doses of rabies vaccine over a 14-day period.[65] The immunoglobulin dose should not exceed 20 units per kilogram body weight. HRIG is expensive and constitutes most of the cost of postexposure treatment, ranging as high as several thousand dollars.[66] As much as possible of this dose should be injected around the bites, with the remainder being given by deep intramuscular injection at a site distant from the vaccination site.[21]
The first dose of rabies vaccine is given as soon as possible after exposure, with additional doses on days three, seven and 14 after the first. Patients who have previously received pre-exposure vaccination do not receive the immunoglobulin, only the postexposure vaccinations on days 0 and 3.[67]
The pain and side effects of modern cell-based vaccines are similar to flu shots. The old nerve-tissue-based vaccinations that require multiple painful injections into the abdomen with a large needle are inexpensive, but are being phased out and replaced by affordable World Health Organization intradermal-vaccination regimens.[50]
Intramuscular vaccination should be given into the deltoid, not the gluteal area, which has been associated with vaccination failure due to injection into fat rather than muscle. In infants, the lateral thigh is recommended.[68]
Awakening to find a bat in the room, or finding a bat in the room of a previously unattended child or mentally disabled or intoxicated person, is regarded as an indication for post-exposure prophylaxis (PEP). The recommendation for the precautionary use of PEP in occult bat encounters where no contact is

recognized has been questioned in the medical literature, based on a cost-benefit analysis.[69] However, a 2002 study has supported the protocol of precautionary administering of PEP where a child or mentally compromised individual has been alone with a bat, especially in sleep areas, where a bite or exposure may occur without the victim being aware.[70] Begun with little or no delay, PEP is 100% effective against rabies.[14] In the case in which there has been a significant delay in administering PEP, the treatment should be administered regardless, as it may still be effective.[21]

Induced coma

In 2004, American teenager Jeanna Giese survived an infection of rabies unvaccinated. She was placed into an induced coma upon onset of symptoms and given ketamine, midazolam, ribavirin, and amantadine. Her doctors administered treatment based on the hypothesis that detrimental effects of rabies were caused by temporary dysfunctions in the brain and could be avoided by inducing a temporary partial halt in brain function that would protect the brain from damage while giving the immune system time to defeat the virus. After 31 days of isolation and 76 days of hospitalization, Giese was released from the hospital.[71] She survived with all higher level brain functions, but an inability to walk and balance.[72] On a podcast of NPR's *Radiolab*, Giese recounted: "I had to learn how to stand and then to walk, turn around, move my toes. I was really, after rabies, a new born baby who couldn't do anything. I had to relearn that all...mentally I knew how to do stuff but my body wouldn't cooperate with what I wanted it to do. It definitely took a toll on me psychologically. You know I'm still recovering. I'm not completely back. Stuff like balance, and I can't run normally."[73]

Giese's treatment regimen became known as the Milwaukee protocol, which has since undergone revision with the second version omitting the use of ribavirin. Two of 25 patients survived when treated under the first protocol. A further 10 patients have been treated under the revised protocol, with a further two survivors.[15]

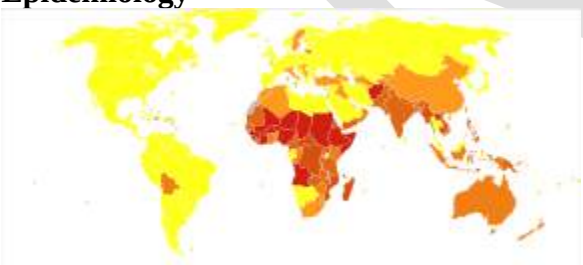
On June 12, 2011, Precious Reynolds, an eight-year-old girl from Humboldt County, California, became the third reported person in the United States to have recovered from rabies without receiving PEP.[74]

Prognosis

In unvaccinated humans, rabies is almost always fatal after neurological symptoms have developed.[75] Vaccination after exposure, PEP, is highly successful in preventing the disease if administered promptly, in general within 6 days of infection. Begun with little or no delay, PEP is 100% effective against rabies.[14] In the case of significant delay in administering PEP, the treatment still has a chance of success.[21]

Five of the first 43 patients (12%) treated with the Milwaukee protocol survived, and those receiving treatment survived longer than those not receiving the treatment.[76]

Epidemiology



Deaths from rabies per million persons in 2012

0-0
1-1
2-4
5-9

10-17
18-69

Rabies-free countries (in green) as of 2013. always rabies-free
rabies eradicated before 1990
rabies eradicated in or after 1990
year of rabies eradication unknown

In 2010, an estimated 26,000 people died from rabies, down from 54,000 in 1990.[77] The majority of the deaths occurred in Asia and Africa.[75] India has the highest rate of human rabies in the world, primarily because of stray dogs,[78] whose number has greatly increased since a 2001 law forbade the killing of dogs.[79] Effective control and treatment of rabies in India is also hindered by a form of mass hysteria known as puppy pregnancy syndrome (PPS). Dog bite victims with PPS (both male and female) become convinced that puppies are growing inside them, and often seek help from faith healers rather than from conventional medical services. In cases where the bite was from a rabid dog, this decision can prove fatal. Dr. Nitai Kishore Marik, former district medical officer of West Midnapur, states "I have seen scores of cases of rabies that reached our hospitals very late because of the intervention of faith healers. We could not save those lives."[80] An estimated 20,000 people die every year from rabies in India — more than a third of the global toll.[79] As of 2015, China had the second-highest number of cases (approximately 6,000), followed by the Democratic Republic of the Congo (5,600).[81]

The rabies virus survives in widespread, varied, rural animal reservoirs. Despite Australia's official rabies-free status,[82] Australian bat lyssavirus (ABLV), discovered in 1996, is a strain of rabies prevalent in native bat populations. There have been three human cases of ABLV in Australia, all of them fatal.

In Asia and in parts of the Americas and Africa, dogs remain the principal host. Mandatory vaccination of animals is less effective in rural areas. Especially in developing countries, pets may not be privately kept and their destruction may be unacceptable. Oral vaccines can be safely distributed in baits, a practice that has successfully reduced rabies in rural areas of Canada, France, and the United States. In Montréal, Quebec, Canada, baits are successfully used on raccoons in the Mont-Royal Park area. Vaccination campaigns may be expensive, and cost-benefit analysis suggests baits may be a cost-effective method of control.[83] In Ontario, a dramatic drop in rabies was recorded when an aerial bait-vaccination campaign was launched.[84]

Rabies is common among wild animals in the US. Bats, raccoons, skunks and foxes account for almost all reported cases (98% in 2009). Rabid bats are found in all 48 contiguous states. Other reservoirs are more limited geographically; for example, the raccoon rabies virus variant is only found in a relatively narrow band along the East Coast. Due to a high public awareness of the virus, efforts at vaccination of domestic animals and curtailment of feral populations, and availability of postexposure prophylaxis, incidents of rabies in humans are very rare. A total of 49 cases of the disease was reported in the country between 1995 and 2011; of these, 11 are thought to have been acquired abroad. Almost all domestically acquired cases are attributed to bat bites.[85]

In Switzerland, the disease has been virtually eradicated after scientists placed chicken heads laced with live attenuated vaccine in the Swiss Alps.[84] The foxes of Switzerland, proven to be the main source of rabies in the country, ate the chicken heads and immunized themselves.[84]

Italy, after being declared rabies-free from 1997 to 2008, has witnessed a reemergence of the disease in wild animals in the Triveneto regions (Trentino-Alto Adige/Südtirol, Veneto and Friuli-Venezia Giulia), due to the spreading of an epidemic in the Balkans that hit Austria too. An extensive wild animals vaccination campaign eradicated the virus from Italy again, and it regained the rabies-free country status in 2013, the last reported case of rabies being reported in a red fox in early 2011.[86][87]

History

Prepared by – Dr. A. A. Arunkumar, Assistant Professor, Department of Microbiology, KAHE.

A woodcut from the Middle Ages showing a rabid dog.

Rabies has been known since around 2000 B.C.[88] The first written record of rabies is in the Mesopotamian Codex of Eshnunna (circa 1930 BC), which dictates that the owner of a dog showing symptoms of rabies should take preventive measure against bites. If another person were bitten by a rabid dog and later died, the owner was heavily fined.[89]

Ineffective folk remedies abounded in the medical literature of the ancient world. The physician Scribonius Largus prescribed a poultice of cloth and hyena skin; Antaeus recommended a preparation made from the skull of a hanged man.[90]

Rabies appears to have originated in the Old World, the first epizootic in the New World occurring in Boston in 1768.[91] It spread from there, over the next few years, to various other states, as well as to the French West Indies, eventually becoming common all across North America.

Rabies was considered a scourge for its prevalence in the 19th century. In France and Belgium, where Saint Hubert was venerated, the "St Hubert's Key" was heated and applied to cauterize the wound. By an application of magical thinking, dogs were branded with the key in hopes of protecting them from rabies. The fear of rabies was almost irrational, due to the insignificant number of vectors (mostly rabid dogs) and the absence of any efficacious treatment. It was not uncommon for a person bitten by a dog but merely suspected of being rabid, to commit suicide or to be killed by others.[92] This gave Louis Pasteur ample opportunity to test postexposure treatments from 1885.[8] In ancient times, the attachment of the tongue (the lingual frenulum, a mucous membrane) was cut and removed as this is where rabies was thought to originate. This practice ceased with the discovery of the actual cause of rabies.[23]

In modern times, the fear of rabies has not diminished, and the disease and its symptoms, particularly agitation has served as an inspiration for several works of zombie or similarly-themed fiction, often portraying rabies as having mutated into a stronger virus which fills humans with murderous rage or uncurable illness, bringing about a devastating, widespread pandemic.[93]

Etymology

The term is derived from the Latin *rabies*, "madness".[94] This, in turn, may be related to the Sanskrit *rabhas*, "to do violence".[*citation needed*] The Greeks derived the word *lyssa*, from *lud* or "violent"; this root is used in the name of the genus of rabies *Lyssavirus*.[92]

Other animals

Main article: Rabies in animals

Rabies is infectious to mammals; three stages are recognized. The first stage is a one- to three-day period characterized by behavioral changes and is known as the prodromal stage. The second is the excitative stage, which lasts three to four days. This stage is often known as "furious rabies" for the tendency of the affected animal to be hyper-reactive to external stimuli and bite at anything near. The third is the paralytic stage and is caused by damage to motor neurons. Incoordination is seen, owing to rear limb paralysis, and drooling and difficulty swallowing is caused by paralysis of facial and throat muscles. Death is usually caused by respiratory arrest.[95]

Research

Rabies has the advantage over other pseudotyping methods for gene delivery in that the cell-targeting (tissue tropism) is more specific for difficult-to-reach sites, such as the central nervous system without invasive delivery methods, as well as being capable of retrograde tracing (i.e., going against the flow of information at synapses) in neuronal circuits.[96]
Evidence indicates artificially increasing the permeability of the blood–brain barrier, which normally does not allow most immune cells across, promotes viral clearanc

Poxviridae

Poxviridae is a family of viruses. Human, vertebrates, and arthropods serve as natural hosts. There are currently 69 species in this family, divided among 28 genera, which are divided into two subfamilies. Diseases associated with this family include smallpox.[1][2]
Four genera of poxviruses may infect humans: orthopoxvirus, parapoxvirus, yatapoxvirus, molluscipoxvirus. **Orthopox**: smallpox virus (variola), vaccinia virus, cowpox virus, monkeypox virus; **Parapox**: orf virus, pseudocowpox, bovine papular stomatitis virus; **Yatapox**: tanapox virus, yaba monkey tumor virus; **Molluscipox**: molluscum contagiosum virus (MCV).[3] The most common are vaccinia (seen on Indian subcontinent) and molluscum contagiosum, but monkeypox infections are rising (seen in west and central African rainforest countries).

Structure

A) Electron micrograph of poxvirus particles in synovium of a big brown bat, northwestern United States. B) Negative staining of poxvirus particles in cell culture supernatant. Scale bar = 100 nm.
Poxviridae viral particles (virions) are generally enveloped (external enveloped virion- EEV), though the intracellular mature virion (IMV) form of the virus, which contains different envelope, is also infectious. They vary in their shape depending upon the species but are generally shaped like a brick or as an oval form similar to a rounded brick because they are wrapped by the endoplasmic reticulum. The virion is exceptionally large, its size is around 200 nm in diameter and 300 nm in length and carries its genome in a single, linear, double-stranded segment of DNA.[4] By comparison, Rhinovirus is 1/10 as large as a typical *Poxviridae* virion.[5]

Genus	Structure	Symmetry	Capsid	Genomic Arrangement	Genomic Segmentation
Betaentomopoxvirus	Ovoid		Enveloped	Linear	Monopartite
Yatapoxvirus	Brick-shaped		Enveloped	Linear	Monopartite
Cervidpoxvirus	Brick-shaped		Enveloped	Linear	Monopartite
Gammaentomopoxvirus	Ovoid		Enveloped	Linear	Monopartite
Leporipoxvirus	Brick-shaped		Enveloped	Linear	Monopartite
Suipoxvirus	Brick-shaped		Enveloped	Linear	Monopartite
Molluscipoxvirus	Brick-shaped		Enveloped	Linear	Monopartite
Crocodylidpoxvirus	Ovoid		Enveloped	Linear	Monopartite

Genus	Structure	Symmetry	Capsid	Genomic Arrangement	Genomic Segmentation
Alphaentomopoxvirus	Ovoid		Enveloped	Linear	Monopartite
Capripoxvirus	Brick-shaped		Enveloped	Linear	Monopartite
Orthopoxvirus	Brick-shaped		Enveloped	Linear	Monopartite
Avipoxvirus	Brick-shaped		Enveloped	Linear	Monopartite
Parapoxvirus	Ovoid		Enveloped	Linear	Monopartite

Replication

Replication of the poxvirus involves several stages. The first thing the virus does is to bind to a receptor on the host cell surface; the receptors for the poxvirus are thought to be glycosaminoglycans (GAGs). After binding to the receptor, the virus enters the cell where it uncoats. Uncoating of the virus is a two step process. Firstly the outer membrane is removed as the particle enters the cell; secondly the virus particle (without the outer membrane) fuses with the cellular membrane to release the core into the cytoplasm. The pox viral genes are expressed in two phases. The early genes encode the non-structural protein, including proteins necessary for replication of the viral genome, and are expressed before the genome is replicated. The late genes are expressed after the genome has been replicated and encode the structural proteins to make the virus particle. The assembly of the virus particle occurs in five stages of maturation that lead to the final exocytosis of the new enveloped virion. After the genome has been replicated, the immature virion (IV) assembles the A5 protein to create the intracellular mature virion (IMV). The protein aligns and the brick-shaped envelope of the intracellular enveloped virion (IEV). These IEV particles are then fused to the cell plasma to form the cell-associated enveloped virion (CEV). Finally, the CEV encounters the microtubules and the virion prepares to exit the cell as an extracellular enveloped virion (EEV). The assembly of the virus particle occurs in the cytoplasm of the cell and is a complex process that is currently being researched to understand each stage in more depth. Considering the fact that this virus is large and complex, replication is relatively quick taking approximately 12 hours until the host cell dies by the release of viruses.

The replication of poxvirus is unusual for a virus with double-stranded DNA genome (dsDNA) because it occurs in the cytoplasm,[6] although this is typical of other large DNA viruses.[7] Poxvirus encodes its own machinery for genome transcription, a DNA dependent RNA polymerase,[8] which makes replication in the cytoplasm possible. Most dsDNA viruses require the host cell's DNA-dependent RNA polymerase to perform transcription. These host DNA are found in the nucleus, and therefore most dsDNA viruses carry out a part of their infection cycle within the host cell's nucleus.

Evolution

The ancestor of the poxviruses is not known but structural studies suggest it may have been an adenovirus or a species related to both the poxviruses and the adenoviruses.[9]

Based on the genome organisation and DNA replication mechanism it seems that phylogenetic relationships may exist between the rudiviruses (*Rudiviridae*) and the large eukaryal DNA viruses: the African swine fever virus (*Asfarviridae*), Chlorella viruses (*Phycodnaviridae*) and poxviruses (*Poxviridae*).[10]

The mutation rate in these genomes has been estimated to be 0.9-1.2 x 10^{-6} substitutions per site per year.[11] A second estimate puts this rate at 0.5-7 × 10^{-6} nucleotide substitutions per site per year.[12] A third estimate places the rate at 4-6 × 10^{-6}.[13]

The last common ancestor of the extant poxviruses that infect vertebrates existed 0.5 million years ago. The genus Avipoxvirus diverged from the ancestor 249 ± 69 thousand years ago. The ancestor of the genus Orthopoxvirus was next to diverge from the other clades at 0.3 million years ago. A second estimate of this divergence time places this event at 166,000 ± 43,000 years ago.[12] The division of the Orthopox into the extant genera occurred ~14,000 years ago. The genus Leporipoxvirus diverged ~137,000 ± 35,000 years ago. This was followed by the ancestor of the genus Yatapoxvirus. The last common ancestor of the Capripoxvirus and Suipoxvirus diverged 111,000 ± 29,000 years ago.
An isolate from a fish - Salmon Gill Poxvirus - appears to be the earliest branch in the Chordopoxvirinae.[14]

Smallpox

The date of the appearance of smallpox is not settled. It most likely evolved from a rodent virus between 68,000 and 16,000 years ago.[15][16] The wide range of dates is due to the different records used to calibrate the molecular clock. One clade was the variola major strains (the more clinically severe form of smallpox) which spread from Asia between 400 and 1,600 years ago. A second clade included both alastrim minor (a phenotypically mild smallpox) described from the American continents and isolates from West Africa which diverged from an ancestral strain between 1,400 and 6,300 years before present. This clade further diverged into two subclades at least 800 years ago.
A second estimate has placed the separation of variola from Taterapox at 3000–4000 years ago.[13] This is consistent with archaeological and historical evidence regarding the appearance of smallpox as a human disease which suggests a relatively recent origin. However, if the mutation rate is assumed to be similar to that of the herpesviruses the divergence date between variola from Taterapox has been estimated to be 50,000 years ago.[13] While this is consistent with the other published estimates it suggests that the archaeological and historical evidence is very incomplete. Better estimates of mutation rates in these viruses are needed.

Taxonomy

Group: dsDNA

Order: Unassigned
The name of the family, *Poxviridae*, is a legacy of the original grouping of viruses associated with diseases that produced poxes in the skin. Modern viral classification is based on phenotypic characteristics; morphology, nucleic acid type, mode of replication, host organisms, and the type of disease they cause. The smallpox virus remains as the most notable member of the family.
The species in the subfamily Chordopoxvirinae infect vertebrates and those in the subfamily Entomopoxvirinae infect insects. There are 10 recognised genera in the Chordopoxvirinae and 3 in the Entomopoxvirinae. Both subfamiles also contain a number of unclassified species for which new genera may be created in the future. Cotia virus is an unusual virus that may belong to a new genus.[17]

Notes

The GC-content of these genomes differs considerably.[18] Avipoxvirus, Capripoxvirus, Cervidpoxvirus, Orthopoxvirus, Suipoxvirus, Yatapoxvirus and one Entomopox genus (Betaentomopoxvirus) along with several other unclassified Entomopoxviruses have a low G+C content while others - Molluscipoxvirus, Orthopoxvirus, Parapoxvirus and some unclassified Chordopoxvirus - have a relatively high G+C content. The reasons for these differences are not known.
Phylogenetic analysis of 26 Chordopoxviruses genomes has shown that the central region of the genome is conserved and contains ~90 genes.[19] The termini in contrast are not conserved between species. Of this group Avipoxvirus is the most divergent. The next most divergent is Molluscipoxvirus. Capripoxvirus, Leporipoxvirus, Suipoxvirus and Yatapoxvirus genera cluster together: Capripoxvirus and Suipoxvirus share a common ancestor and are distinct from the genus Orthopoxvirus. Within the Othopoxvirus genus Cowpox virus strain Brighton Red, Ectromelia virus and Monkeypox virus do not group closely with any other

member. Variola virus and Camelpox virus form a subgroup. Vaccinia virus is most closely related to CPV-GRI-90.

Vaccinia virus

The prototypial poxvirus is vaccinia virus, known for its role as the active agent in the eradication of smallpox. The vaccinia virus is an effective tool for foreign protein expression, as it elicits a strong host immune-response. The vaccinia virus enters cells primarily by cell fusion, although currently the receptor responsible is unknown.

Vaccinia contains three classes of genes: early, intermediate and late. These genes are transcribed by viral RNA polymerase and associated transcription factors. Vaccinia replicates its genome in the cytoplasm of infected cells, and after late-stage gene expression undergoes virion morphogenesis, which produces IMV contained within an envelope membrane. The exact origin of the envelope membrane is still unknown. The IMV is then transported to the Golgi apparatus where it is wrapped with an additional two membranes, becoming the Intracellular Enveloped Virus (IEV). The IEV is transported along cytoskeletal microtubules to reach the cell periphery, where it fuses with the plasma membrane to become the Cell-associated Enveloped Virus (CEV). This triggers actin tails on cell surfaces or is released as EEV.

History

Diseases caused by pox viruses, especially smallpox, have been known about for centuries. One of the earliest suspected cases is that of Egyptian pharaoh Ramses V who is thought to have died from smallpox circa 1150 years BCE.[20][21] Smallpox was thought to have been transferred to Europe around the early 8th century and then to the Americas in the early 16th century. It is widely accepted that the main defeat of the Aztecs was due to a smallpox epidemic and within two years over 3.2 million Aztecs died. This death toll can be attributed to the American population's complete lack of exposure to the virus over millennia. A century after Edward Jenner showed that the less potent cow pox could be used to effectively vaccinate against the more deadly smallpox, a worldwide effort to vaccinate everyone against smallpox began with the ultimate goal to rid the world of the plague-like epidemic. The last case of endemic smallpox occurred in Somalia in 1977. Extensive searches over two years detected no further cases, and in 1979 the World Health Organization (WHO) declared the disease officially eradicated. In 1986, all virus samples were destroyed or transferred to two approved WHO reference labs: at the headquarters of the federal Centers for Disease Control and Prevention (the C.D.C.) in Atlanta, Georgia (the United States) and at the Institute of Virus Preparations in Moscow.[22] Post September 11, 2001 the American and UK governments have had increased concern over the use of smallpox, or a smallpox like disease, in bio-terrorism.

POX virus

Poxviruses (members of the Poxviridae family) can infect both humans and animals. The orthopoxviruses include smallpox (variola), monkeypox, vaccinia, cowpox, buffalopox, cantagalo, and aracatuba viruses. The parapoxviruses include orf virus, bovine papular stomatitis virus, pseudocowpox virus, deerpox virus, and sealpox virus. Yatapoxviruses include tanapox virus and yabapoxviruses, which are found primarily in Africa. Molluscipoxviruses include the human poxvirus, molluscum contagiosum virus.

Smallpox and molluscum contagiosum are specific to humans. The other viruses cause rare zoonotic infections in humans. Vaccinia virus, which has been used for vaccination, can also infect humans.

Infections due to poxviruses have dated back to antiquity. The first evidence of smallpox was found in Egyptian mummies of the 18th Dynasty (1580-1350 BC). Variola became endemic in India in the first millennium BC and spread to Asia and ultimately to Europe in the eighth century. The introduction of smallpox to the New World in the 15th and 16th centuries decimated Native American populations. The British used smallpox as a biological weapon during the French-Indian wars. Smallpox continued to be a major worldwide problem well into the 20th century, accounting for up to a half million deaths per year in

Europe. In the 20th century, through an intense program of vaccination, naturally occurring smallpox was eradicated.

The origins of immunization are grounded in the history of smallpox. The recognition that cutaneous exposure to the dried material of smallpox lesions caused a milder infection and induced permanent immunity led to the practice of variolization. Unfortunately, this practice frequently induced severe smallpox and death. In the 19th century, Jenner observed that inoculation with cowpox virus, a close relative of smallpox, conferred smallpox immunity. This observation established the practice of vaccination, although variolization continued into the 20th century.

The practice of vaccination with vaccinia virus began in the early 20th century. The origins of vaccinia virus remain unknown, but this virus is distinct from both variola and cowpox. Vaccinia virus has recently been shown to be closely related to the New World orthopoxviruses, cantagalo, and aracatuba viruses.

Vaccination was standardized in the mid-20th century. An aggressive program of vaccination eradicated smallpox worldwide. In 1977, the last outbreak of smallpox occurred in Somalia, and the World Health Organization (WHO) certified eradication in 1980. Recently, concern has been raised over the potential of smallpox as an agent in bioterrorism. For an excellent discussion of the subject, refer to the article by Richard Preston, "The Demon in the Freezer."[1]

Following the WHO certification of smallpox eradication in 1980, only 2 known stocks of variola virus were permitted to exist. One is kept at the US Centers for Disease Control and Prevention (CDC) in Atlanta, Georgia, and the other is kept in the former USSR. Evidence suggests that the former USSR expanded their stocks of variola and experimented with it for use as a biological weapon. Concern also exists that samples of these virus stocks have been transferred to other countries.

Molluscum contagiosum is also a poxvirus unique to humans. This virus is spread via close contact, often through sexual contact.

Other human poxvirus infections result from either zoonotic exposure to animal poxviruses or planned or accidental vaccinia administration. Notable examples of zoonotic spread to humans have recently been reported. In 2003, the first outbreak of monkeypox in North America occurred in the Midwest, with 81 cases. These infections were linked to skin exposure to pets, notably prairie dogs. The origin of the infection was ultimately traced to exotic rodents imported from Africa.[2]

Laboratory exposures that have led to infection with vaccinia and tanapox viruses, which are commonly used as vectors for experimental vaccines, have recently been documented. The smallpox vaccination program of civilian and military personnel resulted in numerous infections due to transfer to contacts.

Pathophysiology

Poxviruses are the largest and most complex viruses. They are linear double-stranded DNA viruses of 130-300 kilobase pair.[3] The 200-400 nm virion is oval or brick-shaped and can be visualized on light microscopy. The extracellular virion possesses 2 envelopes, while the intracellular virus has only one envelope. The virion contains a large number of proteins, at least 10 of which possess enzymatic activity needed for genomic replication.

The replication of poxviruses is equally complex.[4] Infection is initiated by attachment of the poxvirus to one of several cellular receptors. The virus can then enter the cell via numerous mechanisms. Unlike other DNA viruses, poxviruses replicate in the cytoplasm. The virus contains all the elements for genomic replication, but cellular functions appear necessary for complete viral maturation.

Smallpox infections are initiated by inhalational exposure of nasal, oral, or pharyngeal droplets. The incubation period is 10-14 days. Smallpox viruses replicate locally and spread to the local lymph nodes. An asymptomatic viremia ensues on day 3-4, with spread to the bone marrow and spleen. A secondary viremia begins on approximately day 8. This secondary viremia is associated with generalized symptoms of fever

and a toxic appearance. The virus in leukocytes then becomes localized in the blood vessels of the dermis. The characteristic rash of smallpox then develops.
Maculopapular lesions appear on the buccal and pharyngeal mucosa and on the face and extremities and move to the trunk. Over several days, these lesions first form vesicles, which are firm and imbedded in the epidermis. They then slowly form pustules. Approximately 8 days after onset, the pustules umbilicate. Scab formation follows. At this stage, mucosal lesions ulcerate, with the release of infectious virus into secretions. The smallpox rash is characterized by skin lesions that are in the same stage of evolution. These lesions are in contrast to chickenpox, in which lesions appear in successive waves and various forms (ie, vesicles, pustules, scabs) that can develop simultaneously. In addition, smallpox causes a significantly worse fever and toxicity prior to the rash than chickenpox. The smallpox lesions then heal, although they characteristically lead to significant scarring.
Other poxviruses are introduced by cutaneous or ocular inoculation. Vaccinia virus used as a vaccine replicates at the site of inoculation, forming local erythematous maculopapules. These maculopapules then vesiculate (ie, jennerian vesicles), scar, and heal over 10-14 days. The virus also spreads to regional lymph nodes, which is often associated with tenderness and fever. Resolution of the lesions involves pustule formation followed by scabbing and healing. This resolution is associated with the development of immunity to variola infection that persists for up to 10 years.
Other poxviruses generally follow the same pattern of evolution, with primarily localized disease. An exception is monkeypox infection, which leads to a clinical syndrome similar to variola. Monkeypox infections can range from mild with few lesions, as in the North American outbreak, to severe systemic illness that resembles smallpox. Molluscum contagiosum virus also replicates at the site of inoculation, but the character of the skin lesions is distinct.

Epidemiology
Frequency
United States
The last reported cases of wild-type smallpox occurred in 1977 in Somalia. No reporting system exists for molluscum contagiosum, but its transmission as a sexually transmitted disease is fairly common. Infections involving the other poxviruses are rare. In 2003, a monkeypox outbreak occurred, involving 81 cases related to the importation of exotic animals from Africa and subsequent spread to prairie dogs that were purchased as pets.[2]
International
With the exception of molluscum contagiosum, poxvirus infections are uncommon. The last cases of smallpox occurred in the late 1970s. Infections with the other poxviruses are due to animal exposures, laboratory infections, or spread following vaccinia immunization.
Mortality/Morbidity
Variola major carries a mortality rate of 25-30%, while the fatality rate associated with variola minor is less than 1%. Morbidity and mortality due to vaccinia infections are uncommon, but infection can be spread by autoinoculation or by close contact with someone who is infected. Poxvirus infections tend to be more severe in persons with eczema and/or immunodeficiency (eg, leukemia). Molluscum contagiosum rarely causes morbidity, although persons with immunodeficiency who develop molluscum contagiosum tend to develop multiple skin lesions. Other poxvirus infections are rare and generally cause only localized scarring. The exception is monkeypox infection. Mortality rates in African monkeypox outbreaks have been as high as 17%. No deaths were reported in the 81 cases in the United States.
Race
Poxvirus infections have no racial predilection.
Prepared by – Dr. A. A. Arunkumar, Assistant Professor, Department of Microbiology, KAHE.

Sex
Poxvirus infections have no sexual predilection.
Age
Poxvirus infections have no age predilection.
History
Among poxvirus infections, variola and molluscum contagiosum are diseases of humans. Vaccinia results from either vaccination or accidental laboratory exposure. Other poxvirus infections are zoonoses, resulting from close animal exposure.
Smallpox
Smallpox generally presents in 2 clinical forms, variola major (25-30% fatality rate) and a similar but milder disease known as variola minor (< 1% fatality rate).
Patients with smallpox initially present with nonspecific symptoms, including fever and a toxic appearance. These symptoms are followed by a slow developing maculopapular rash, which generally develops on the face and extremities and spreads to the trunk. The rash evolves rapidly into vesicles, followed by pustules, scabs, and healing.
Some patients present with unusual forms of variola. Flat smallpox is a severe form in which the pustules remain relatively flat. Hemorrhagic variola is a syndrome that appears clinically similar to meningococcemia. This form is invariably fatal.
Molluscum contagiosum
Patients infected with molluscum contagiosum develop small pearly epidermal nodules (1-2 mm in diameter) that have a characteristic central pit known as an umbilication.
This condition generally resolves over time. However, persons with immunodeficiency (eg, HIV infection) who develop molluscum contagiosum may develop chronic and extensive skin lesions.
Vaccinia
Vaccinia infections result from iatrogenic or accidental inoculation of the virus.
Infections have been described at multiple sites, including the eyes. On the skin, the infection initially appears as localized maculopapular lesions that evolve into vesicles and pustules, which then form a scab. Healing may be associated with significant scarring. The CDC has provided an excellent training program on vaccinia vaccination and adverse events (Smallpox Vaccination and Adverse Events Training Module).
Patients with vaccinia infections may have fever and regional lymphadenopathy.
In patients with eczema (ie, active or inactive), vaccinia can cause eczema vaccinatum. Infection involves the eczematous skin, and areas become intensely inflamed. The infection may disseminate. Constitutional symptoms are severe, with high fever and generalized lymphadenopathy. Death is common.
In immunodeficient patients, vaccinia is known to cause progressive vaccinia. The initial site of inoculation develops a progressive unrelenting lesion known as vaccinia gangrenosum. Dissemination of vaccinia can occur with generalized lesions. Death is common in these patients. See the images below.

Poxviruses. Following vaccination for smallpox, this patient with chronic lymphocytic leukemia developed vaccinia gangrenosum. Poxviruses. Following vaccination for smallpox, a patient with chronic lymphocytic leukemia developed vaccinia gangrenosum. The lesion was on the left shoulder. As the lesion progressed, the patient also developed evidence of dissemination. This image shows a vaccinia pustule on the foot.
Monkeypox
Monkeypox infection can produce a disease similar to variola minor characterized by a disseminated rash or relatively localized lesions. Clinically, disseminated monkeypox infection cannot be distinguished from smallpox. Monkeypox infections generally occur in villages in tropical regions of western and central

Africa. Most of the monkeypox infections that occurred during the US outbreak in 2003 were characterized by localized lesions

Other human poxvirus infections

Other human poxvirus infections include cowpox, orf (ie, contagious pustular dermatitis), bovine papular stomatitis, pseudocowpox (milker's nodule), sealpox, tanapox, and yabapox. These are rare zoonotic infections that are caused by cutaneous inoculation due to the close proximity of humans to animals. Cowpox causes a localized pustular skin lesion that follows a course similar to that of uncomplicated vaccinia infection. The remainder of the infections produce a localized nodular lesion that resolves over time.

Physical

Poxvirus infections cause either a localized or a generalized vesicular exanthem. The lesions of smallpox, vaccinia, monkeypox, and cowpox evolve from a papule to a vesicle. The vesicles then form pustules, followed by scabbing and healing. The remaining viruses cause localized nodules at the site of inoculation. Individual viruses cause characteristic clinical syndromes. With the exception of smallpox, regional lymphadenopathy is common.

Causes

Exposure to poxviruses (members of the Poxviridae family) causes these infections

Herpes simplex virus

Herpes simplex virus 1 and **2** (**HSV-1** and **HSV-2**), also known as **human herpesvirus 1** and **2** (**HHV-1** and **HHV-2**), are two members of the herpesvirus family, Herpesviridae, that infect humans.[1] Both HSV-1 (which produces most cold sores) and HSV-2 (which produces most genital herpes) are ubiquitous and contagious. They can be spread when an infected person is producing and shedding the virus.

Symptoms of herpes simplex virus infection include watery blisters in the skin or mucous membranes of the mouth, lips or genitals.[1] Lesions heal with a scab characteristic of herpetic disease. Sometimes, the viruses cause very mild or atypical symptoms during outbreaks. However, they can also cause more troublesome forms of herpes simplex. As neurotropic and neuroinvasive viruses, HSV-1 and -2 persist in the body by becoming *latent* and hiding from the immune system in the cell bodies of neurons. After the initial or *primary* infection, some infected people experience sporadic episodes of viral *reactivation* or *outbreaks*. In an outbreak, the virus in a nerve cell becomes active and is transported via the neuron's axon to the skin, where virus replication and shedding occur and cause new sores.[2] It is one of the most common sexually transmitted infections.[3]

Transmission

HSV-1 and -2 are transmitted by contact with an infected area of the skin during reactivations of the virus. Herpes simplex virus (HSV)-2 is periodically shed in the human genital tract, most often asymptomatically, and most sexual transmissions occur during asymptomatic shedding.[4] Asymptomatic reactivation means that the virus causes atypical, subtle or hard to notice symptoms that are not identified as an active herpes infection. In one study, daily genital swab samples found HSV-2 at a median of 12–28% of days among those who have had an outbreak, and 10% of days among those suffering from asymptomatic infection, with many of these episodes occurring without visible outbreak ("subclinical shedding").[5]

In another study, 73 subjects were randomized to receive valaciclovir 1 g daily or placebo for 60 days each in a 2-way crossover design. A daily swab of the genital area was self-collected for HSV-2 detection by polymerase chain reaction, in order to compare the effect of valaciclovir 1 g once daily for 60 days versus placebo on asymptomatic viral shedding in immunocompetent, HSV-2 seropositive subjects without a

history of symptomatic genital herpes infection. The study found that valaciclovir significantly reduced shedding during subclinical days compared to placebo, showing a 71% reduction. 84% of subjects had no shedding while receiving valaciclovir versus 54% of subjects on placebo. 88% of patients treated with valaciclovir had no recognized signs or symptoms versus 77% for placebo.[6]
For HSV-2, subclinical shedding may account for most of the transmission, and one study found that infection occurred after a median of 40 sex acts.[5] Atypical symptoms are often attributed to other causes such as a yeast infection.[7][8] HSV-1 is often acquired orally during childhood. It may also be sexually transmitted, including contact with saliva, such as kissing and mouth-to-genital contact (oral sex).[9] HSV-2 is primarily a sexually transmitted infection, but rates of HSV-1 genital infections are increasing.[7]
Both viruses may also be transmitted vertically during childbirth, although the real risk is very low.[10] The risk of infection is minimal if the mother has no symptoms or exposed blisters during delivery. The risk is considerable when the mother is infected with the virus for the first time during late pregnancy.[11]
Herpes simplex viruses can affect areas of skin exposed to contact with an infected person. An example of this is herpetic whitlow which is a herpes infection on the fingers. This was a common affliction of dental surgeons prior to the routine use of gloves when conducting treatment on patients.

Viral structure

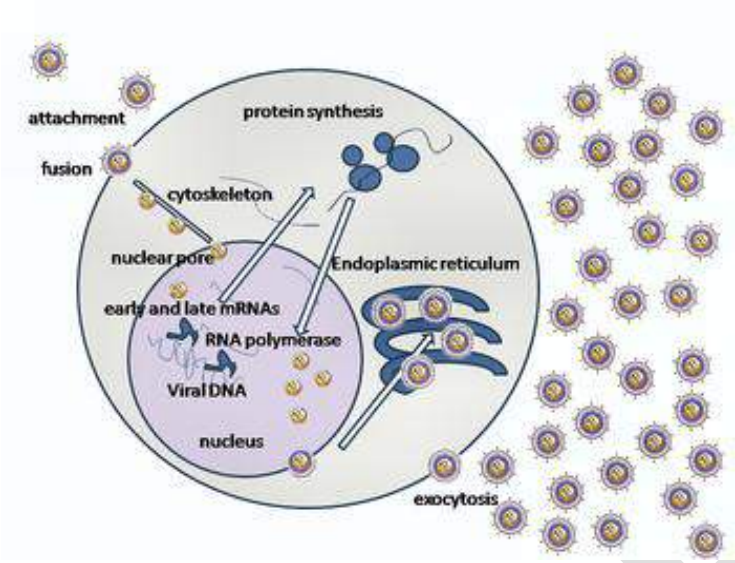
3D reconstruction and animation of a tail-like assembly in HSV-1 virion

3D reconstruction of Herpes simplex virus type 1 (HSV-1)

Animal herpes viruses all share some common properties. The structure of herpes viruses consists of a relatively large double-stranded, linear DNA genome encased within an icosahedral protein cage called the capsid, which is wrapped in a lipid bilayer called the envelope. The envelope is joined to the capsid by means of a tegument. This complete particle is known as the virion.[12] HSV-1 and HSV-2 each contain at least 74 genes (or open reading frames, ORFs) within their genomes,[13] although speculation over gene crowding allows as many as 84 unique protein coding genes by 94 putative ORFs.[14] These genes encode a variety of proteins involved in forming the capsid, tegument and envelope of the virus, as well as controlling the replication and infectivity of the virus. These genes and their functions are summarized in the table below.
The genomes of HSV-1 and HSV-2 are complex and contain two unique regions called the long unique region (U_L) and the short unique region (U_S). Of the 74 known ORFs, U_L contains 56 viral genes, whereas U_S contains only 12.[13] Transcription of HSV genes is catalyzed by RNA polymerase II of the infected host.[13] Immediate early genes, which encode proteins that regulate the expression of *early* and *late* viral genes, are the first to be expressed following infection. Early gene expression follows, to allow the synthesis

of enzymes involved in DNA replication and the production of certain envelope glycoproteins. Expression of late genes occurs last; this group of genes predominantly encode proteins that form the virion particle.[13] Five proteins from (U_L) form the viral capsid; UL6, UL18, UL35, UL38 and the major capsid protein UL19.[12]

Cellular entry



A simplified diagram of HSV replication

Entry of HSV into the host cell involves interactions of several glycoproteins on the surface of the enveloped virus, with receptors on the surface of the host cell. The envelope covering the virus particle, when bound to specific receptors on the cell surface, will fuse with the host cell membrane and create an opening, or *pore*, through which the virus enters the host cell.

The sequential stages of HSV entry are analogous to those of other viruses. At first, complementary receptors on the virus and the cell surface bring the viral and cell membranes into proximity. In an intermediate state, the two membranes begin to merge, forming a *hemifusion state.* Finally, a stable *entry pore* is formed through which the viral envelope contents are introduced to the host cell.[15] The virus can also be endocytosed after binding to the receptors, and the fusion could occur at the endosome.

In the case of a herpes virus, initial interactions occur when two viral envelope glycoprotein called glycoprotein C (gC) and glycoprotein B (gB) bind to a cell surface particle called heparan sulfate. Next, the major receptor binding protein, glycoprotein D (gD), binds specifically to at least one of three known entry receptors.[16] These cell receptors include herpesvirus entry mediator (HVEM), nectin-1 and 3-O sulfated heparan sulfate. The receptor provides a strong, fixed attachment to the host cell. These interactions bring the membrane surfaces into mutual proximity and allow for other glycoproteins embedded in the viral envelope to interact with other cell surface molecules. Once bound to the HVEM, gD changes its conformation and interacts with viral glycoproteins H (gH) and L (gL), which form a complex. The interaction of these membrane proteins results in the hemifusion state. Afterward, gB interaction with the gH/gL complex creates an entry pore for the viral capsid.[15] gB interacts with glycosaminoglycans on the surface of the host cell.[*citation needed*]

Genetic inoculation

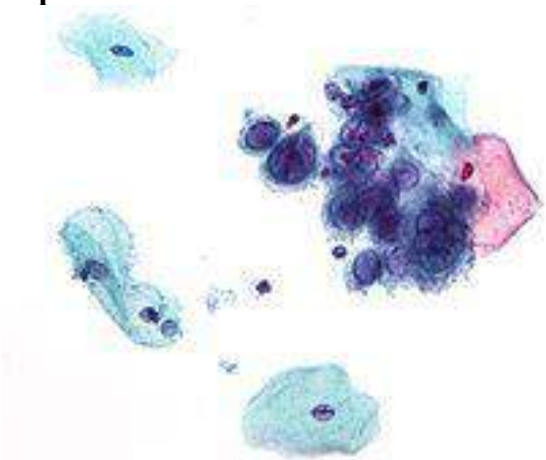
After the viral capsid enters the cellular cytoplasm, it is transported to the cell nucleus. Once attached to the nucleus at a *nuclear entry pore*, the capsid ejects its DNA contents via the capsid *portal*. The capsid portal is formed by twelve copies of portal protein, UL6, arranged as a ring; the proteins contain a leucine zipper

sequence of amino acids which allow them to adhere to each other.[17] Each icosahedral capsid contains a single portal, located in one vertex.[18][19] The DNA exits the capsid in a single linear segment.[20]

Immune evasion

HSV evades the immune system through interference with MHC class I antigen presentation on the cell surface, by blocking **TAP** or the transporter associated with antigen processing induced by the secretion of ICP-47 by HSV.[21] In the host cell, **TAP** transports digested viral antigen epitope peptides from the cytosol to the endoplasmic reticulum, allowing these epitopes to be combined with MHC class I molecules and presented on the surface of the cell. Viral epitope presentation with MHC class I is a requirement for activation of cytotoxic T-lymphocytes (CTLs), the major effectors of the cell-mediated immune response against virally-infected cells. ICP-47 prevents initiation of a CTL-response against HSV, allowing the virus to survive for a protracted period in the host.

Replication



Micrograph showing the viral cytopathic effect of HSV (multi-nucleation, ground glass chromatin).

Following infection of a cell, a cascade of herpes virus proteins, called *immediate-early*, *early*, and *late*, are produced. Research using flow cytometry on another member of the herpes virus family, Kaposi's sarcoma-associated herpesvirus, indicates the possibility of an additional lytic stage, *delayed-late*.[22] These stages of lytic infection, particularly *late lytic*, are distinct from the latency stage. In the case of HSV-1, no protein products are detected during latency, whereas they are detected during the lytic cycle.

The early proteins transcribed are used in the regulation of genetic replication of the virus. On entering the cell, an α-TIF protein joins the viral particle and aids in immediate-early transcription. The virion host shutoff protein (VHS or UL41) is very important to viral replication.[23] This enzyme shuts off protein synthesis in the host, degrades host mRNA, helps in viral replication, and regulates gene expression of viral proteins. The viral genome immediately travels to the nucleus but the VHS protein remains in the cytoplasm.[24][25]

The late proteins form the capsid and the receptors on the surface of the virus. Packaging of the viral particles — including the genome, core and the capsid - occurs in the nucleus of the cell. Here, concatemers of the viral genome are separated by cleavage and are placed into pre-formed capsids. HSV-1 undergoes a process of primary and secondary envelopment. The primary envelope is acquired by budding into the inner nuclear membrane of the cell. This then fuses with the outer nuclear membrane releasing a naked capsid into the cytoplasm. The virus acquires its final envelope by budding into cytoplasmic vesicles.[26]

Latent infection

HSVs may persist in a quiescent but persistent form known as *latent infection*, notably in neural ganglia.[1] HSV-1 tends to reside in the trigeminal ganglia, while HSV-2 tends to reside in the sacral ganglia, but these

are tendencies only, not fixed behavior. During latent infection of a cell, HSVs express latency associated transcript (LAT) RNA. LAT regulates the host cell genome and interferes with natural cell death mechanisms. By maintaining the host cells, LAT expression preserves a reservoir of the virus, which allows subsequent, usually symptomatic, periodic recurrences or "outbreaks" characteristic of non-latency. Whether or not recurrences are symptomatic, viral shedding occurs to infect a new host. A protein found in neurons may bind to herpes virus DNA and regulate latency. Herpes virus DNA contains a gene for a protein called ICP4, which is an important transactivator of genes associated with lytic infection in HSV-1.[27] Elements surrounding the gene for ICP4 bind a protein known as the human neuronal protein Neuronal Restrictive Silencing Factor (NRSF) or human Repressor Element Silencing Transcription Factor (REST). When bound to the viral DNA elements, histone deacetylation occurs atop the ICP4 gene sequence to prevent initiation of transcription from this gene, thereby preventing transcription of other viral genes involved in the lytic cycle.[27][28] Another HSV protein reverses the inhibition of ICP4 protein synthesis. ICP0 dissociates NRSF from the *ICP4* gene and thus prevents silencing of the viral DNA.[29]

The virus can be reactivated by illnesses such as colds and influenza, eczema, emotional and physical stress, gastric upset, fatigue or injury, by menstruation and possibly exposure to bright sunlight. Genital Herpes may be reactivated by friction.[*citation needed*]

Evolution

The herpes simplex 1 genomes can be classified into six clades.[34] Four of these occur in East Africa, one in East Asia and one in Europe and North America. This suggests that the virus may have originated in East Africa. The most recent common ancestor of the Eurasian strains appears to have evolved ~60,000 years ago.[35] The East Asian HSV-1 isolates have an unusual pattern that is currently best explained by the two waves of migration responsible for the peopling of Japan.[*citation needed*]

The mutation rate has been estimated to be ~1.38×10^{-7} substitutions/site/year.[34] In clinical setting, the mutations in either the thymidine kinase gene or DNA polymerase gene has caused resistance to aciclovir. However, most of the mutations occur in the thymidine kinase gene rather than the DNA polymerase gene.[36]

Treatment

Herpes viruses establish lifelong infections, and the virus cannot yet be eradicated from the body. Treatment usually involves general-purpose antiviral drugs that interfere with viral replication, reduce the physical severity of outbreak-associated lesions, and lower the chance of transmission to others. Studies of vulnerable patient populations have indicated that daily use of antivirals such as aciclovir[37] and valaciclovir can reduce reactivation rates.[8]

Alzheimer's disease

In the presence of a certain gene variation (APOE-epsilon4 allele carriers), a possible link between HSV-1 (i.e., the virus that causes cold sores or oral herpes) and Alzheimer's disease was reported in 1979.[38] HSV-1 appears to be particularly damaging to the nervous system and increases one's risk of developing Alzheimer's disease. The virus interacts with the components and receptors of lipoproteins, which may lead to the development of Alzheimer's disease.[39] This research identifies HSVs as the pathogen most clearly linked to the establishment of Alzheimer's.[40] According to a study done in 1997, without the presence of the gene allele, HSV-1 does not appear to cause any neurological damage or increase the risk of Alzheimer's.[41] However, a more recent prospective study published in 2008 with a cohort of 591 people showed a statistically significant difference between patients with antibodies indicating recent reactivation of HSV and those without these antibodies in the incidence of Alzheimer's disease, without direct correlation to the APOE-epsilon4 allele.[42] It should be noted that the trial had a small sample of patients who did not have the antibody at baseline, so the results should be viewed as highly uncertain. In 2011 Manchester University

scientists showed that treating HSV1-infected cells with antiviral agents decreased the accumulation of β-amyloid and P-tau, and also decreased HSV-1 replication.[43]

Multiplicity reactivation

Multiplicity reactivation (MR) is the process by which viral genomes containing inactivating damage interact within an infected cell to form a viable viral genome. MR was originally discovered with the bacterial virus bacteriophage T4, but was subsequently also found with pathogenic viruses including influenza virus, HIV-1, adenovirus simian virus 40, vaccinia virus, reovirus, poliovirus and herpes simplex virus.[44]

When HSV particles are exposed to doses of a DNA damaging agent that would be lethal in single infections, but are then allowed to undergo multiple infection (i.e. two or more viruses per host cell), MR is observed. Enhanced survival of HSV-1 due to MR occurs upon exposure to different DNA damaging agents, including methyl methanesulfonate,[45] trimethylpsoralen (which causes inter-strand DNA cross-links),[46][47] and UV light.[48] After treatment of genetically marked HSV with trimethylpsoralen, recombination between the marked viruses increases, suggesting that trimethylpsoralen damages stimulate recombination.[46] MR of HSV appears to partially depend on the host cell recombinational repair machinery since skin fibroblast cells defective in a component of this machinery (i.e. cells from Bloom's syndrome patients) are deficient in MR.[48] These observations suggest that MR in HSV infections involves genetic recombination between damaged viral genomes resulting in production of viable progeny viruses. HSV-1, upon infecting host cells, induces inflammation and oxidative stress.[49] Thus it appears that the HSV genome may be subjected to oxidative DNA damage during infection, and that MR may enhance viral survival and virulence under these conditions.

Use as an anti-cancer agent

Main article: Oncolytic herpes virus

Herpes simplex virus is considered as a potential therapy for cancer and has been extensively clinically tested to assess its oncolytic (cancer killing) ability.[50] Interim overall survival data from Amgen's phase 3 trial of a genetically-attenuated herpes virus suggests efficacy against melanoma.[51]

Use in neuronal connection tracing

Herpes simplex virus is also used as a transneuronal tracer defining connections among neurons by virtue of traversing synapses.[52]

Other related outcomes

Herpes simplex virus is likely the most common cause of Mollaret's meningitis,[53] and, in worse case scenarios, can lead to a potentially fatal case of herpes simplex encephalitis.[54]

Research

There exist commonly used vaccines to some herpesviruses, but only veterinary, such as HVT/LT (Turkey herpesvirus vector laryngotracheitis vaccine), interestingly however, it prevents atherosclerosis (which histologically mirrors atherosclerosis in humans) in target animals vaccinated.[55][56]

What Is Herpes Simplex?

The herpes simplex virus, also known as HSV, is an infection that causes herpes. Herpes can appear in various parts of the body, most commonly on the genitals or mouth. There are two types of the herpes simplex virus. HSV-1, also known as oral herpes, can cause cold sores and fever blisters around the mouth and on the face. HSV-2 is generally responsible for genital herpes outbreaks.

What Causes Herpes Simplex?

The herpes simplex virus is a contagious virus that can be passed from person to person through direct contact. Children will often contract HSV-1 from early contact with an infected adult. They then carry the virus with them for the rest of their life.

Prepared by – Dr. A. A. Arunkumar, Assistant Professor, Department of Microbiology, KAHE.

Infection with HSV-1 can happen from general interactions such as eating from the same utensils, sharing lip balm, or kissing. The virus spreads more quickly when an infected person is experiencing an outbreak. Additionally, it is possible to get genital herpes from HSV-1 if the individual has had cold sores and performed sexual activities during that time.

HSV-2 is contracted through forms of sexual contact with a person who has HSV-2. It is estimated that around 20 percent of sexually active adults within the United States have been infected with HSV-2, according to the American Academy of Dermatology (AAD). (AAD) While HSV-2 infections are spread by coming into contact with a herpes sore, the AAD reports that most people get HSV-1 from an infected person who is asymptomatic, or does not have sores.

Who Is At Risk of Developing Herpes Simplex Infections?

Anyone can be infected with HSV, regardless of age. Your risk is determined almost entirely based on exposure to the infection.

In cases of sexually transmitted HSV, people are more at risk when they participate in risky sexual behavior without the use of protection, such as condoms. Other risk factors for HSV-2 include:

- having multiple sex partners
- being female
- having another sexually transmitted infection (STI)
- having a weakened immune system

If a mother is having an outbreak of genital herpes at the time of childbirth, it can expose the baby to both types of HSV, and may put them at risk for serious complications.

Recognizing the Signs of Herpes Simplex

It is important to understand that although someone may not have visible sores or symptoms, they may still be infected by the virus and may transmit the virus to others. Some of the symptoms associated with this virus include:

- blistering sores (in the mouth or on the genitals)
- pain during urination (genital herpes)
- itching

Additionally, you may experience many symptoms that are similar to the flu. These symptoms can include fever, swollen lymph nodes, headaches, tiredness, and lack of appetite. HSV can also spread to the eyes, causing a condition called herpes keratitis. This can cause symptoms such as eye pain, discharge, and a gritty feeling in the eye.

How Is Herpes Simplex Diagnosed?

This type of virus is generally diagnosed with a physical exam. Your doctor may check your body for sores and ask you about some of your current symptoms. Your doctor may also request HSV testing, also known as a herpes culture, to confirm the diagnosis if you have sores on your genitals. During this test, your doctor will take a swab sample of fluid from the sore and then send it to a laboratory for testing.

Blood tests looking for antibodies to HSV-1 and HSV-2 may also be used to diagnose these infections. This is especially helpful when there are no sores present.

How Is Herpes Simplex Treated?

There is currently no cure for this virus. Treatment focuses on getting rid of sores and limiting outbreaks. It is possible that your sores will disappear without treatment. However, your doctor may determine that you need one or more of the following medications:

- acyclovir
- famciclovir
- valacyclovir

These medications can help infected individuals reduce the risk of spreading the virus to other people. The medications also help to lower the intensity and frequency of outbreaks. These medications may come in oral (pill) form, or may be applied as a cream. For severe outbreaks, these medications may also be administered by injection.

What Is the Long-Term Outlook for Herpes Simplex?

People who become infected with HSV will have the virus for the rest of their lives. Even if it does not manifest symptoms, the virus will continue to live in an infected person's nerve cells. Some people may experience regular outbreaks. Others will only experience one outbreak after they have been infected, after which the virus may become dormant. Even if a virus is dormant, an outbreak can be triggered by certain stimuli, such as:

- stress
- menstrual periods
- fever or illness
- sun exposure or sunburn

It is believed that the outbreaks may become less intense over time because the body starts creating antibodies. If a generally healthy individual has been infected with the virus, there are usually no complications.

Preventing the Spread of Herpes Simplex Infections

Although there is no cure for herpes, you can take precautionary measures to avoid becoming infected, or to prevent spreading HSV to another person.

If you are experiencing an outbreak of HSV-1, try to avoid direct physical contact with other people. Do not share any items that can pass the virus around, such as cups, towels, silverware, clothing, makeup, or lip balm. Doctors also recommend that infected individuals should not participate in oral sex, kissing, or any other type of sexual activity, during an outbreak. Additionally, if your hands have come into contact with your sores, you should wash them thoroughly and apply medication with cotton swabs to reduce contact.

Individuals with HSV-2 should avoid any type of sexual activity with other people during an outbreak. If the individual is not experiencing symptoms but has previously been diagnosed with the virus, a condom should be used during intercourse. Although a condom may be used, it may still be possible to pass herpes to your partner from uncovered skin. Women who are pregnant and infected may have to take medicine to prevent the virus from infecting their unborn babies.

Herpes simplex viruses -- more commonly known as herpes -- are categorized into two types: herpes type 1 (HSV-1, or oral herpes) and herpes type 2 (HSV-2, or genital herpes). Most commonly, herpes type 1 causes sores around the mouth and lips (sometimes called fever blisters or cold sores). HSV-1 can cause genital herpes, but most cases of genital herpes are caused by herpes type 2. In HSV-2, the infected person may have sores around the genitals or rectum. Although HSV-2 sores may occur in other locations, these sores usually are found below the waist.

What Causes Herpes Infections and Outbreaks?

Herpes simplex type 1, which is transmitted through oral secretions or sores on the skin, can be spread through kissing or sharing objects such as toothbrushes or eating utensils. In general, a person can only get herpes type 2 infection during sexual contact with someone who has a genital HSV-2 infection. It is important to know that both HSV-1 and HSV-2 can be spread even if sores are not present.

Pregnant women with genital herpes should talk to their doctor, as genital herpes can be passed on to the baby during childbirth.

Treating & Preventing Cold Sores

For many people with the herpes virus, which can go through periods of being dormant, attacks (or outbreaks) can be brought on by the following conditions:

- General illness (from mild illnesses to serious conditions)
- Fatigue
- Physical or emotional stress
- Immunosuppression due to AIDS or such medications as chemotherapy or steroids
- Trauma to the affected area, including sexual activity
- Menstruation

What Are the Symptoms of Herpes Simplex?

Symptoms of herpes simplex virus typically appear as a blister or as multiple blisters on or around affected areas -- usually the mouth, genitals, or rectum. The blisters break, leaving tender sores.

How Is Herpes Simplex Diagnosed?

Often, the appearance of herpes simplex virus is typical and no testing is needed to confirm the diagnosis. If a health care provider is uncertain, herpes simplex can be diagnosed with lab tests, including DNA -- or PCR -- tests and virus cultures.

How Is Herpes Simplex Treated?

Although there is no cure for herpes, treatments can relieve the symptoms. Medication can decrease the pain related to an outbreak and can shorten healing time. They can also decrease the total number of outbreaks. Drugs including Famvir, Zovirax, and Valtrex are among the drugs used to treat the symptoms of herpes. Warm baths may relieve the pain associated with genital sores.

How Painful Is Herpes Simplex?

Some people experience very mild genital herpes symptoms or no symptoms at all. Frequently, people infected with the virus don't even know they have it. However, when it causes symptoms, it can be described as extremely painful. This is especially true for the first outbreak, which is often the worst. Outbreaks are described as aches or pains in or around the genital area or burning, pain, or difficulty urinating. Some people experience discharge from the vagina or penis.

Oral herpes lesions (cold sores) usually cause tingling and burning just prior to the breakout of the blisters. The blisters themselves can also be painful.

Can Herpes Be Cured?

There is no cure for herpes simplex. Once a person has the virus, it remains in the body. The virus lies inactive in the nerve cells until something triggers it to become active again.

Influenza

Influenza, commonly known as "the **flu**", is an infectious disease caused by an influenza virus.[1] Symptoms can be mild to severe.[2] The most common symptoms include: a high fever, runny nose, sore throat, muscle pains, headache, coughing, and feeling tired. These symptoms typically begin two days after exposure to the virus and most last less than a week. The cough, however, may last for more than two weeks.[1] In children, there may be nausea and vomiting, but these are not common in adults. Nausea and vomiting occur more commonly in the unrelated infection gastroenteritis, which is sometimes inaccurately referred to as "stomach flu" or "24-hour flu".[3] Complications of influenza may include viral pneumonia, secondary bacterial pneumonia, sinus infections, and worsening of previous health problems such as asthma or heart failure.[2][4] Three types of influenza viruses affect people, called Type A, Type B, and Type C.[4] Usually, the virus is spread through the air from coughs or sneezes.[1] This is believed to occur mostly over relatively short distances.[5] It can also be spread by touching surfaces contaminated by the virus and then touching the mouth or eyes.[2][5] A person may be infectious to others both before and during the time they are showing symptoms.[2] The infection may be confirmed by testing the throat, sputum, or nose for the virus. A number

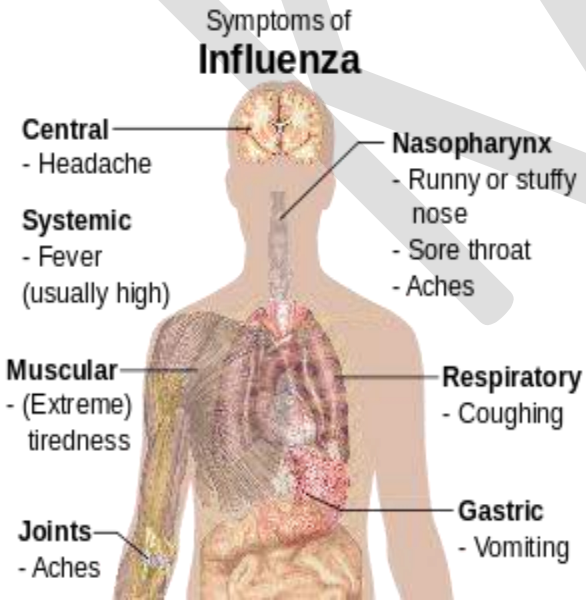
of rapid tests are available; however, people may still have the infection if the results are negative. A type of polymerase chain reaction that detects the virus's RNA is more accurate.[4]
Frequent hand washing reduces the risk of infection because the virus is inactivated by soap.[6] Wearing a surgical mask is also useful.[6] Yearly vaccinations against influenza are recommended by the World Health Organization for those at high risk. The vaccine is usually effective against three or four types of influenza.[1] It is usually well tolerated. A vaccine made for one year may not be useful in the following year, since the virus evolves rapidly. Antiviral drugs such as the neuraminidase inhibitor oseltamivir, among others, have been used to treat influenza.[1] Their benefits in those who are otherwise healthy do not appear to be greater than their risks.[7] No benefit has been found in those with other health problems.[7][8]
Influenza spreads around the world in a yearly outbreak, resulting in about three to five million cases of severe illness and about 250,000 to 500,000 deaths.[1] In the Northern and Southern parts of the world outbreaks occur mainly in winter while in areas around the equator outbreaks may occur at any time of the year.[1] Death occurs mostly in the young, the old and those with other health problems.[1] Larger outbreaks known as pandemics are less frequent.[4] In the 20th century three influenza pandemics occurred: Spanish influenza in 1918, Asian influenza in 1958, and Hong Kong influenza in 1968, each resulting in more than a million deaths.[9] The World Health Organization declared an outbreak of a new type of influenza A/H1N1 to be a pandemic in June 2009.[10] Influenza may also affect other animals, including pigs, horses and birds.[11]

Signs and symptoms

Most sensitive symptoms for diagnosing influenza[12]

Symptom:	sensitivity	specificity
Fever	68–86%	25–73%
Cough	84–98%	7–29%
Nasal congestion	68–91%	19–41%

- All three findings, especially fever, were less sensitive in people over 60 years of age.



Prepared by – Dr. A. A. Arunkumar, Assistant Professor, Department of Microbiology, KAHE.

Symptoms of influenza,[13] with fever and cough the most common symptoms.[12]
Approximately 33% of people with influenza are asymptomatic.[14]
Symptoms of influenza can start quite suddenly one to two days after infection. Usually the first symptoms are chills or a chilly sensation, but fever is also common early in the infection, with body temperatures ranging from 38 to 39 °C (approximately 100 to 103 °F).[15] Many people are so ill that they are confined to bed for several days, with aches and pains throughout their bodies, which are worse in their backs and legs.[16] Symptoms of influenza may include:

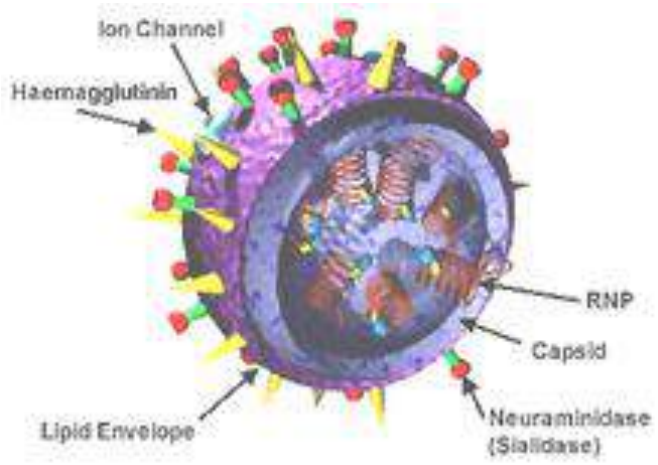
- Fever and extreme coldness (chills shivering, shaking (rigor))
- Cough
- Nasal congestion
- Runny nose
- Sneezing
- Body aches, especially joints and throat
- Fatigue
- Headache
- Irritated, watering eyes
- Reddened eyes, skin (especially face), mouth, throat and nose
- Petechial rash[17]
- In children, gastrointestinal symptoms such as diarrhea and abdominal pain,[18][19] (may be severe in children with influenza B)[20]

It can be difficult to distinguish between the common cold and influenza in the early stages of these infections,[21] but a flu can be identified by a high fever with a sudden onset and extreme fatigue. Influenza is a mixture of symptoms of common cold and pneumonia, body ache, headache, and fatigue. Diarrhea is not normally a symptom of influenza in adults,[12] although it has been seen in some human cases of the H5N1 "bird flu"[22] and can be a symptom in children.[18] The symptoms most reliably seen in influenza are shown in the table to the right.[12]
Since antiviral drugs are effective in treating influenza if given early (see treatment section, below), it can be important to identify cases early. Of the symptoms listed above, the combinations of fever with cough, sore throat and/or nasal congestion can improve diagnostic accuracy.[23] Two decision analysis studies[24][25] suggest that *during local outbreaks* of influenza, the prevalence will be over 70%,[25] and thus patients with any of these combinations of symptoms may be treated with neuraminidase inhibitors without testing. Even in the absence of a local outbreak, treatment may be justified in the elderly during the influenza season as long as the prevalence is over 15%.[25]
The available laboratory tests for influenza continue to improve. The United States Centers for Disease Control and Prevention (CDC) maintains an up-to-date summary of available laboratory tests.[26] According to the CDC, rapid diagnostic tests have a sensitivity of 50–75% and specificity of 90–95% when compared with viral culture.[27] These tests may be especially useful during the influenza season (prevalence=25%) but in the absence of a local outbreak, or peri-influenza season (prevalence=10%[25]).
Occasionally, influenza can cause severe illness including primary viral pneumonia or secondary bacterial pneumonia.[28][29] The obvious symptom is trouble breathing. In addition, if a child (or presumably an adult) seems to be getting better and then relapses with a high fever, that is a danger sign since this relapse can be bacterial pneumonia.[30]

Virology

Types of virus

Prepared by – Dr. A. A. Arunkumar, Assistant Professor, Department of Microbiology, KAHE.



Structure of the influenza virion. The hemagglutinin (HA) and neuraminidase(NA) proteins are shown on the surface of the particle. The viral RNAs that make up the genome are shown as red coils inside the particle and bound to Ribonuclear Proteins (RNPs).

In virus classification influenza viruses are RNA viruses that make up three of the five genera of the family Orthomyxoviridae:[31]

- Influenzavirus A
- Influenzavirus B
- Influenzavirus C

These viruses are only distantly related to the human parainfluenza viruses, which are RNA viruses belonging to the paramyxovirus family that are a common cause of respiratory infections in children such as croup,[32] but can also cause a disease similar to influenza in adults.[33]

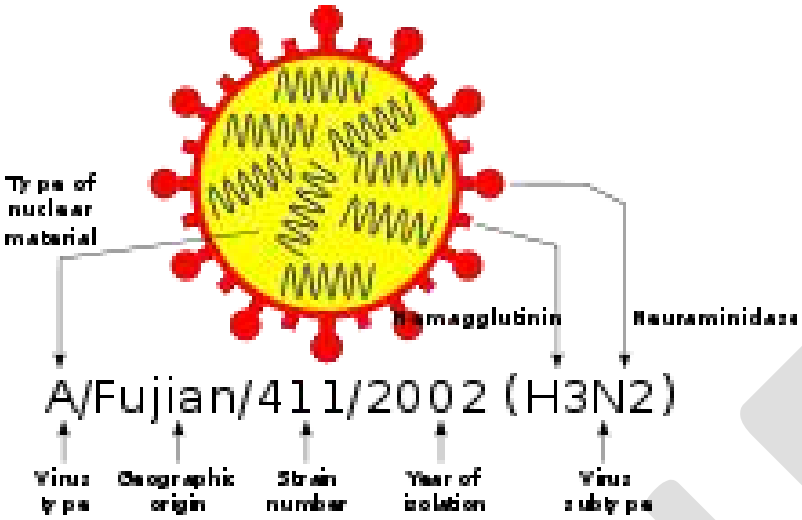
A fourth family of influenza viruses has been proposed - influenza D.[34][35][36][37][38][39][40] The type species for this family is Bovine Influenza D virus which was first isolated in 2012.

Influenzavirus A

This genus has one species, influenza A virus. Wild aquatic birds are the natural hosts for a large variety of influenza A. Occasionally, viruses are transmitted to other species and may then cause devastating outbreaks in domestic poultry or give rise to human influenza pandemics.[41] The type A viruses are the most virulent human pathogens among the three influenza types and cause the severest disease. The influenza A virus can be subdivided into different serotypes based on the antibody response to these viruses.[42] The serotypes that have been confirmed in humans, ordered by the number of known human pandemic deaths, are:

- H1N1, which caused Spanish Flu in 1918, and Swine Flu in 2009
- H2N2, which caused Asian Flu in 1957
- H3N2, which caused Hong Kong Flu in 1968
- H5N1, which caused Bird Flu in 2004
- H7N7, which has unusual zoonotic potential[43]
- H1N2, endemic in humans, pigs and birds
- H9N2
- H7N2
- H7N3
- H10N7
- H7N9

Influenzavirus B



Influenza virus nomenclature (for a Fujian flu virus)

This genus has one species, influenza B virus. Influenza B almost exclusively infects humans[42] and is less common than influenza A. The only other animals known to be susceptible to influenza B infection are the seal[44] and the ferret.[45] This type of influenza mutates at a rate 2–3 times slower than type A[46] and consequently is less genetically diverse, with only one influenza B serotype.[42] As a result of this lack of antigenic diversity, a degree of immunity to influenza B is usually acquired at an early age. However, influenza B mutates enough that lasting immunity is not possible.[47] This reduced rate of antigenic change, combined with its limited host range (inhibiting cross species antigenic shift), ensures that pandemics of influenza B do not occur.[48]

Influenzavirus C

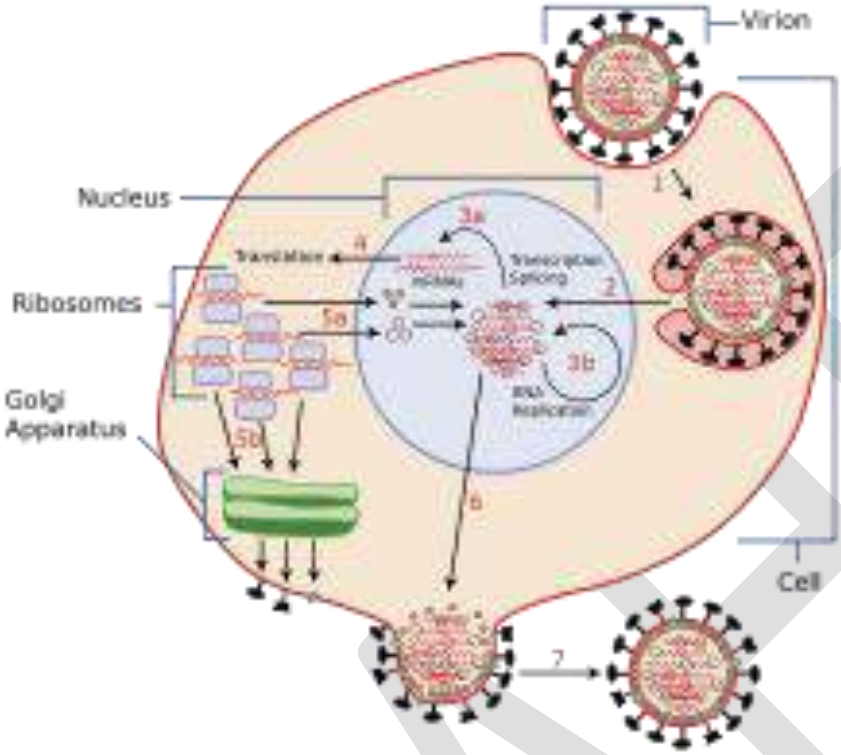
This genus has one species, influenza C virus, which infects humans, dogs and pigs, sometimes causing both severe illness and local epidemics.[49][50] However, influenza C is less common than the other types and usually only causes mild disease in children.[51][52]

Structure, properties, and subtype nomenclature

Influenzaviruses A, B and C are very similar in overall structure.[53] The virus particle is 80–120 nanometers in diameter and usually roughly spherical, although filamentous forms can occur.[54][55] These filamentous forms are more common in influenza C, which can form cordlike structures up to 500 micrometers long on the surfaces of infected cells.[56] However, despite these varied shapes, the viral particles of all influenza viruses are similar in composition.[56] These are made of a viral envelope containing two main types of glycoproteins, wrapped around a central core. The central core contains the viral RNA genome and other viral proteins that package and protect this RNA. RNA tends to be single stranded but in special cases it is double.[55] Unusually for a virus, its genome is not a single piece of nucleic acid; instead, it contains seven or eight pieces of segmented negative-sense RNA, each piece of RNA containing either one or two genes, which code for a gene product (protein).[56] For example, the influenza A genome contains 11 genes on eight pieces of RNA, encoding for 11 proteins: hemagglutinin (HA), neuraminidase (NA), nucleoprotein (NP), M1, M2, NS1, NS2 (NEP: nuclear export protein), PA, PB1 (polymerase basic 1), PB1-F2 and PB2.[57] Hemagglutinin (HA) and neuraminidase (NA) are the two large glycoproteins on the outside of the viral particles. HA is a lectin that mediates binding of the virus to target cells and entry of the viral genome into the target cell, while NA is involved in the release of progeny virus from infected cells, by cleaving sugars that bind the mature viral particles.[58] Thus, these proteins are targets for antiviral drugs.[59] Furthermore, they are antigens to which antibodies can be raised. Influenza A viruses are classified into subtypes based on antibody responses to HA and NA. These different types of HA and NA form the basis of the *H* and *N*

distinctions in, for example, *H5N1*.[60] There are 16 H and 9 N subtypes known, but only H 1, 2 and 3, and N 1 and 2 are commonly found in humans.[61]

Replication



Host cell invasion and replication by the influenza virus. The steps in this process are discussed in the text.

Viruses can replicate only in living cells.[62] Influenza infection and replication is a multi-step process: First, the virus has to bind to and enter the cell, then deliver its genome to a site where it can produce new copies of viral proteins and RNA, assemble these components into new viral particles, and, last, exit the host cell.[56]

Influenza viruses bind through hemagglutinin onto sialic acid sugars on the surfaces of epithelial cells, typically in the nose, throat, and lungs of mammals, and intestines of birds (Stage 1 in infection figure).[63] After the hemagglutinin is cleaved by a protease, the cell imports the virus by endocytosis.[64]

The intracellular details are still being elucidated. It is known that virions converge to the microtubule organizing center, interact with acidic endosomes and finally enter the target endosomes for genome release.[65]

Once inside the cell, the acidic conditions in the endosome cause two events to happen: First, part of the hemagglutinin protein fuses the viral envelope with the vacuole's membrane, then the M2 ion channel allows protons to move through the viral envelope and acidify the core of the virus, which causes the core to disassemble and release the viral RNA and core proteins.[56] The viral RNA (vRNA) molecules, accessory proteins and RNA-dependent RNA polymerase are then released into the cytoplasm (Stage 2).[66] The M2 ion channel is blocked by amantadine drugs, preventing infection.[67]

These core proteins and vRNA form a complex that is transported into the cell nucleus, where the RNA-dependent RNA polymerase begins transcribing complementary positive-sense vRNA (Steps 3a and b).[68] The vRNA either is exported into the cytoplasm and translated (step 4) or remains in the nucleus. Newly synthesized viral proteins are either secreted through the Golgi apparatus onto the cell surface (in the case of

neuraminidase and hemagglutinin, step 5b) or transported back into the nucleus to bind vRNA and form new viral genome particles (step 5a). Other viral proteins have multiple actions in the host cell, including degrading cellular mRNA and using the released nucleotides for vRNA synthesis and also inhibiting translation of host-cell mRNAs.[69]
Negative-sense vRNAs that form the genomes of future viruses, RNA-dependent RNA polymerase, and other viral proteins are assembled into a virion. Hemagglutinin and neuraminidase molecules cluster into a bulge in the cell membrane. The vRNA and viral core proteins leave the nucleus and enter this membrane protrusion (step 6). The mature virus buds off from the cell in a sphere of host phospholipid membrane, acquiring hemagglutinin and neuraminidase with this membrane coat (step 7).[70] As before, the viruses adhere to the cell through hemagglutinin; the mature viruses detach once their neuraminidase has cleaved sialic acid residues from the host cell.[63] After the release of new influenza viruses, the host cell dies.
Because of the absence of RNA proofreading enzymes, the RNA-dependent RNA polymerase that copies the viral genome makes an error roughly every 10 thousand nucleotides, which is the approximate length of the influenza vRNA. Hence, the majority of newly manufactured influenza viruses are mutants; this causes antigenic drift, which is a slow change in the antigens on the viral surface over time.[71] The separation of the genome into eight separate segments of vRNA allows mixing or reassortment of vRNAs if more than one type of influenza virus infects a single cell. The resulting rapid change in viral genetics produces antigenic shifts, which are sudden changes from one antigen to another. These sudden large changes allow the virus to infect new host species and quickly overcome protective immunity.[60] This is important in the emergence of pandemics, as discussed below in the section on Epidemiology.

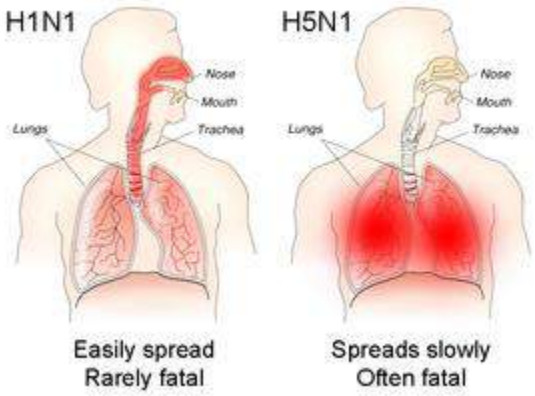
Mechanism

Transmission

When an infected person sneezes or coughs more than half a million virus particles can be spread to those close by.[72] In otherwise healthy adults, influenza virus shedding (the time during which a person might be infectious to another person) increases sharply one-half to one day after infection, peaks on day 2 and persists for an average total duration of 5 days—but can persist as long as 9 days.[73] In those who develop symptoms from experimental infection (only 67% of healthy experimentally infected individuals), symptoms and viral shedding show a similar pattern, but with viral shedding preceding illness by one day.[73] Children are much more infectious than adults and shed virus from just before they develop symptoms until two weeks after infection.[74] In immunocompromised people, viral shedding can continue for longer than two weeks.[75]
Influenza can be spread in three main ways:[76][77] by direct transmission (when an infected person sneezes mucus directly into the eyes, nose or mouth of another person); the airborne route (when someone inhales the aerosols produced by an infected person coughing, sneezing or spitting) and through hand-to-eye, hand-to-nose, or hand-to-mouth transmission, either from contaminated surfaces or from direct personal contact such as a hand-shake. The relative importance of these three modes of transmission is unclear, and they may all contribute to the spread of the virus.[5] In the airborne route, the droplets that are small enough for people to inhale are 0.5 to 5 μm in diameter and inhaling just one droplet might be enough to cause an infection.[76] Although a single sneeze releases up to 40,000 droplets,[78] most of these droplets are quite large and will quickly settle out of the air.[76] How long influenza survives in airborne droplets seems to be influenced by the levels of humidity and UV radiation, with low humidity and a lack of sunlight in winter aiding its survival.[76]
As the influenza virus can persist outside of the body, it can also be transmitted by contaminated surfaces such as banknotes,[79] doorknobs, light switches and other household items.[16] The length of time the virus will persist on a surface varies, with the virus surviving for one to two days on hard, non-porous surfaces such as plastic or metal, for about fifteen minutes from dry paper tissues, and only five minutes on skin.[80]

However, if the virus is present in mucus, this can protect it for longer periods (up to 17 days on banknotes).[76][79] Avian influenza viruses can survive indefinitely when frozen.[81] They are inactivated by heating to 56 °C (133 °F) for a minimum of 60 minutes, as well as by acids (at pH <2).[81]

Pathophysiology



The different sites of infection (shown in red) of seasonal H1N1 versus avian H5N1. This influences their lethality and ability to spread.

The mechanisms by which influenza infection causes symptoms in humans have been studied intensively. One of the mechanisms is believed to be the inhibition of adrenocorticotropic hormone (ACTH) resulting in lowered cortisol levels.[82] Knowing which genes are carried by a particular strain can help predict how well it will infect humans and how severe this infection will be (that is, predict the strain's pathophysiology).[50][83]

For instance, part of the process that allows influenza viruses to invade cells is the cleavage of the viral hemagglutinin protein by any one of several human proteases.[64] In mild and avirulent viruses, the structure of the hemagglutinin means that it can only be cleaved by proteases found in the throat and lungs, so these viruses cannot infect other tissues. However, in highly virulent strains, such as H5N1, the hemagglutinin can be cleaved by a wide variety of proteases, allowing the virus to spread throughout the body.[83]

The viral hemagglutinin protein is responsible for determining both which species a strain can infect and where in the human respiratory tract a strain of influenza will bind.[84] Strains that are easily transmitted between people have hemagglutinin proteins that bind to receptors in the upper part of the respiratory tract, such as in the nose, throat and mouth. In contrast, the highly lethal H5N1 strain binds to receptors that are mostly found deep in the lungs.[85] This difference in the site of infection may be part of the reason why the H5N1 strain causes severe viral pneumonia in the lungs, but is not easily transmitted by people coughing and sneezing.[86][87]

Common symptoms of the flu such as fever, headaches, and fatigue are the result of the huge amounts of proinflammatory cytokines and chemokines (such as interferon or tumor necrosis factor) produced from influenza-infected cells.[21][88] In contrast to the rhinovirus that causes the common cold, influenza does cause tissue damage, so symptoms are not entirely due to the inflammatory response.[89] This massive immune response might produce a life-threatening cytokine storm. This effect has been proposed to be the cause of the unusual lethality of both the H5N1 avian influenza,[90] and the 1918 pandemic strain.[91][92] However, another possibility is that these large amounts of cytokines are just a result of the massive levels of viral replication produced by these strains, and the immune response does not itself contribute to the disease.[93]

Prevention

Vaccination

The influenza vaccine is recommended by the World Health Organization and United States Centers for Disease Control and Prevention for high-risk groups, such as children, the elderly, health care workers, and people who have chronic illnesses such as asthma, diabetes, heart disease, or are immuno-compromised among others.[94][95] In healthy adults it is modestly effective in decreasing the amount of influenza-like symptoms in a population.[96] Evidence is supportive of a decreased rate of influenza in children over the age of two.[97] In those with chronic obstructive pulmonary disease vaccination reduces exacerbations,[98] it is not clear if it reduces asthma exacerbations.[99] Evidence supports a lower rate of influenza-like illness in many groups who are immunocompromised such as those with: HIV/AIDS, cancer, and post organ transplant.[100] In those at high risk immunization may reduce the risk of heart disease.[101] Whether immunizing health care workers affects patient outcomes is controversial with some reviews finding insufficient evidence[102][103] and others finding tentative evidence.[104][105]

Due to the high mutation rate of the virus, a particular influenza vaccine usually confers protection for no more than a few years. Every year, the World Health Organization predicts which strains of the virus are most likely to be circulating in the next year (see Historical annual reformulations of the influenza vaccine), allowing pharmaceutical companies to develop vaccines that will provide the best immunity against these strains.[106] The vaccine is reformulated each season for a few specific flu strains but does not include all the strains active in the world during that season. It takes about six months for the manufacturers to formulate and produce the millions of doses required to deal with the seasonal epidemics; occasionally, a new or overlooked strain becomes prominent during that time.[107] It is also possible to get infected just before vaccination and get sick with the strain that the vaccine is supposed to prevent, as the vaccine takes about two weeks to become effective.[108]

Vaccines can cause the immune system to react as if the body were actually being infected, and general infection symptoms (many cold and flu symptoms are just general infection symptoms) can appear, though these symptoms are usually not as severe or long-lasting as influenza. The most dangerous adverse effect is a severe allergic reaction to either the virus material itself or residues from the hen eggs used to grow the influenza; however, these reactions are extremely rare.[109]

The cost-effectiveness of seasonal influenza vaccination has been widely evaluated for different groups and in different settings.[110] It has generally been found to be a cost-effective intervention, especially in children[111] and the elderly,[112] however the results of economic evaluations of influenza vaccination have often been found to be dependent on key assumptions.[113][114]

Infection control

Further information: Influenza prevention

Reasonably effective ways to reduce the transmission of influenza include good personal health and hygiene habits such as: not touching your eyes, nose or mouth;[115] frequent hand washing (with soap and water, or with alcohol-based hand rubs);[116] covering coughs and sneezes; avoiding close contact with sick people; and staying home yourself if you are sick. Avoiding spitting is also recommended.[117] Although face masks might help prevent transmission when caring for the sick,[118][119] there is mixed evidence on beneficial effects in the community.[117][120] Smoking raises the risk of contracting influenza, as well as producing more severe disease symptoms.[121][122]

Since influenza spreads through both aerosols and contact with contaminated surfaces, surface sanitizing may help prevent some infections.[123] Alcohol is an effective sanitizer against influenza viruses, while quaternary ammonium compounds can be used with alcohol so that the sanitizing effect lasts for longer.[124] In hospitals, quaternary ammonium compounds and bleach are used to sanitize rooms or equipment that have been occupied by patients with influenza symptoms.[124] At home, this can be done effectively with a diluted chlorine bleach.[125]

During past pandemics, closing schools, churches and theaters slowed the spread of the virus but did not have a large effect on the overall death rate.[126][127] It is uncertain if reducing public gatherings, by for example closing schools and workplaces, will reduce transmission since people with influenza may just be moved from one area to another; such measures would also be difficult to enforce and might be unpopular.[117] When small numbers of people are infected, isolating the sick might reduce the risk of transmission.[117]

Treatment

People with the flu are advised to get plenty of rest, drink plenty of liquids, avoid using alcohol and tobacco and, if necessary, take medications such as acetaminophen (paracetamol) to relieve the fever and muscle aches associated with the flu.[128] Children and teenagers with flu symptoms (particularly fever) should avoid taking aspirin during an influenza infection (especially influenza type B), because doing so can lead to Reye's syndrome, a rare but potentially fatal disease of the liver.[129] Since influenza is caused by a virus, antibiotics have no effect on the infection; unless prescribed for secondary infections such as bacterial pneumonia. Antiviral medication may be effective, if given early, but some strains of influenza can show resistance to the standard antiviral drugs and there is concern about the quality of the research.[130]

Antivirals

The two classes of antiviral drugs used against influenza are neuraminidase inhibitors (oseltamivir and zanamivir) and M2 protein inhibitors (adamantane derivatives).

Neuraminidase inhibitors

Overall the benefits of neuraminidase inhibitors in those who are otherwise healthy do not appear to be greater than the risks.[7] There does not appear to be any benefit in those with other health problems.[7] In those believed to have the flu, they decreased the length of time symptoms were present by slightly less than a day but did not appear to affect the risk of complications such as needing hospitalization or pneumonia.[8] Previous to 2013 the benefits were unclear as the manufacturer (Roche) refused to release trial data for independent analysis.[131] Increasingly prevalent resistance to neuraminidase inhibitors has led to researchers to seek alternative antiviral drugs with different mechanisms of action.[132]

M2 inhibitors

The antiviral drugs amantadine and rimantadine inhibit a viral ion channel (M2 protein), thus inhibiting replication of the influenza A virus.[67] These drugs are sometimes effective against influenza A if given early in the infection but are ineffective against influenza B viruses, which lack the M2 drug target.[133] Measured resistance to amantadine and rimantadine in American isolates of H3N2 has increased to 91% in 2005.[134] This high level of resistance may be due to the easy availability of amantadines as part of over-the-counter cold remedies in countries such as China and Russia,[135] and their use to prevent outbreaks of influenza in farmed poultry.[136][137] The CDC recommended against using M2 inhibitors during the 2005–06 influenza season due to high levels of drug resistance.[138]

Prognosis

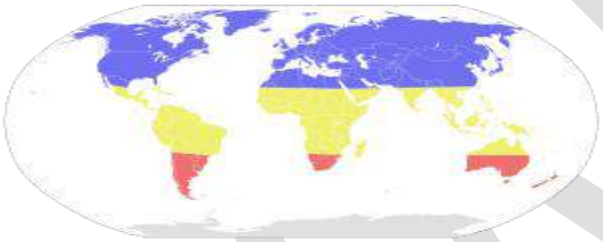
Influenza's effects are much more severe and last longer than those of the common cold. Most people will recover completely in about one to two weeks, but others will develop life-threatening complications (such as pneumonia). Thus, influenza can be deadly, especially for the weak, young and old, or chronically ill.[60] People with a weak immune system, such as people with advanced HIV infection or transplant patients (whose immune systems are medically suppressed to prevent transplant organ rejection), suffer from particularly severe disease.[139] Pregnant women and young children are also at a high risk for complications.[140]

The flu can worsen chronic health problems. People with emphysema, chronic bronchitis or asthma may experience shortness of breath while they have the flu, and influenza may cause worsening of coronary heart

disease or congestive heart failure.[141] Smoking is another risk factor associated with more serious disease and increased mortality from influenza.[142]
According to the World Health Organization: "Every winter, tens of millions of people get the flu. Most are only ill and out of work for a week, yet the elderly are at a higher risk of death from the illness. We know the worldwide death toll exceeds a few hundred thousand people a year, but even in developed countries the numbers are uncertain, because medical authorities don't usually verify who actually died of influenza and who died of a flu-like illness."[143] Even healthy people can be affected, and serious problems from influenza can happen at any age. People over 50 years old, very young children and people of any age with chronic medical conditions are more likely to get complications from influenza, such as pneumonia, bronchitis, sinus, and ear infections.[108]
In some cases, an autoimmune response to an influenza infection may contribute to the development of Guillain–Barré syndrome.[144] However, as many other infections can increase the risk of this disease, influenza may only be an important cause during epidemics.[144][145] This syndrome has been believed to also be a rare side effect of influenza vaccines. One review gives an incidence of about one case per million vaccinations.[146] Getting infected by influenza itself increases both the risk of death (up to 1 in 10,000) and increases the risk of developing GBS to a much higher level than the highest level of suspected vaccine involvement (approx. 10 times higher by recent estimates).[147][148]

Epidemiology

Seasonal variations



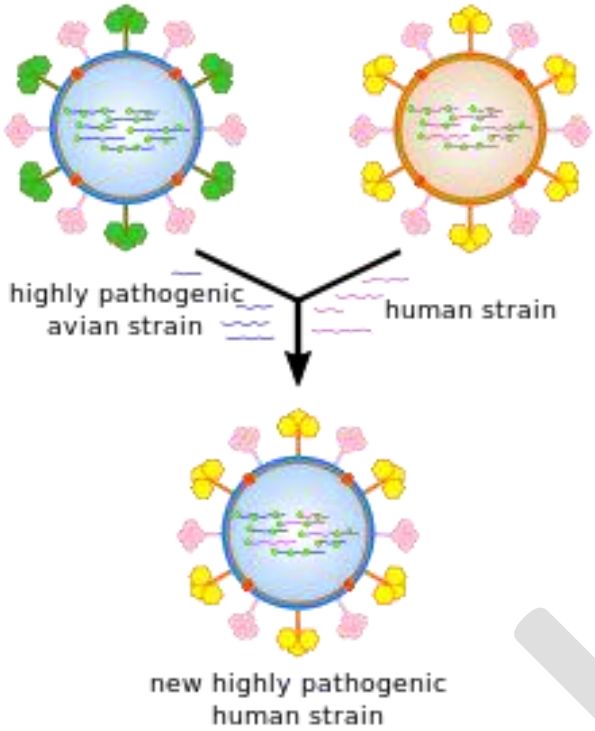
Seasonal risk areas for influenza: November–April (blue), April–November (red), and year-round (yellow).
Influenza reaches peak prevalence in winter, and because the Northern and Southern Hemispheres have winter at different times of the year, there are actually two different flu seasons each year. This is why the World Health Organization (assisted by the National Influenza Centers) makes recommendations for two different vaccine formulations every year; one for the Northern, and one for the Southern Hemisphere.[106]
A long-standing puzzle has been why outbreaks of the flu occur seasonally rather than uniformly throughout the year. One possible explanation is that, because people are indoors more often during the winter, they are in close contact more often, and this promotes transmission from person to person. Increased travel due to the Northern Hemisphere winter holiday season may also play a role.[149] Another factor is that cold temperatures lead to drier air, which may dehydrate mucus, preventing the body from effectively expelling virus particles. The virus also survives longer on surfaces at colder temperatures and aerosol transmission of the virus is highest in cold environments (less than 5 °C) with low relative humidity.[150] The lower air humidity in winter seems to be the main cause of seasonal influenza transmission in temperate regions.[151][152]
However, seasonal changes in infection rates also occur in tropical regions, and in some countries these peaks of infection are seen mainly during the rainy season.[153] Seasonal changes in contact rates from school terms, which are a major factor in other childhood diseases such as measles and pertussis, may also play a role in the flu. A combination of these small seasonal effects may be amplified by dynamical resonance with the endogenous disease cycles.[154] H5N1 exhibits seasonality in both humans and birds.[155]

An alternative hypothesis to explain seasonality in influenza infections is an effect of vitamin D levels on immunity to the virus.[156] This idea was first proposed by Robert Edgar Hope-Simpson in 1965.[157] He proposed that the cause of influenza epidemics during winter may be connected to seasonal fluctuations of vitamin D, which is produced in the skin under the influence of solar (or artificial) UV radiation. This could explain why influenza occurs mostly in winter and during the tropical rainy season, when people stay indoors, away from the sun, and their vitamin D levels fall.

Epidemic and pandemic spread

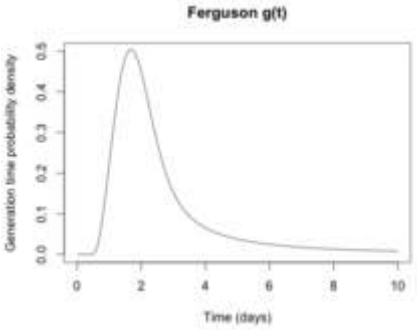
Antigenic drift creates influenza viruses with slightly modified antigens, while antigenic shift generates viruses with entirely novel antigens.

As influenza is caused by a variety of species and strains of viruses, in any given year some strains can die out while others create epidemics, while yet another strain can cause a pandemic. Typically, in a year's normal two flu seasons (one per hemisphere), there are between three and five million cases of severe illness and around 500,000 deaths worldwide,[158] which by some definitions is a yearly influenza epidemic.[159] Although the incidence of influenza can vary widely between years, approximately 36,000 deaths and more than 200,000 hospitalizations are directly associated with influenza every year in the United States.[160][161] One method of calculating influenza mortality produced an estimate of 41,400 average deaths per year in the United States between 1979 and 2001.[162] Different methods in 2010 by the Centers for Disease Control and Prevention (CDC) reported a range from a low of about 3,300 deaths to a high of 49,000 per year.[163] Roughly three times per century, a pandemic occurs, which infects a large proportion of the world's population and can kill tens of millions of people (see pandemics section). One study estimated that if a strain with similar virulence to the 1918 influenza emerged today, it could kill between 50 and 80 million people.[164]



Antigenic shift, or reassortment, can result in novel and highly pathogenic strains of human influenza

New influenza viruses are constantly evolving by mutation or by reassortment.[42] Mutations can cause small changes in the hemagglutinin and neuraminidase antigens on the surface of the virus. This is called antigenic drift, which slowly creates an increasing variety of strains until one evolves that can infect people who are immune to the pre-existing strains. This new variant then replaces the older strains as it rapidly sweeps through the human population, often causing an epidemic.[165] However, since the strains produced by drift will still be reasonably similar to the older strains, some people will still be immune to them. In contrast, when influenza viruses reassort, they acquire completely new antigens—for example by reassortment between avian strains and human strains; this is called antigenic shift. If a human influenza virus is produced that has entirely new antigens, everybody will be susceptible, and the novel influenza will spread uncontrollably, causing a pandemic.[166] In contrast to this model of pandemics based on antigenic drift and shift, an alternative approach has been proposed where the periodic pandemics are produced by interactions of a fixed set of viral strains with a human population with a constantly changing set of immunities to different viral strains.[167]



The generation time for influenza (the time from one infection to the next) is very short (only 2 days). This explains why influenza epidemics start and finish in a short time scale of only a few months.[168]

Prepared by – Dr. A. A. Arunkumar, Assistant Professor, Department of Microbiology, KAHE.

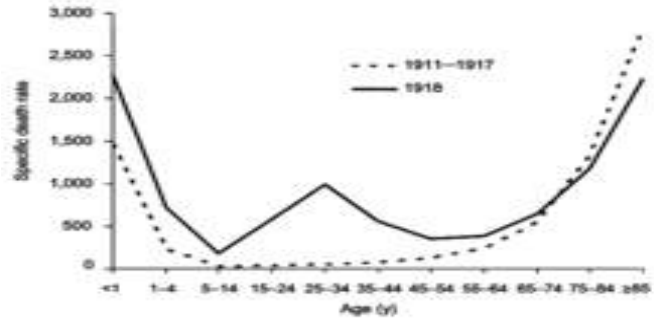
From a public health point of view, flu epidemics spread rapidly and are very difficult to control. Most influenza virus strains are not very infectious and each infected individual will only go on to infect one or two other individuals (the basic reproduction number for influenza is generally around 1.4). However, the generation time for influenza is extremely short: the time from a person becoming infected to when he infects the next person is only two days. The short generation time means that influenza epidemics generally peak at around 2 months and burn out after 3 months: the decision to intervene in an influenza epidemic therefore has to be taken early, and the decision is therefore often made on the back of incomplete data. Another problem is that individuals become infectious before they become symptomatic, which means that putting people in quarantine after they become ill is not an effective public health intervention.[168] For the average person, viral shedding tends to peak on day two whereas symptoms peak on day three.[14]

History

Etymology

The word *Influenza* comes from the Italian language meaning "influence" and refers to the cause of the disease; initially, this ascribed illness to unfavorable astrological influences.[169] Changes in medical thought led to its modification to *influenza del freddo*, meaning "influence of the cold". The word *influenza* was first used in English to refer to the disease we know today in 1703 by J. Hugger of the University of Edinburgh in his thesis *De Catarrho epidemio, vel Influenza, prout in India occidentali sese ostendit*.[170] Archaic terms for influenza include *epidemic catarrh*, *grippe* (from the French, first used by Molyneaux in 1694 [171]), *sweating sickness*, and *Spanish fever* (particularly for the 1918 flu pandemic strain).[172]

Pandemics



The difference between the influenza mortality age distributions of the 1918 epidemic and normal epidemics. Deaths per 100,000 persons in each age group, United States, for the interpandemic years 1911–1917 (dashed line) and the pandemic year 1918 (solid line).[173]

Thermal imaging camera and screen, photographed in an airport terminal in Greece during the 2009 flu pandemic. Thermal imaging can detect elevated body temperature, one of the signs of swine flu.

The symptoms of human influenza were clearly described by Hippocrates roughly 2,400 years ago.[174][175] Although the virus seems to have caused epidemics throughout human history, historical data on influenza are difficult to interpret, because the symptoms can be similar to those of other respiratory diseases.[176][177]

The disease may have spread from Europe to the Americas as early as the European colonization of the Americas; since almost the entire indigenous population of the Antilles was killed by an epidemic resembling influenza that broke out in 1493, after the arrival of Christopher Columbus.[178][179]
The first convincing record of an influenza pandemic was of an outbreak in 1580, which began in Russia and spread to Europe via Africa. In Rome, over 8,000 people were killed, and several Spanish cities were almost wiped out. Pandemics continued sporadically throughout the 17th and 18th centuries, with the pandemic of 1830–1833 being particularly widespread; it infected approximately a quarter of the people exposed.[177]
The most famous and lethal outbreak was the 1918 flu pandemic (Spanish flu pandemic) (type A influenza, H1N1 subtype), which lasted from 1918 to 1919. It is not known exactly how many it killed, but estimates range from 50 to 100 million people.[173][180][181] This pandemic has been described as "the greatest medical holocaust in history" and may have killed as many people as the Black Death.[177] This huge death toll was caused by an extremely high infection rate of up to 50% and the extreme severity of the symptoms, suspected to be caused by cytokine storms.[181] Symptoms in 1918 were so unusual that initially influenza was misdiagnosed as dengue, cholera, or typhoid. One observer wrote, "One of the most striking of the complications was hemorrhage from mucous membranes, especially from the nose, stomach, and intestine. Bleeding from the ears and petechial hemorrhages in the skin also occurred."[180] The majority of deaths were from bacterial pneumonia, a secondary infection caused by influenza, but the virus also killed people directly, causing massive hemorrhages and edema in the lung.[182]
The 1918 flu pandemic (Spanish flu pandemic) was truly global, spreading even to the Arctic and remote Pacific islands. The unusually severe disease killed between 2 and 20% of those infected, as opposed to the more usual flu epidemic mortality rate of 0.1%.[173][180] Another unusual feature of this pandemic was that it mostly killed young adults, with 99% of pandemic influenza deaths occurring in people under 65, and more than half in young adults 20 to 40 years old.[183] This is unusual since influenza is normally most deadly to the very young (under age 2) and the very old (over age 70). The total mortality of the 1918–1919 pandemic is not known, but it is estimated that 2.5% to 5% of the world's population was killed. As many as 25 million may have been killed in the first 25 weeks; in contrast, HIV/AIDS has killed 25 million in its first 25 years.[180]
Later flu pandemics were not so devastating. They included the 1957 Asian Flu (type A, H2N2 strain) and the 1968 Hong Kong Flu (type A, H3N2 strain), but even these smaller outbreaks killed millions of people. In later pandemics antibiotics were available to control secondary infections and this may have helped reduce mortality compared to the Spanish Flu of 1918.

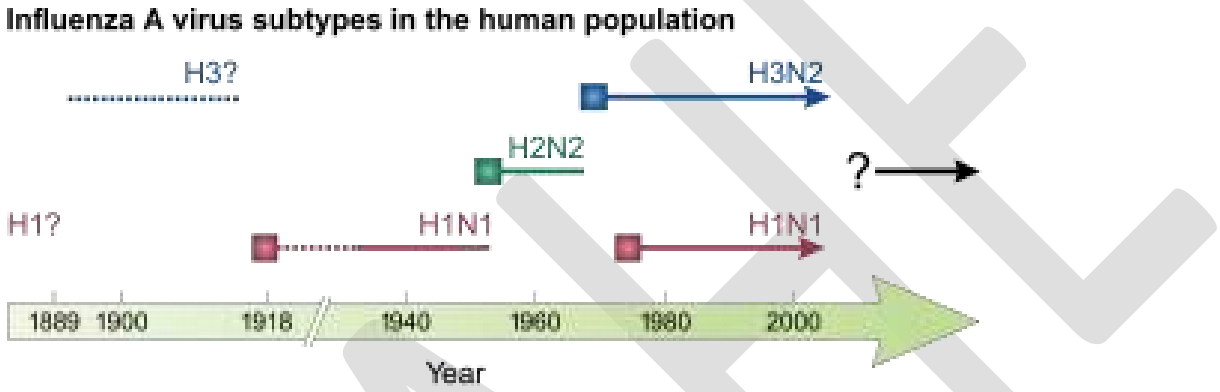
Known flu pandemics

Name of pandemic	Date	Deaths	Case fatality rate	Subtype involved	Pandemic Severity Index
1889–1890 flu pandemic (Asiatic or Russian Flu)[184]	1889–1890	1 million	0.15%	possibly H3N8 or H2N2	N/A
1918 flu pandemic (Spanish flu)[185]	1918–1920	20 to 100 million	2%	H1N1	5
Asian Flu	1957–1958	1 to 1.5 million	0.13%	H2N2	2
Hong Kong Flu	1968–1969	0.75 to 1 million	<0.1%	H3N2	2

Prepared by – Dr. A. A. Arunkumar, Assistant Professor, Department of Microbiology, KAHE.

Russian flu	1977–1978	no accurate count	N/A	H1N1	N/A
2009 flu pandemic[186]	2009–2010	105,700-395,600[187]	0.03%	H1N1	N/A

The first influenza virus to be isolated was from poultry, when in 1901 the agent causing a disease called "fowl plague" was passed through Chamberland filters, which have pores that are too small for bacteria to pass through.[188] The etiological cause of influenza, the Orthomyxoviridae family of viruses, was first discovered in pigs by Richard Shope in 1931.[189] This discovery was shortly followed by the isolation of the virus from humans by a group headed by Patrick Laidlaw at the Medical Research Council of the United Kingdom in 1933.[190] However, it was not until Wendell Stanley first crystallized tobacco mosaic virus in 1935 that the non-cellular nature of viruses was appreciated.



The main types of influenza viruses in humans. Solid squares show the appearance of a new strain, causing recurring influenza pandemics. Broken lines indicate uncertain strain identifications.[191]

The first significant step towards preventing influenza was the development in 1944 of a killed-virus vaccine for influenza by Thomas Francis, Jr.. This built on work by Australian Frank Macfarlane Burnet, who showed that the virus lost virulence when it was cultured in fertilized hen's eggs.[192] Application of this observation by Francis allowed his group of researchers at the University of Michigan to develop the first influenza vaccine, with support from the U.S. Army.[193] The Army was deeply involved in this research due to its experience of influenza in World War I, when thousands of troops were killed by the virus in a matter of months.[180] In comparison to vaccines, the development of anti-influenza drugs has been slower, with amantadine being licensed in 1966 and, almost thirty years later, the next class of drugs (the neuraminidase inhibitors) being developed.[61]

Society and culture

Influenza produces direct costs due to lost productivity and associated medical treatment, as well as indirect costs of preventative measures. In the United States, influenza is responsible for a total cost of over $10 billion per year, while it has been estimated that a future pandemic could cause hundreds of billions of dollars in direct and indirect costs.[194] However, the economic impacts of past pandemics have not been intensively studied, and some authors have suggested that the Spanish influenza actually had a positive long-term effect on per-capita income growth, despite a large reduction in the working population and severe short-term depressive effects.[195] Other studies have attempted to predict the costs of a pandemic as serious as the 1918 Spanish flu on the U.S. economy, where 30% of all workers became ill, and 2.5% were killed. A 30% sickness rate and a three-week length of illness would decrease the gross domestic product by 5%. Additional costs would come from medical treatment of 18 million to 45 million people, and total economic costs would be approximately $700 billion.[196]

Preventative costs are also high. Governments worldwide have spent billions of U.S. dollars preparing and planning for a potential H5N1 avian influenza pandemic, with costs associated with purchasing drugs and vaccines as well as developing disaster drills and strategies for improved border controls.[197] On 1 November 2005, United States President George W. Bush unveiled the National Strategy to Safeguard Against the Danger of Pandemic Influenza[198] backed by a request to Congress for $7.1 billion to begin implementing the plan.[199] Internationally, on 18 January 2006, donor nations pledged US$2 billion to combat bird flu at the two-day International Pledging Conference on Avian and Human Influenza held in China.[200]

In an assessment of the 2009 H1N1 pandemic on selected countries in the Southern Hemisphere, data suggest that all countries experienced some time-limited and/or geographically isolated socio/economic effects and a temporary decrease in tourism most likely due to fear of 2009 H1N1 disease. It is still too early to determine whether the H1N1 pandemic has caused any long-term economic impacts.[201]

Research

Research on influenza includes studies on molecular virology, how the virus produces disease (pathogenesis), host immune responses, viral genomics, and how the virus spreads (epidemiology). These studies help in developing influenza countermeasures; for example, a better understanding of the body's immune system response helps vaccine development, and a detailed picture of how influenza invades cells aids the development of antiviral drugs. One important basic research program is the Influenza Genome Sequencing Project, which is creating a library of influenza sequences; this library should help clarify which factors make one strain more lethal than another, which genes most affect immunogenicity, and how the virus evolves over time.[202]

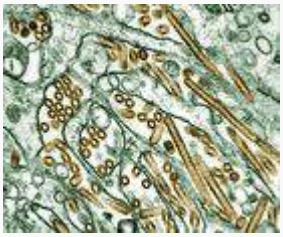
Research into new vaccines is particularly important, as current vaccines are very slow and expensive to produce and must be reformulated every year. The sequencing of the influenza genome and recombinant DNA technology may accelerate the generation of new vaccine strains by allowing scientists to substitute new antigens into a previously developed vaccine strain.[203] New technologies are also being developed to grow viruses in cell culture, which promises higher yields, less cost, better quality and surge capacity.[204]

Research on a universal influenza A vaccine, targeted against the external domain of the transmembrane viral M2 protein (M2e), is being done at the University of Ghent by Walter Fiers, Xavier Saelens and their team[205][206][207] and has now successfully concluded Phase I clinical trials. There has been some research success towards a "universal flu vaccine" that produces antibodies against proteins on the viral coat which mutate less rapidly, and thus a single shot could potentially provide longer-lasting protection.[208][209][210]

A number of biologics, therapeutic vaccines and immunobiologics are also being investigated for treatment of infection caused by viruses. Therapeutic biologics are designed to activate the immune response to virus or antigens. Typically, biologics do not target metabolic pathways like anti-viral drugs, but stimulate immune cells such as lymphocytes, macrophages, and/or antigen presenting cells, in an effort to drive an immune response towards a cytotoxic effect against the virus. Influenza models, such as murine influenza, are convenient models to test the effects of prophylactic and therapeutic biologics. For example, Lymphocyte T-Cell Immune Modulator inhibits viral growth in the murine model of influenza.[211]

Other animals

H5N1



- Influenza A virus
  - subtype H5N1
- Genetic structure

- Infection
- Human mortality
- Global spread
  - in 2004
  - in 2005
  - in 2006
  - in 2007
- Social impact
- Pandemic
- Vaccine

Influenza infects many animal species, and transfer of viral strains between species can occur. Birds are thought to be the main animal reservoirs of influenza viruses.[212] Sixteen forms of hemagglutinin and nine forms of neuraminidase have been identified. All known subtypes (HxNy) are found in birds, but many subtypes are endemic in humans, dogs, horses, and pigs; populations of camels, ferrets, cats, seals, mink, and whales also show evidence of prior infection or exposure to influenza.[47] Variants of flu virus are sometimes named according to the species the strain is endemic in or adapted to. The main variants named using this convention are: bird flu, human flu, swine flu, horse flu and dog flu. (Cat flu generally refers to feline viral rhinotracheitis or feline calicivirus and not infection from an influenza virus.) In pigs, horses and dogs, influenza symptoms are similar to humans, with cough, fever and loss of appetite.[47] The frequency of animal diseases are not as well-studied as human infection, but an outbreak of influenza in harbor seals caused approximately 500 seal deaths off the New England coast in 1979–1980.[213] However, outbreaks in pigs are common and do not cause severe mortality.[47] Vaccines have also been developed to protect poultry from avian influenza. These vaccines can be effective against multiple strains and are used either as part of a preventative strategy, or combined with culling in attempts to eradicate outbreaks.[214]

Bird flu

Flu symptoms in birds are variable and can be unspecific.[215] The symptoms following infection with low-pathogenicity avian influenza may be as mild as ruffled feathers, a small reduction in egg production, or weight loss combined with minor respiratory disease.[216] Since these mild symptoms can make diagnosis in the field difficult, tracking the spread of avian influenza requires laboratory testing of samples from infected birds. Some strains such as Asian H9N2 are highly virulent to poultry and may cause more extreme symptoms and significant mortality.[217] In its most highly pathogenic form, influenza in chickens and turkeys produces a sudden appearance of severe symptoms and almost 100% mortality within two days.[218] As the virus spreads rapidly in the crowded conditions seen in the intensive farming of chickens and turkeys, these outbreaks can cause large economic losses to poultry farmers.

An avian-adapted, highly pathogenic strain of H5N1 (called HPAI A(H5N1), for "highly pathogenic avian influenza virus of type A of subtype H5N1") causes H5N1 flu, commonly known as "avian influenza" or simply "bird flu", and is endemic in many bird populations, especially in Southeast Asia. This Asian lineage strain of HPAI A(H5N1) is spreading globally. It is epizootic (an epidemic in non-humans) and panzootic (a disease affecting animals of many species, especially over a wide area), killing tens of millions of birds and spurring the culling of hundreds of millions of other birds in an attempt to control its spread. Most references in the media to "bird flu" and most references to H5N1 are about this specific strain.[219][220]

At present, HPAI A(H5N1) is an avian disease, and there is no evidence suggesting efficient human-to-human transmission of HPAI A(H5N1). In almost all cases, those infected have had extensive physical

contact with infected birds.[221] In the future, H5N1 may mutate or reassort into a strain capable of efficient human-to-human transmission. The exact changes that are required for this to happen are not well understood.[222] However, due to the high lethality and virulence of H5N1, its endemic presence, and its large and increasing biological host reservoir, the H5N1 virus was the world's pandemic threat in the 2006–07 flu season, and billions of dollars are being raised and spent researching H5N1 and preparing for a potential influenza pandemic.[197]

Chinese inspectors on an airplane, checking passengers for fevers, a common symptom of swine flu

In March 2013, the Chinese government reported three cases of H7N9 influenza infections in humans. Two of whom had died and the third was critically ill. Although the strain of the virus is not thought to spread efficiently between humans,[223][224] by mid-April, at least 82 persons had become ill from H7N9, of which 17 had died. These cases include three small family clusters in Shanghai and one cluster between a neighboring girl and boy in Beijing, raising at least the possibility of human-to-human transmission. WHO points out that one cluster did not have two of the cases lab confirmed and further points out, as a matter of baseline information, that some viruses are able to cause limited human-to-human transmission under conditions of close contact but are not transmissible enough to cause large community outbreaks.[225][226]

Swine flu

In pigs swine influenza produces fever, lethargy, sneezing, coughing, difficulty breathing and decreased appetite.[227] In some cases the infection can cause abortion. Although mortality is usually low, the virus can produce weight loss and poor growth, causing economic loss to farmers.[227] Infected pigs can lose up to 12 pounds of body weight over a 3- to 4-week period.[227] Direct transmission of an influenza virus from pigs to humans is occasionally possible (this is called zoonotic swine flu). In all, 50 human cases are known to have occurred since the virus was identified in the mid-20th century, which have resulted in six deaths.[228]

In 2009, a swine-origin H1N1 virus strain commonly referred to as "swine flu" caused the 2009 flu pandemic, but there is no evidence that it is endemic to pigs (i.e. actually a swine flu) or of transmission from pigs to people, instead the virus is spreading from person to person.[229][230] This strain is a reassortment of several strains of H1N1 that are usually found separately, in humans, birds, and pigs.

Practice Essentials

Influenza, one of the most common infectious diseases, is a highly contagious airborne disease that occurs in seasonal epidemics and manifests as an acute febrile illness with variable degrees of systemic symptoms, ranging from mild fatigue to respiratory failure and death. Influenza causes significant loss of workdays, human suffering, and mortality.

The US Centers for Disease Control and Prevention (CDC) estimates that seasonal influenza is responsible for an average of more than 20,000 deaths annually.[1]Mortality is highest in infants and the elderly.

Signs and symptoms

The presentation of influenza virus infection varies, but it usually includes many of the following signs and symptoms:

- Fever
- Sore throat
- Myalgias

- Frontal or retro-orbital headache
- Nasal discharge
- Weakness and severe fatigue
- Cough and other respiratory symptoms
- Tachycardia
- Red, watery eyes

The incubation period of influenza is 2 days long on average but may range from 1 to 4 days in length. Aerosol transmission may occur 1 day before the onset of symptoms[3]; thus, it may be possible for transmission to occur via asymptomatic persons or persons with subclinical disease, who may be unaware that they have been exposed to the disease.[4, 5]

See Presentation for more detail.

Diagnosis

Influenza has traditionally been diagnosed on the basis of clinical criteria, but rapid diagnostic tests, which have a high degree of specificity but only moderate sensitivity, are becoming more widely used. The criterion standard for diagnosing influenza A and B is a viral culture of nasopharyngeal samples or throat samples. In elderly or high-risk patients with pulmonary symptoms, chest radiography should be performed to exclude pneumonia.

Avian influenza

Avian influenza (H5N1) is rare in humans in developed countries (see the image below). Unless advised by the CDC or regional health departments, clinicians do not routinely need to test for avian influenza.

Management

Prevention

Prevention of influenza is the most effective management strategy. Influenza A and B vaccine is administered each year before flu season. The CDC analyzes the vaccine subtypes each year and makes any necessary changes on the basis of worldwide trends.

Traditionally, the vaccine is trivalent (ie, designed to provide protection against 3 viral subtypes, generally an A-H1, an A-H3, and a B). The first quadrivalent vaccines, which also provide coverage against a second influenza B subtype, were approved in 2012 and were made available for the 2013-2014 flu season.[6, 7]

The FDA has approved a vaccine for H5N1 influenza. It is available only to government agencies and for stockpiles.[8]

The following are influenza vaccine recommendations by the Advisory Committee on Immunization Practices for 2016-2017:[2]

- All persons aged 6 months or older should receive influenza vaccine annually. Influenza vaccination should not be delayed to procure a specific vaccine preparation if an appropriate one is already available.
- For healthy children aged 2-8 years who have no contraindications or precautions, either live attenuated influenza vaccine (LAIV) or inactivated influenza vaccine (IIV) is an appropriate option. No preference is expressed for LAIV or IIV for any person aged 2-49 years for whom either vaccine is appropriate. An age-appropriate formulation of vaccine should be used.
- LAIV should not be used in the following populations: Persons younger than 2 years or older than 49 years; children aged 2-17 years who are receiving aspirin or aspirin-containing products; persons who have experienced severe allergic reactions to the vaccine or any of its components or to a previous dose of any influenza vaccine; pregnant women; immunocompromised persons; persons with a history of egg allergy; children aged 2-4 years who have asthma or who have had a wheezing episode noted in the medical record within the past 12 months; or persons who have taken influenza antiviral medications within the previous 48 hours.
- Persons with a history of egg allergy who have experienced only hives after exposure to egg should receive influenza vaccine. Because relatively few data are available for use of LAIV in this setting,

IIV or trivalent recombinant influenza vaccine (RIV3) should be used. RIV3 may be used for persons aged 18 years or older who have no other contraindications.

- Regardless of allergy history, all vaccines should be administered in settings in which personnel and equipment for rapid recognition and treatment of anaphylaxis are available.
- A previous severe allergic reaction to influenza vaccine, regardless of the component suspected of being responsible for the reaction, is a contraindication to future receipt of the vaccine.

In addition to vaccination, other public health measures are also effective in limiting influenza transmission in closed environments. Enhanced surveillance with daily temperature taking and prompt reporting with isolation through home medical leave and segregation of smaller subgroups decrease the spread of influenza.[9]

Treatment

In the United States, the following prescription antiviral drugs have been approved for treatment and chemoprophylaxis of influenza and are active against recently circulating subtypes of influenza:

- Oseltamivir
- Zanamivir

See Treatment and Medication for more detail.

Background

Influenza, one of the most common infectious diseases, is a highly contagious airborne disease that occurs in seasonal epidemics and manifests as an acute febrile illness with variable degrees of systemic symptoms, ranging from mild fatigue to respiratory failure and death. Influenza causes significant loss of workdays, human suffering, and mortality.

Although the seasonal strains of influenza virus that circulate in the annual influenza cycle constitute a substantial public health concern, far more lethal influenza strains than these have emerged periodically. These deadly strains produced 3 global pandemics in the last century, the worst of which occurred in 1918. Called the Spanish flu (though cases appeared earlier in the United States and elsewhere in Europe), this pandemic killed an estimated 20-50 million persons, with 549,000 deaths in the United States alone.[10]

Besides humans, influenza also infects a variety of animal species. Some of these influenza strains are species-specific, but new strains may spread from other animals to humans (see Pathophysiology). The term avian influenza, in this context, refers to zoonotic human infection with an influenza strain that primarily affects birds. Swine influenza refers to infections from strains derived from pigs. The 2009 influenza pandemic was a recombinant influenza involving a mix of swine, avian, and human gene segments (see H1N1 Influenza [Swine Flu]).

The signs and symptoms of influenza overlap with those of many other viral upper respiratory tract infections (URIs). A number of viruses, including human parainfluenza virus, adenoviruses, enteroviruses, and paramyxoviruses, may initially cause influenzalike illness. The early presentation of mild or moderate cases of flavivirus infections (eg, dengue) may initially mimic influenza. For example, some cases of West Nile fever acquired in New York in 1999 were clinically misdiagnosed as influenza.[4] (See DDx.)

When influenza viruses are circulating in the community, clinicians can often diagnose influenza on the basis of clinical criteria alone (see Presentation). Rapid diagnostic tests for influenza that can provide results within 30 minutes and can help confirm the diagnosis.

It should be kept in mind, however, that these rapid tests have limited sensitivities and predictive values; false-negative results are common, especially when influenza activity is high, and false-positive results can also occur, especially when influenza activity is low.[11] Nevertheless, influenza virus testing may be considered if the results will change the clinical care of the patient (especially if the patient is hospitalized or has a high-risk condition) or influence the care of other patients.[11]

The criterion standard for confirming influenza virus infection is reverse transcription-polymerase chain reaction (RT-PCR) testing or viral culture of nasopharyngeal or throat secretions. However, culture may

require 3-7 days, yielding results long after the patient has left the clinic, office, or emergency department and well past the time when drug therapy could be efficacious.
Prevention of influenza is the most effective strategy. Each year in the United States, a vaccine that contains antigens from the strains most likely to cause infection during the winter flu season is produced. The vaccine provides reasonable protection against immunized strains, becoming effective 10-14 days after administration. Antiviral agents are also available that can prevent some cases of influenza; when given after the development of influenza, they can reduce the duration and severity of illness. (See Treatment.)
For information on influenza in children, see Pediatric Influenza. For patient education information, see Colds, Flu in Adults, and Flu in Children.

Pathophysiology

Influenza viruses are enveloped, negative-sense, single-stranded RNA viruses of the family Orthomyxoviridae. The core nucleoproteins are used to distinguish the 3 types of influenza viruses: A, B, and C. Influenza A viruses cause most human and all avian influenza infections. The RNA core consists of 8 gene segments surrounded by a coat of 10 (influenza A) or 11 (influenza B) proteins. Immunologically, the most significant surface proteins include hemagglutinin (H) and neuraminidase (N).
Hemagglutinin and neuraminidase are critical for virulence, and they are major targets for the neutralizing antibodies of acquired immunity to influenza. Hemagglutinin binds to respiratory epithelial cells, allowing cellular infection. Neuraminidase cleaves the bond that holds newly replicated virions to the cell surface, permitting the infection to spread.[12]
Major typing of influenza A occurs through identification of both H and N proteins. Seventeen H and 9 N types have been identified. All hemagglutinins and neuraminidases infect wild waterfowl, and the various combinations of H and N yield 144 potential subtypes of influenza.
The hemagglutinin and neuraminidase variants are used to identify influenza A virus subtypes. For example, influenza A subtype H3N2 expresses hemagglutinin 3 and neuraminidase 2. The most common subtypes of human influenza virus identified to date contain only hemagglutinins 1, 2, and 3 and neuraminidases 1 and 2. H3N2 and H1N1 are the most common prevailing influenza A subtypes that infect humans. Each year, the trivalent vaccine used worldwide contains influenza A strains from H1N1 and H3N2, along with an influenza B strain.
Because the viral RNA polymerase lacks error-checking mechanisms, the year-to-year antigenic drift is sufficient to ensure that there is a significant susceptible host population each year. However, the segmented genome also has the potential to allow reassortment of genome segments from different strains of influenza in a coinfected host.

Interspecies spread

In addition to humans, influenza also infects a variety of animal species. More than 100 types of influenza A infect most species of birds, pigs, horses, dogs, and seals. Influenza B has also been reported in seals, and influenza C has been reported, though rarely, in pigs.
Some of these influenza strains are species-specific. The species specificity of influenza strains is partly due to the ability of a given hemagglutinin to bind to different sialic acid receptors on respiratory tract epithelial cells. Avian influenza viruses generally bind to alpha-2,3-sialic acid receptors, whereas human influenza viruses bind to alpha-2,6-sialic acid receptors.
In this context, the term avian influenza (or “bird flu”) refers to zoonotic human infection with an influenza strain that primarily affects birds. Swine influenza refers to infections from strains derived from pigs.
New strains of influenza may spread from other animal species to humans, however. Alternatively, an existing human strain may pick up new genes from a strain that usually infects birds or pigs.

Antigenic drift and shift

Influenza A is a genetically labile virus, with mutation rates as high as 300 times that of other microbes.[13]
Changes in its major functional and antigenic proteins occur by means of 2 well-described mechanisms: antigenic drift and antigenic shift.

Antigenic drift is the process by which inaccurate viral RNA polymerase frequently produces point mutations in certain error-prone regions in the genes. These mutations are ongoing and are responsible for the ability of the virus to evade annually acquired immunity in humans. Drift can also alter the virulence of the strain. Drift occurs within a set subtype (eg, H2N2). For example, AH2N2 Singapore 225/99 may reappear with a slightly altered antigen coat as AH2N2 New Delhi 033/01.

Antigenic shift is less frequent than antigenic drift. In a shift event, influenza genes between 2 strains are reassorted, presumably during coinfection of a single host. Segmentation of the viral genome, which consists of 10 genes on 8 RNA molecules, facilitates genetic reassortment. Because pigs have been susceptible to both human and avian influenza strains, many experts believe that combined swine and duck farms in some parts of Asia may have facilitated antigenic shifts and the evolution of previous pandemic influenza strains. The reassortment of an avian strain with a mammalian strain may produce a chimeric virus that is transmissible between mammals; such mutation products may contain H or N proteins that are unrecognizable to the immune systems of mammals. This antigenic shift results in a much greater population of susceptible individuals in whom more severe disease is possible.

Such an antigenic shift can result in a virulent strain of influenza that possesses the triad of infectivity, lethality, and transmissibility and can cause a pandemic. Three major influenza pandemics have been recorded:

- The Spanish influenza pandemic of 1918 (H1N1)
- The pandemic of 1957 (H2N2)
- The pandemic of 1968 (H3N2)

Smaller outbreaks occurred in 1947, 1976, 1977, and 2009.

Transmission and infection

Transmission of influenza from poultry or pigs to humans appears to occur predominantly as a result of direct contact with infected animals. The risk is especially high during slaughter and preparation for consumption; eating properly cooked meat poses no risk. Avian influenza can also be spread through exposure to water and surfaces contaminated by bird droppings.[14]

Influenza viruses spread from human to human via aerosols created when an infected individual coughs or sneezes. Infection occurs after an immunologically susceptible person inhales the aerosol. If not neutralized by secretory antibodies, the virus invades airway and respiratory tract cells.

Once the virus is within host cells, cellular dysfunction and degeneration occur, along with viral replication and release of viral progeny. As in other viral infections, systemic symptoms result from release of inflammatory mediators.

The incubation period of influenza ranges from 1 to 4 days. Aerosol transmission may occur 1 day before the onset of symptoms[3]; thus, it may be possible for transmission to occur via asymptomatic persons or persons with subclinical disease, who may be unaware that they have been exposed to the disease.[4, 5, 15]

Viral shedding

Viral shedding occurs at the onset of symptoms or just before the onset of illness (0-24 hours). Shedding continues for 5-10 days. Young children may shed virus longer, placing others at risk for contracting infection. In highly immunocompromised persons, shedding may persist for weeks to months.[15]

H5N1 avian influenza

To date, avian influenza (H5) remains a zoonosis. The vast majority of cases of avian influenza have been acquired from direct contact with live poultry, with no sustained human-to-human transmission. Hemagglutinin type 5 attaches well to avian respiratory cells and thus spreads easily among avian species. However, attachment to human cells and resultant infection is more difficult.

Avian viruses tend to prefer sialic acid alpha(2-3) galactose, which, in humans, is found in the terminal bronchi and alveoli. Conversely, human viruses prefer sialic acid alpha(2-6) galactose, which is found on

epithelial cells in the upper respiratory tract.[16] Although this results in a more severe respiratory infection, it probably explains why few, if any, definite human-to-human transmissions of avian influenza have been reported: infection of the upper airways is probably required for efficient spread via coughing and sneezing. Most human deaths from bird flu have occurred in Indonesia. Sporadic outbreaks among humans have continued elsewhere, including China, Egypt, Thailand, and Cambodia.[17]

In theory, however, mutation of the hemagglutinin protein through antigenic drift could result in a virus capable of binding to upper and lower respiratory epithelium, creating a strain that is easily transferred from human to human and thus could cause a worldwide pandemic.

Etiology

Influenza results from infection with 1 of 3 basic types of influenza virus: A, B, or C. Influenza A is generally more pathogenic than influenza B. Epidemics of influenza C have been reported, especially in young children.[18] In the United States, during the 2011-2012 influenza season, H3N2 viruses predominated overall, but H1N1 and influenza B viruses also circulated widely.[19] Influenza viruses are classified within the family Orthomyxoviridae.

Avian influenza (ie, human infection with avian H5N1 influenza virus) is transmitted primarily through direct contact with diseased or deceased birds infected with the virus. Contact with excrement from infected birds or contaminated surfaces or water are also considered mechanisms of infection. Close and prolonged contact of a caregiver with an infected person is believed to have resulted in at least 1 case. Other specific risk factors are not apparent, given the few cases to date.

Epidemiology

In tropical areas, influenza occurs throughout the year. In the Northern Hemisphere, the influenza season typically starts in early fall, peaks in mid-February, and ends in the late spring of the following year. The duration and severity of influenza epidemics vary, however, depending on the virus subtype involved.

The World Health Organization estimates that worldwide, annual influenza epidemics result in about 3-5 million cases of severe illness and about 250,000 to 500,000 deaths.[20] In the United States, individual cases of seasonal flu and flu-related deaths in adults are not reportable illnesses; consequently, mortality is estimated by using statistical models.[1]

The US Centers for Disease Control and Prevention (CDC) estimates that flu-associated deaths in the US ranged from about 3000 to 49,000 annually between 1976 and 2006. The CDC notes that the often-cited figure of 36,000 annual flu-related deaths was derived from years when the predominant virus subtype was H3N2, which tends to be more lethal than H1N1.[1]

Unlike adult flu-related deaths, pediatric flu-related deaths are reportable in the United States. (See Pediatric Influenza.) For the 2011-2012 influenza season, which was mild in comparison with preceding years, 26 laboratory-confirmed influenza-associated pediatric deaths were reported.[19] The 2012-2013 season, in which the predominant virus subtype was an H3N2, was notable for widespread disease and a higher mortality than the previous years. By March 3, 2013, a total of 87 influenza-associated pediatric deaths had been reported.[21]

The following statistics are offered for comparison:

- The 1918 H1N1 influenza pandemic caused 500,000-700,000 deaths in the United States—almost 200,000 of them in October 1918 alone—and an estimated 30-40 million deaths worldwide, mostly among people aged 15-35 years
- The 1957 H2N2 influenza pandemic (Asian flu) caused an estimated 70,000 deaths in the United States and 1-2 million fatalities worldwide
- The 1968 H3N2 influenza pandemic (Hong Kong flu) caused an estimated 34,000 deaths in the United States and 700,000 to 1 million fatalities worldwide

In contrast to typical influenza seasons, the 2009-2010 influenza season was affected by the H1N1 (“swine flu”) influenza epidemic, the first wave of which hit the United States in the spring of 2009, followed by a second, larger wave in the fall and winter; activity peaked in October and then quickly declined to below

baseline levels by January, but small numbers of cases were reported through the spring and summer of 2010.[22]
In addition, the effect of H1N1 influenza across the lifespan differed from that of typical influenza. Disease was more severe among people younger than 65 years than in nonpandemic influenza seasons, with significantly higher pediatric mortality and higher rates of hospitalizations in children and young adults. Of the 477 reported H1N1-associated deaths from April to August 2009, 36 were in children younger than 18 years; 67% of those children had 1 or more high-risk medical conditions.[22]
No cases of the highly pathogenic H5N1 influenza have been reported in humans or birds in the United States. Frequently updated information on H5N1 avian influenza cases and pandemic flu preparedness is available from the CDC.[23] Two case reports describe humans infected with another avian influenza virus, H7N2, one in Virginia in 2002 and the other in New York in 2003. The patients had no characteristic symptoms, but the first had positive serologic results and the second had mild respiratory symptoms.
As of June 2013, 630 cases of avian influenza had been reported by the World Health Organization (WHO) worldwide, with 375 deaths.[17] Currently, reporting from areas with poor access to health care may be limited to clinically severe cases; illness that does not fulfill WHO diagnostic criteria is not reported.[24]
Most cases have been in eastern Asia; some cases have been reported in Eastern Europe and North Africa (see the image below). Underreporting has been a concern, particularly in China, but the prevailing attitude about the need to suspect, test, and report cases of avian influenza is growing. In 2013, cases were reported in Cambodia, Vietnam, China, Egypt, and Bangladesh.
Countries where avian influenza has been reported. Image courtesy of the World Health Organization.

Prognosis

In patients without comorbid disease who contract seasonal influenza, the prognosis is very good. However, some patients have a prolonged recovery time and remain weak and fatigued for weeks. Mortality from seasonal influenza is highest in infants and the elderly.
The prognosis for patients with avian influenza is related to the degree and duration of hypoxemia. Cases to date have exhibited a 60% mortality; however, Wang et al suggest that this may be an overestimate stemming from the underreporting of mild cases.[24]
The risk of mortality from avian influenza depends on the degree of respiratory disease rather than on the bacterial complications (pneumonia). Mortality is significantly lower among patients cared for in more-developed nations. Little evidence is available regarding the long-term effects of disease among survivors.
Diabetes increases the risk of severe flu-related illness. In a cohort study of 166,715 individuals in Manitoba, Canada, Lau and colleagues found that adults with diabetes are at significantly greater risk for serious illness related to influenza compared with those without diabetes; this justifies guideline recommendations for influenza vaccination in this population. After controlling for age, sex, socioeconomic status, location of residence, comorbidities, and vaccination, adults with diabetes had a significant increase (6%) in all-cause hospitalizations associated with influenza (P = .044). Only 16% of the patients with diabetes in the cohort and 7% of the patients without diabetes had been vaccinated.[25, 26]

2013-2014 season

In December 2013, the CDC issued a health advisory based on reports of severe respiratory illness among young and middle-aged adults, including a number who were infected with influenza A (H1N1) pdm09 (pH1N1) virus, the strain responsible for the 2009 influenza pandemic. Concerns have been raised that if the virus continues to circulate widely during the 2013-14 flu season, young and middle-aged adults will be disproportionately affected.[27, 28] According to the CDC, evidence from previous flu seasons, including from the 2009 pandemic, indicates that antiviral medications initiated as early as possible after the onset of illness reduce severe influenza outcomes.

KARPAGAM ACADEMY OF HIGHER EDUCATION					
DEPARTMENT OF MICROBIOLOGY					
17MBP201 - VIROLOGY					
UNIT - I	**Option A**	**Option B**	**Option C**	**Option D**	**Answers**
Nucleic acid contains _____ genes.	3 - 400	2 - 100	2	1	3 - 400
Capsomeres are the unit of _____ .	Envelope	Spikes	Capsid	DNA	Capsid
Antigenic glycoprotein projections are called_____ .	Protomeres	Spikes	Capsomeres	Capsid	Spikes
The weight of virus particles ranges from _____Daltons.	4 – 400 x 10^6	3 - 800 x 106	3 - 800 x 10^6	4 – 400 x 10^6	3 - 800 x 10^6
One drop of water contain _____virus particles.	3 x 10^4	3 x 10^2	3 x 10^3	3 x 10^5	3 x 10^4
The basic unit of Svedberg(S) value is _____se	a. 10^{-11}	b. 10^{-13}	c. 10^{-12}	d. 10^{-14}	b. 10^{-13}
An example for spherical virus is_____ .	Poliovirus	Rabies virus	TMV	Smallpox	Poliovirus
Core of the virus is_____ .	Capsomere	Envelope	Nucleocapsid	Protomere	Nucleocapsid
Infectious virus particles with (or)without envelop is_____ .	Virion	Nucleocapsid	Envelop	Capsid	Virion
An example for enveloped virus is_____.	Retrovirus	Papovavirus	Adenovirus	Parvovirus	Retrovirus
An example for non-enveloped virus is_____ .	Togavirus	Flavivirus	Poxvirus	Poliovirus	Poliovirus

Envelope with glycoprotein subunit is known as_____ .	Peplomer	Protomere	Hexamere	Capsomer	Peplomer
The size of viruses ranges from _____ diameter.	10-20 nm	20-400nm	30-40nm	10-40nm	20-400nm
The smallest viruses is_____ .	Poxviridae	Paramyxoviridae	Parvoviridae	Reoviridae	Parvoviridae
The size of Parvoviridae ranges from_____ .	20nm	300nm	150nm	60nm	20nm
The largest viruses is_____	Polioviruses	Smallpox viruses	Adenoviruses	Bunyaviruses	Smallpox viruses
The size of smallpox viruses _____ in diameter.	100nm	150nm	200nm	250nm	200nm
Adenoviruse _____ in size	25nm	35nm	45nm	75nm	75nm
The size of poliovirus is_____ in diameter.	28nm	38nm.	48nm	58nm	28nm
_____ except in RNA viruses has double stranded genome.	Paramyxoviruses	Arenaviruses	Reoviruses	Flaviviruses	Reoviruses
_____ expect in DNA viruses has a single –stranded genome.	Herpesviruses	Parvoviruses	Papovaviruses	Adenoviruses	Parvoviruses
Viruses genomes range in size from _____ nucleotides	3,200	3,000	4,200	4,000	3,200
An example for the virus having ambisense genome is_____ .	Hepadnavirus	Influenza virus	Mimivirus	Bunyavirus	Bunyavirus
An example for segmented genome containing virus _____ .	Gemini virus	Influenza virus	Poxvirus	Herpes virus	Influenza virus

Geminivirus is an example for _____ genomes possessing viruses.	Segmented	Multipartite	(+)Strand	(-)Strand	Multipartite
The size of virus particles is measured by _____ membrane filters.	Seitz	Nitrocellulose	Asbestos	Collodion	Collodion
VAP is_____ .	Viral attachament protein	virus attaching phage	viral anchored phage	virus anchored particles	Viral attachament protein
RDE is_____ .	Receptor Decting Enzyme	Receptor Determining Envelope	RNA Detecting Enzyme	Receptor Destroying Enzyme	Receptor Destroying Enzyme
Receptor destroying enzyme is_____ .	Ligase	Neuraminidase	Polymerase	Reverse Transcriptase.	Neuraminidase
Rabies virus haemagglutinate pigeon red cells at _____ .	37° C	50° C	4° C	100° C	4° C
Reovirus haemagglutinate human erythrocytes at _____ .	37° C	50° C	4° C	100° C	37° C
Viral envelop is made up of _____ .	Matrix and Glycoprotein	Calcium	Phosphorous	Sodium	Matrix and Glycoprotein
The specialized enzyme required for viral replication is _____ .	Exonucleases	S_1 nuclease	Kinases	Endonucleases	Endonucleases
Each cell possess _____ receptors.	5,000	50,000	5,00,000	50,00,000	5,00,000
Fusion determining membrane glycoproteins is present in _____ .	Retrovirus	Adenovirus	Poliovirus	Reovirus	Retrovirus
Receptor – mediated endocytosis is otherwise known as _____ .	RDE	VAP	Eclipse phase	Viropoxis	Viropoxis

The time interval between the entry of viral nucleic acid into the host cell and then the appearance of first infectious virus particle is known as _____ .	Penetration	Uncoating	Eclipse phase	End phase.	Eclipse phase
Assembly of DNA virus occurs in _____ of the host.	Nucleus	Cytoplasm	Capsid	Envelope	Nucleus
Assembly of POX virus occurs in _____ of the host.	Cytoplasm	Nucleus	Capsid	Envelope	Cytoplasm
RNA viruses get assembled in _____ of the host.	Capsid	Nucleus	Cytoplasm	Envelope	Cytoplasm
Enveloped viruses are released from the host cell by the process of _____ .	Lysogeny	Lysis	Budding	Endocytosis	Budding
Naked viruses are released from the host cell by _____.	Cell Lysis	Budding	Endocytosis	Phaging	Cell Lysis
The process of wrong selection of host cell by the virus is known as _____.	Incomplete infection	Abortive infection	Complete infection	Sexduction	Abortive infection
The process of 'Von Magnus Phenomenon' is observed in _____.	Influenza viruses	Complete viruses	Pseudoviruses Abnormal viruses		Influenza viruses
The process of production of high haemagglutination titre with low infectivity is known as	Eclipse phase	Haemagglutination	Von magnus phenomenon Uncoating		Von magnus phenomenon Uncoating
Electron microscopy is one of the diagnostic method in	Inclusion Bodies	Morphology	Genome	Virus isolation	Morphology

virology to observe _____.					
IgM antibody is detected in _____.	Secondary Infection	Tissues	Body fluids	Primary infection	Primary infection
Haemagglutination inhibition test is a _____ metho	Novel	Subordinate	Classical	Temporary	Classical
RIA is _____ .	Radical Induction Assay	Radar Immuno Activity	Reading Immuno Assay	Radio Immuno Assay	Radio Immuno Assay
SRH is abbreviated as _____ .	Single Radial Analysis	Single Radial Heat	Single Radial Haemolysis	Single Reading Haemolysis	Single Radial Haemolysis
Viral genomes is detected by _____ .	Immunofluorescence	ELISA	chick embryo	Molecular Techniques	Molecular Techniques
RIBA is _____ .	Radio Immunosorbant Assay	Recobinant Immuno Blot Assay	Radio Immuno Blot Assay		Recobinant Immuno Blot Assay
For visualization, electron microscopy requires _____ virus particle / ml of the sample.	103	105	104	102	105
The magnification used in electron microscopy is _____ .	50	500	50,000	5,00,000	50,000
The virus particles in the given sample is concentrated using _____ metho	Solar drying	Agar diffusion	Dessication	Dilution	Agar diffusion
SPIEM is _____ .	Simple Immuno Electron Microscopy	Super Immuno electron Microscopy	Solid Phase Immuno Electron Microscopy	Simple Immuno electron Microscopy	Solid Phase Immuno Electron Microscopy
Prions composed of a single sialoglycoprotein known as _____ .	PrP23 – 27	PrP27 – 30	PrP28 – 29	PrP24 – 30	PrP27 – 30

PrP gene in prions is found on _____ humans.	Chromosome 20	Chromosome 30	Chromosome 31	Chromosome 10	Chromosome 20
Subviral particles is known as _____ .	Capsid	Envelope	Spikes	Viroids	Viroids
Viroids are catalytic RNA's known as _____ .	Ribozymes	Enzymes	Nucleases	Proteases.	Ribozymes
UNIT - II	**Option A**	**Option B**	**Option C**	**Option D**	**Answers**
Picorna Viruses are small _____ viruses.	DNA	RNA	Obligate	Plant	RNA
Poliovirus comes under _____ genera of Picornaviruses.	Rhinoviruses	Aphthoviruses	Cardioviruses	Enteroviruses	Enteroviruses
Total antigenic types of poliovirus include _____ types.	4	2	3	5	2
The route of entry of poliovirus is _____ .	Food	skin	Utensils	Mouth	Mouth
Incubation period of poliovirus ranges from _____ days.	9 – 12	1 – 2	5 – 6	6 – 7	6 – 7
Flaccid paralysis occurs due to the damage of _____ .	Large Intestine	Small Intestine	Liver	Motor Neurons	Motor Neurons
IPV is _____ .	Induced Polio Vaccine	Induced Polio Virus	Inactivated Polio Vaccine	Inactivated Part Virus	Inactivated Polio Vaccine
OPV is _____ .	Oral Polio vaccine	Old Polio Vaccine	Oral Polio Virus	Old Polio Virus	Oral Polio vaccine
_____- contain formalized strains of poliovirus type 1, 2 and 3.	Sabin Vaccine	Salk Vaccine	Subunit Vaccine	Bacterial Vaccine	Salk Vaccine
_____ contain Live, attenuated strains of poliovirus type 1, 2 and 3.	Salk Vaccine	Subunit vaccine	Sabin Vaccine	Cholera Vaccine	Sabin Vaccine

The common name of Hepatitis A is _____ .	Serum Hepatitis	Delta Hepatitis	Enteric Hepatitis	Infectious Hepatitis	Infectious Hepatitis
Hepatitis A belong to the family _____ .	Picorna Viridae	Flaviviridae	Calciviridae	Retroviridae	Serum Hepatitis
The common name of Hepatitis B is _____ .	Infectious Hepatitis	Serum Hepatitis	Enteric Hepatitis	Delta Hepatitis	Flaviviridae
Hepatitis B belong to the family _____ .	Reoviridae	Flaviviridae	Calciviridae	Hepadna viridae	Hepadna viridae
The virion size of Hepatitis A is _____ .	20 nm	27 nm	21 nm	19 nm	27 nm
The serum bilirubin level reaches _____ with the appearance of jaundice in Hepatitis A infection.	70 mg/dl	50 mg/dl	5 – 20 mg / dl	30 mg / dl	5 – 20 mg / dl
The virion size of Hepatitis B is _____ .	27 nm	42 nm	20 nm	21 nm	42 nm
The incubation period of Hepatitis – A infection ranges from _____ weeks.	10 - 20	10 - 12	2 - 6	1 – 2	2 - 6
ALT is _____ .	Alanine Amino Transferase	Amino Labile Transferase	Alanine Transferase	Alanine Labile Transferase	Alanine Amino Transferase
The virion Hepatitis B is also called _____ particle.	Delta	Dane	Dawn	Double	Dane
HDV is a _____ virus	Dominant	Direct	Defective	Induced	Dominant
Hepatitis D virus is also known as _____ virus.	Dane	Delta	Deep	Drop	Delta
An example for bullet-shaped enveloped viruses is _____ .	Adenovirus	Reovirus	Rabies	Papovavirus	Rabies

Rabies occurring in wild animals is referred as _____ rabies.	Sylvatic	Urban	Mokola	Duvernhage	Sylvatic
In Rabies Infection specific Cytoplasmic inclusion bodies in neurons is known as	Quarnieri bodies	Councilman	Bollinger	Negri Bodies	Negri Bodies
Another name for rabies is _____	Hydrophilia	Anaemia	Hydrophobia	Anaphylaxia	Hydrophobia
Negri bodies are demonstrated in _____- during postmortem diagnosis.	Lung	Brain	Skin	Leg	Brain
Sample vaccine was developed by sample in _____ .	1900	1811	1800	1911	1911
BPL is _____ .	Betaproline	Betapropane	Betapropiolactone	Betaprolactane	Betapropiolactone
HDVC is _____ .	Human Diploid cell Strain vaccine	Human Direct Cell vaccine	Highly diploid cell vaccine	Highly direct cell strain vaccine	Human Diploid cell Strain vaccine
PCEC is _____ .	Particle circularized Eliminated culture	Particle Embryonated cell culture	Purified circularized embryonated culture	Purified chick embryo cell culture	Purified chick embryo cell culture
HRIG is _____ .	Highly Rich Immuno Globulin	Human Rabies Immuno Globulin	Human Rich Immuno Globulin	Highly Reverse Immuno Globulin	Human Rabies Immuno Globulin
Influenza virus belongs to the family of _____ .	Paramyxoviridae	Rhabdoviridae	Orthomyxoviridae	Picornaviridae	Orthomyxoviridae
In 1933 _____ virus was first isolated from throat washings.	Mumps	Measles	Rabies	Influenza Virus type A	Influenza Virus type A

Influenza virus is a _____ type of virus.	Spherical	Irregular	Cubical	Rod	Spherical
The diameter of influenza virus ranges between _____ .	20 – 30 nm	80 – 110 nm	50 – 70 nm	60 – 70 nm	80 – 110 nm
The genome of influenza C has _____ segments.	2	5	7	8	7
The M_1 protein of influenza virus is a _____ protein.	Non-structural	Capsid	Membrane	Matrix	Matrix
The old designation of H_1N_1 influenza A subtype is _____ .	A2	A_2 (HongKong)	Aswine	A3	A3
The old designation of H_2N_2 influenza A subtype is _____ .	Aswine	A3	A2	A0	A0
Influenza viruses are grouped into _____ types on the basis of antigen differences in Nucleoprotein and Matrix protein	3	4	2	5	3
In 1968 _____ influenza A subtype was originated from HongKong influenza strain.	H1N1	H3N3	H2N2	H0N0	H3N3
MDCK is _____ cells.	Madin – Duck	Madin – Delbruck Kidney	Madin – duck Canine Kidney	Madin – Darby Canine Kidney	Madin – Darby Canine Kidney
Amantadine acts on _____ of Influenza virus.	M4 Protein	M1 Protein	M2 Protein	M5 Protein	M2 Protein
Rimantadine is an analogue of _____ .	Amantadine	Rifampicin	Ampicillin	Penicillin	Amantadine

Mumps virus belongs to the family _____ .	Orthornyxoviridae	Retroviridae	Rioviridae	Paramyxoviridae	Paramyxoviridae
Incubation period of Mumps virus infection is _____ .	12 – 14 days	16 – 18 days	11 – 12 days	10 – 12 days	16 – 18 days
Mumps virus has _____ serotypes.	2	6	Only one	5	Only one
The live attenuated vaccine for Mumps infection is _____ .	RA / 273 Strain	Teryl Lynn Strain	Edmonston & Strain A / Hongkong / 168.	Honkong Strain	Teryl Lynn Strain
Measles virus is otherwise known as _____ virus.	Mumps	Rubella	Rabies	Rubella	Rube1la
Measles virus belongs to _____ gener	Hepatitis virus	Paramyxoviruses	Morbilli viruses	Rubella	Morbilli viruses
CDV is _____ .	Cubical Dermatities Virus	Councilman Dietemper Virus	Canine Distemper Virus	canine Dermatitis Virus	Canine Distemper Virus
Prodromal phase of Measles infection includes running nose otherwise known as _____.	Cornea	Coryza	Calphta	Cordae	Coryza
_____ is a redspot appears in measles infection	Morbilliform	Coryza	Canine	Koplik’s Spot	Koplik’s Spot
Measles pneumonia is also referred as _____ .	Mumps pneumonia	Rubella pneumonia	Rubeola pneumonia	Giant Cell pneumonia	Giant Cell pneumonia
ISPE is _____ .	Subacute Sclerosing pan encephalitis	Sub single pan encephalitis	Subacute, single pan encephalitis	Sub sclerosing pan encephalitis	Subacute Sclerosing pan encephalitis
The inclusion bodies found in measles infection is _____ .	Guarnieri Bodies	Bollinger bodies	Cowdry type A	Councilman type A	Cowdry type A
Incubation of measles virus infection is _____ days.	8 – 10	9 – 12	5 – 6	14 – 16	9 – 12

MMR is _____ vaccine.	Myxo Mumps Rubella	Mumps Measles Rubeola	Mumps Measles Rubella	Myxo Mumps Rubeola	Mumps Measles Rubella
Edmonston B strain is a _____ vaccine.	Killed	subunit	Vector	Live Attenuated	Live Attenuated
An example of brick-shaped enveloped virus is _____ .	Adenovirus	Picornavirus	Pox virus	Calcivirus	Pox virus
The name _____ was given to the virus causing classical small pox.	Variola Minor	Variola Major	Variola zoster Variola Irregular		Variola Major
UNIT - III	**Option A**	**Option B**	**Option C**	**Option D**	**Answers**
The family Herpesviridae is divided into _____ subfamilites.	3	2	1	4	3
The size of Herpes viruses are _____ nm in diameter.	160	140	130	150	150
The space between the envelope and the capsid is known as _____ .	Legument	Tegument	Regument	Aegument	Tegument
Herpes viruses were described as _____ .	Hot sores	Hot spots	Cold sores	Cold spots	Cold sores
The disease caused by HSV – I is _____ .	Neonatal herpes	Cervical carcinoma	Vulvular carcinoma	Eczema herpeticum	Eczema herpeticum
Herpetic whitlow is an infection of _____ .	Neck	Leg	Hair	Finger	Finger
Tzank cells are otherwise known as _____ .	Multinuclear giant cells	Kupffer cells	Latent cells	Vesicular endothelial cells	Multinuclear giant cells
Famciclovir is a drug administered _____ to HSV infections.	Intraneously	Subcutaneously	Orally	Cutaneously	Cutaneously

Acyclovir should be given within _____ to all HSV infections.	24 hrs	12 hrs	48 hrs	72 hrs	72 hrs
Shingles are caused by _____ virus.	Pox	Varicella	Herpes-zoster	Influenza	Herpes-zoster
VZIG is _____ .	Viral zidovidine Immuno globulin	Varicella – zoster Immuno globulin	Viral zoster Immuno globulin	Varicella zidovidine Immunoglobulin	Varicella – zoster Immuno globulin
_____ drug is given to CMV infections.	Ganciclovir	Vamciflovir	Acyclovir	Valacyclovir	Ganciclovir
'OWL's eye' type large intranuclear inclusion bodies is found in _____ virus.	TMV	EBV	CaMV	CMV	CMV
TORCH is panel of test including _____ .	Toxoplasmic cauliflower mosaic HSV	Toxoplasmosis Other cytomegalovirus HSV	Toxoplasmosis Oriented cytomegalo HSV	Toxoplasmosis Other cystic HSV	Toxoplasmosis Other cytomegalovirus HSV
Tumour of jaw is known as _____ .	Kaposi's Sarcoma	Firboma	Burkittt's Lymphoma	Lymphosarcoma	Burkittt's Lymphoma
Paul – Bunnel test is a _____ metho	Direct Examination	Indirect examination Molecular Serological	Molecular	Serological	Indirect examination Molecular Serological
VCA is _____ .	Vero Cell Antigen	Viral Capsid Antigen	Viral core A	Viral Capsid A	Vero Cell Antigen
EA is _____ .	Early Antigen	E-type Antibody	Type E Antigen Epstein Antibody		Type E Antigen Epstein Antibody
EBNA is _____ .	Enveloped B type nucleic acid	Enveloped Bunyavirus Nuclear – Ag	Epstein – Barr Nuclear Antigen	Epstein type B Nucleic acid	Epstein type B Nucleic acid

_____ a benign exanthematous disease of childhoo	Burkitt's Lymphoma	Kaposi's Sarcoma	Leukoplakia	Roseola Infantum	Kaposi's Sarcoma
In KSHV, 'KS' Stands for _____ Herpes Virus.	Koplik's spot	Kaposi's Sarcoma	Klebsiella sensitive	Kenyen S-type	Kenyen S-type
In _____ Rowe and his associates first detected adenoviruses in adenoid tissue.	1923	1933	1943	1953	1943
Adenovirus contain icosohedral capsid with _____ capsomers.	212	222	252	242	212
Total Number of human adenovirus serotypes is _____ .	47	48	46	49	46
Incubation period ranges between _____ days in adenovirus infection.	1 - 2	2 - 3	5 - 7	8 – 9	2 - 3
Adenovirus associated virus is known as _____ .	Picornaviruses	Dependoviruses	Reoviruses	Retroviruses	Picornaviruses
Burkitt's Lymphoma is caused by _____ family.	Herpesviridae	Papoviridae	Flaviviridae	Reoviridae	Papoviridae
Apoptosis is _____ .	Generation Time	Programmed cell death	Programmed cell proliferation	MIC	Programmed cell death
_____ is the only human oncogenic retrovirus that encodes a 'tax' protein.	Hepatitis B Virus	HTLV – I	HTLV – II	HCV	HTLV – II
'V-one' gene commonly referred as _____ .	Cellular genes	Regulatory genes	Cancer genes	Structural genes	Cancer genes
Loss of 'Rb' gene leads to development of _____ .	Myelomas	Sarcomas	Carcinomas	Retinoblastomas	Retinoblastomas

In _____, Peyton Rous first described Retroviruses.	1911	1901	1910	1912	1911
_____ genes codes for envelope glycoprotein.	V-onc	O onc	Pol	EnV	EnV
_____ gene codes for nucleocapsid shell.	V – onc	Gag	Pol	EnV	Gag
_____ gene codes for reverse transcriptase.	Pol	Gag	EnV	O onc	Pol
_____ gene encodes the products responsible for cell transformation.	V-onc	O onc	C – Src	V – Src	O onc
In _____ , Robert Gallo isolated a retrovirus, HTLV – III from AIDS patient.	1982	1983	1984	1985	1984
The size of HIV ranges between _____ nm diameter.	60 - 80	50 - 70	190 - 220	90 – 120	90 – 120
_____ is cleared into two envelope components gp120 and gp41.	gp121	gp140	gp160	gp141	gp160
The tat gene codes for a protein _____ .	p19	p16	p18	p20	p16
_____ protein promotes the expression of HIV – I structural proteins.	p16	p18	p19	p20	p19
_____ gene codes a protein that downregulate the transcription of HIV genome.	tat	rev	vif	nef	nef
Vif is _____ gene.	Virus flexible	virion infectivity factor	virus infection	Virion including factor	virion infectivity factor
_____ gene represents the promoting region of the virus.	vpr	vif	vpu	vpx	vpr

PGL is also known as _____ .	Burkitt's syndrome	Lymphadenopathy syndrome	Nasopharyngeal syndrome	Polyma like syndrome	Lymphadenopathy syndrome
The interval between HIV infection and appearance of antibodies in serum is known as _____ .	Eclipse period	early Eclipse period	Window Period	Late Eclipse period	Window Period
Zydovudine is also known as _____ .	Dideoxyinosine	Azidideoxycytidine	Azidodideoxy thymidine	Dideoxyadenine	Azidodideoxy thymidine
Which of the following is not the protease inhibitor?	Squinavir	Ritonavir	Indianavir	Ganciclovir	Ganciclovir
The Cowpea Mosaic Virus is a Plant mosaic virus belongs to _____ group.	Enterovirus	Rhinovirus	Comovirus	Hepadnavirus	Comovirus
CPMV can be readily isolated from_____	Animals	Insects	Human	Plants	Plants
_____Particls are used to amplify signals in microarray based sensors	HSV	EBV	HBV	CPMV	CPMV
When a Satellite Subviral Agent encodes the coat protein in which it is encapsulated,it is known as ---------- virus.	Polyoma	Satellite	Adeno	Herpes	Satellite
MSV is _____	Maize Streak Virus	Master Standard Virus	Mixed Standard Virus	Mixed Steak Virus	Maize Streak Virus
MSV is transmitted by a variety of _____ species.	Stemhopper	Leafhopper	Roothopper	Flowerhopper	Leafhopper
TYDV is _____	Tri Yield Dwaff Virus	Tobacco Yellow Dwarf Virus	Tri Yellow Dwarf Virus	Tobacco Yield Dwarf Virus	Tobacco Yellow Dwarf Virus
The Mastrevirus genome contains	3	4	2	5	2

_____intergenic regions located at opposite sides of the viral genome					
For smooth entry into the plant cells ,Tmv produces protein called_____	P30	P20	P40	P50	P30
In TMV ,there are_____RNA nucleotides per protein monomer.	2	3	4	5	3
In _____ d' Herelle suggested the name Bacteriophage to bacteria eaters.	1911	1917	1918	1916	1917
UNIT - IV	**Option A**	**Option B**	**Option C**	**Option D**	**Answers**
Mostly phages are _____ in their shape.	Helical	Icosahedra	tad-pole like	Linear	tad-pole like
The head of phage T_4 has a diameter of _____ nm.	65	35	45	55	65
The tail has a terminal base plate with totally _____ tail fibres.	3	4	5	6	6
The tail of phage T_4 is _____ in length.	10	1000	100	10000	100
_____ phage produces lysis of infected cells releasing large number of progeny viruses.	Temperate	Lysogenic	Tryptic	Virulent	Virulent
In _____ phage, phage DNA gets integrated into the bacterial chromosome.	Temperate	Lysogenic	Cryptic	Virulent	Temperate

The integrated phage is known as _____ .	Coliphage	Prophage	Lytic phage	Prephage	Prophage
The bacteria with intetgrated phage is known as _____ .	Lysogen	Colin	Plasmin	Dolphin	Lysogen
Bacterial Iysis occurs without phage multiplication is called _____ .	Lysis	Lysis from without	Lysogenic	Helper phage	Lysis from without
_____ phage enzymes weakens the cell wall during replication of phage.	Neuraminidase	Polymerase	Muramidase	cellulose	Muramidase
The time interval between the infection of host cell and sudden increase in extracellular virus is called _____.	Eclipse period	Window period	Dormant period	Latent period	Latent period
_____ of phage is defined as the highest dilution of phage preparation required to produce confluent lysis.	STD	ISD	RTD	TTD	RTD
When phages are applied on a lawn culture of susceptible strain resulting in clear zone, known as _____ after incubation.	Phages	Pock	Plaques	Lysis	Plaques
Bacterial cell acquiring new properties after phage infection by the process known as _____ .	Phage Lysis	Phage inversion	Phage immersion	Phage conversion	Phage conversion
_____ converts prophage to lytic cycle.	H_2O_2	H_2O	O_2	HO_2	H_2O_2

Virus takes _____ time interval from cell adsorption to appearance of newly formed viruses.	20 min	10 min	40 min	60 min	40 min
Maintenance of Lysogenic infection is dependent on _____ .	Protein inducer	Protein adsorber	Protein adheser	Protein repressor	Protein repressor
The duration of eclipse phase is about _____ in phages.	5-10 min	10-12 min	15-30 min	1-5 min	5-10 min
The tail fibre is _____ nm long.	130	120	110	140	130
Mostly phages range in size from ---------- nm in length.	20-22	24-200	14-20	15-20	24-200
The number of particles released per infected bacteria may be as high as ----------	2000	500	1000	100	1000
The infectious particle that give rise to a plaque is known as ----------	Plaque forming unit	Pock forming unit	Colony forming unit	Cell forming unit	Plaque forming unit
Pfu is ----------	pock forming unit	Plaque forming unit	Plasmid forming unit	Probe forming unit	Plaque forming unit
---------- is a phage – coded protein which binds to an operation site on the phage DN	Inducer.	Regulator	Repressor	Operator	Repressor
When a lysogenic bacterium is exposed to adverse conditions, the lysogenic state can be ferminated by the process known as ----------	Repression	Regulation	Conversion	Induction	Induction

Adverse conditions lead to the production ----------	rec B protein	rec A protein	re C protein	rec D protein	rec A protein
Rec A protein is a ---------- enzyme in its activity.	Nucleases	Ligases	Proteases	Kinase	Proteases
The lambda phge to enter the lytic (on lysogenic cycle m a cell is determined by the Concentration of the repressor and another phage protein called ---------- in the cell.	Cl	Cro	OR 1	OR 2	Cro
A ---------- Protein turns off the synthesis of the repressor and prevents the establishment of lysogeny.	Cl	CRT	Cro	Or 1	Cro
In ---------- the United States Food and Drug Administration (FDA) approved using bacteriophages on certain meats to kill the Listeria monocytogenes bacteria, giving GRAS Status	August 2006	September 2006	April 2006	May 2006	August 2006
GRAS is ----------	Globally Recorded As Superior	Globally Recorded As safe	Generally recognized as safe	Globally Recognized As safe	Generally recognized as safe
Dr. Angela Belcher, founder cambrios Technologies, pioneered the use of ---------- bacteriophage to create nanowires and electrodes.	λ	¢x1 74	Ms 2	M 13	λ

The integration of phage λ takes place at a special attachment site in the bacterial genome Called ----------	N	Cro	aHλ	int	aHλ
IHF is ----------	Interrupted H factor	Integration host factor	Integrated High factor	Interrupted host factor	Integration host factor
Lambda phage was discovered by ---------- in 1951.	Angela Belcher	Fred Sanger	Waher fries	Esther Lederberg	Esther Lederberg
---------- is a dimer also known cl protein that regulates the transcription of cl protein and cro protein.	Lambda regulator	Lambda repressor	Lambda inducer	Lambda co inducer	Lambda repressor
M 13, a filamentous bacteriophage is ---------- nucleotide long	6207	6307	6407	6507	6407
The phage coat is assembled from a 50 amino acid protein called ---------- which is encoded in the phage genome.	pVI	pVIII	p VII	PvI	pVIII
The ---------- bacteriophage infects specifically Bacillus Subtitles.	M13	Ms 1	Ms 2	M 12	Ms 2
The infection of a bacterium by the naked phage nucleic acid is known as ----------	Transcription	Translation	Transfect ion	Transformative	Transfect ion
The presence of high concentrations of upto ---------- per ml of phage particles	10^{-2}	10^{-8}	10^{-3}	10^{-4}	10^{-8}

control bacterial population in the particular environment.					
Adsorption is mediated by cofactors such as ----------	Enzyme	Protein	Anions	Cations	Cations
Penetration is facilitated by the presence of ---------- on the phage tail that produces a hole in the bacterial wall for the entry of phage DNA	Ribozyme	Lysozyme	Lipase	Ligase	Lysozyme
The period in which the number of infectious phages released increases is referred as ---------- Perio	Window	Dormant	Rise	Latent	Rise
A lysogenic bacterium is resistant to reinfection by the same (or) related phages is known as	Suppressor immunity	Super infection immunity	Sustainable immunity	Secondary immunity	Super infection immunity
The average yield of Progeny phages per infected bacterial cell is known as ----------	Latent period	Small size	Large size	burst size	burst size
Assembly of M 13 phage is associated with the membrane of bacteria and requires one Bacterial protein, ----------	redoxin	Bacteriocin	Colicin	thioredoxin	thioredoxin
The bacteriophage MS 2 is otherwise known as ----------	Staplylococcus phage M 2	Balillus phage M 2	E. wli phage M 2	Shikgella phage M 2	Balillus phage M 2
A ---------- was the first organism to have its DN-based genome to be sequenced in 1977.	M 13 phage	¢ x 714	Ms 2	λ phage	¢ x 714

In ---------- Walter fires demonstrated the physical and covalently closed circularity of ¢ x174 DN	1960	1961	1962	1963	1962
¢x174 is made of ---------- genes.	11	10	12	13	11
---------- is one of the longest DNA-s in phages having icosahedral rea	phage x	phage T4	M 13	M 2	M 2
LTF is ----------	Long Tumor formers	Long Terminal fibre	Long Tail fibre	Long Technical former	Long Terminal fibre
The lytic life cycle of Bacteriophage T_4 tokes place within ---------- minutes.	10	30	20	2	2
A ---------- Bacteriophage has been used as a model in synthetic biology.	T1	T2	T4	T7	T1
A ---------- was the first phage typing method developed in 1938.	Vi-phage typing	Bacteriocin typing	HLA typing	MHC typing	Vi-phage typing
DVS is ----------	Dissected variable strain	Degenerated variable strain	Degraded Vi-Strains	Desseminated Vi-strains	Degraded Vi-Strains
In 1975 ---------- developed a scheme for biotyping to study salmonella typhimunium infections.	Barker et al.	Old et al.	Dudguid et al.	Scarlata et al.	Dudguid et al.
There are about ---------- different phage – types of Salmonella typhimurium have been distinguished	252	242	262	232	232
UNIT - V	**Option A**	**Option B**	**Option C**	**Option D**	**Answers**

An example for natural antiviral compounds is _____ .	Zydovudine	Interferon	Acyclovir	Protein kinase	Interferon
An example for synthetic antiviral agents is _____ .	Interferon	Interleukin	Acyclovir	Polymerase	Acyclovir
In _____, Isaacs and Lindenmann discovered interferon.	1957	1967	1947	1937	1957
_____ Interferon is produced by monocytes and B lymphocytes.	β	γ	δ	α	α
_____ interferon is produced by fibroblasts and epithelial cells.	γ	β	δ	α	β
_____ interferon is produced by activated T-cells.	α	δ	γ	ζ	γ
The IFN - β lies on chromosome _____ .	2	3	9	6	9
The IFN – γ lies on chromosome _____ .	10	12	11	13	12
Interferons are glycoprotein with molecular weight of _____ .	10,000 - 15,000	20,000 - 40,000	50,000 - 60,000	5,000 - 15,000	20,000 - 40,000
Interferons is inactivated by _____ enzymes.	polymerase	Ligase	Restriction	Proteolytic	Proteolytic
_____ interferon is a lymphokine.	β	α	δ	γ	γ
There are totally _____ gene codes for IFN – α	24	23	1	2	23

There are totally _____ gene codes for IFN – β	23	2	1	25	2
There are totally _____ gene codes for IFN – γ	1	2	3	4	1
The molecular weight of IFN – α is _____.	11,000	22,000	14,000	17,000	17,000
The molecular weight of IFN – β is _____.	11,000	17,000	16,000	15,000	17,000
The molecular weight of IFN – γ is _____.	17,000	11,000	15,000	16,000	17,000
The best inducers of α and β interferons production is _____ .	ss DNA	ds DNA	ss RNA	ds RNA	ds RNA
Example for mitogens is _____ .	Exotoxins	Endotoxins	Mutant	Aflatoxins	Endotoxins
Immune stimulators is otherwise referred as _____ .	Polymerase	Sterile Soil	Sterile water	Mitogens	Mitogens
Synthesis of IFN – α is and INF – β takes _____ hours after infection and attaining maximum level of synthesis.	2-5	6-12	15-25	16-24	6-12
Type I interferon inhibit _____ .	Cellular protein synthesis	viral protein synthesis	DNA synthesis	RNA synthesis	viral protein synthesis
Type II interferon possess _____ activity.	antibacterial	antifungal	antiviral	antihaemolytic	antiviral
_____ protein kinase inactivates elongation factor – 2 in protein synthesis.	72 kd	62 kd	52 kd	68 kd	68 kd
Induction of _____ inhibit viral replication.	Sodium oxide synthetase	Hydrogen peroxide synthetase	Potassium oxide synthetase	Nitric oxide synthetase	Nitric oxide synthetase

_____ bind to gp120 in HIV.	CD4	CD8	Recombinant CD4	Rec CD8	Recombinant CD4
ICAM is _____, blocks infectivity of common cold viruses.	Induced choroallantric membrane	Intracellular cell adhesion molecule	Indian council of Atomic membership	Indian council of Academic Microbiology	Intracellular cell adhesion molecule
_____ is used as an Immunostimulant.	IFN - β	IFN – γ	IFN – δ	IFN - α	IFN – γ
_____ inhibit the fusion of viral envelope with endosome membrance preventing the release of nucleocapsid into the cytoplasm.	Amanladine	Zydovudine	Ganciclovir	Rifampicin	Amanladine
_____ agent has a territory structure similar to deoxyadenosine.	Idoxuridine	Amantadine	Xydovudine	Vidarabine	Vidarabine
IDU is _____ .	Induced Dermi Uridine	Idoxuridine	Indoluridine	idiotopic uridine	Idoxuridine
_____ is a derivative of acyclovir.	Ganciclovir	Valacyclovir	famciclovir	Vidarabine	Ganciclovir
_____ is an antiviral drug that cause termination of DNA chain.	Valacyclovir	Amantadine	ICAM	type I IFN	Valacyclovir
The enzyme _____ is expressed in HSV infected cells soon after its infection.	Viral polymerase	Viral Ligase	Viral thymidine kinase	Viral kinase	Viral thymidine kinase
Zydovudine is otherwise known as _____ .	Acyclovir	Zakitabine	Ribavirin	Azido thymidine	Azido thymidine
_____ was the first drug used for the treatment of HIV infection.	Zalcitabine	Zidovudine	Stavudine	Nevirapine	Zidovudine
_____ is a nucleoside analogue of thymidine that	Retrovir	Zalcitabine	Idoxuridine	ribavirin	Zalcitabine

has greater affinity for reverse transcriptase.					
AZT, thymidine analogue lacks _____ essential for the formation of 5’ – 3’ diester linkages between nucleic acid molecules.	5’ P	3’ P	5’ OH	3’ OH	3’ OH
Dideoxycytidine is otherwise known as _____ .	Retrovir	Zalcitabine	Idoxuridine	Ribavirin	Zalcitabine
Dideoxyinosine is otherwise known as _____ .	Idoxuridine	Azidothymidine	Didanosine	Foscarnet	Didanosine
_____ is an inhibitor of HIV protease.	Retrovir	Acyclovir	Ritonavir	Famciclovir	Ritonavir
_____ is also known as phasphonoformic aci	Foscarnet	Ribavirin	Retrovir	Ritonavir	Foscarnet
_____ is an “Broad Spectrum” nucleoside analogue that acts on cellular enzymes important to viral replication.	Zalcitabine	Ribavirin	Acyclovir	Didanosine	Ribavirin
Enzyme producing GTP is the initial compound necessary for capping of _____ synthesis.	r RNA	m RNA	s RNA	t RNA	m RNA
_____ drug used in the early stages of Hantavirus infection.	Zidovudine	Zalcitabine	Ribavirin	Didanosine	Ribavirin
A ---------- deactivates virus particles outside the body.	Fungicide	Bacteriocide	Viricide	Gellicide	Viricide
The first experimental antiviral were developed in --	1920s	1960s	1950s	1970s	1950s

---------- to be dealt with Herpesviruses.					
Vaccines attack viruses when they are in ---------- Stage.	Minute particle	Small unit	incomplete particle	complete particle	complete particle
Entry blocking drug is ---------- is used to combat influento.	pleconaril	streptomycin	ketaconazole	Amantadine	Amantadine
The entry blocker against rninoviruses is ----------.	Streptomycin	Amantadine	Pleconaril	Glucanazole	Pleconaril
A ---------- is the first successful antiviral drug effective against herpes virus infection	Acyclovir	amantadine	Lamivudine	pleconaril.	Acyclovir
The first antiviral drug to be approved for treating HIV is ----------.	Amantadine	Gluconazole	Zidovudine	Pleconaril	Zidovudine
A ---------- is a component of reverse transcriptase that splits the synthesized DNA from the original viral RN	polymerase	Ligase	DNase H	RNase H	RNase H
The enzyme ---------- Splices the synthesized DNA into the host cell genome.	integrase	Ligase	polymerase	protease	integrase
---------- is used to treat opportunistic eye infections in AIDS patient caused by Cytomegalovirus.	Streptomycin	fomivirsen	Lamivudine	pleconaril	fomivirsen
Relenta is the other name for ---------- drug.	Acyclovir	Ribavirin	Zanamivir	Oseltamivir	Zanamivir

Tamiflu is otherwise known as ----------	Acyclovir	Oseltamivir	Zanamivir	Ribavirin	Oseltamivir
MMR vaccine is ecommended by CDC for Children at the age group between_____	0-1	0 to 9	0 - 6	11 to 12	0 - 6
BCG is given for infants at the time of_____	Disease	Birth	Emergency	Maturity	Birth
MCV is _____	Measles Vaccine	Mumps Cornea Virus	Measles Cornea Virus	Meningococcal Vaccine	Meningococcal Vaccine

POSSIBLE QUESTIONS

1. Write any two general properties of viruses
2. Define i) viroids ii) prions
3. Give few examples of RNA virus.
4. Write about the structure of viruses and its types
5. Give a detailed note on viral genome and viral nomenclature.
6. Explain viral replication with clear diagram.
7. Discuss on old and modern classification of viruses.
8. Give a detailed note on viral symmetry.
9. Write an essay about the DNA viruses.
10. Explain in short about the viral cultivation methods
11. Write in detail about the old and modern classification of viruses
12. Give a detailed note on viral symmetry
13. What is the structure of viruses and its types?
14. What is Bacteriophage(T4)? Add a note on its replication
15. Give a brief note on filamentous phage (Ø X174)
16. Explain in detail about the general properties of the viroids and prions
17. Explain in short about Rabies vaccines preparation and its immunization
18. Give a detailed note on TMV
19. Explain in detail about the HIV
20. Write an essay about the DNA viruses
21. Explain in short about the viral purification.
22. Write in detail about the assay of viruses based on serology.
23. Give a detailed note on viral replication
24. What are the structure of viruses and its symmetry?
25. What are bacterial viruses? How they infect their host?
26. Give a brief note on reproduction of T4 phage.
27. Explain in detail about the plant viruses
28. Give the general properties of the viruses
29. Give a detailed note on Influenza virus.
30. Explain in detail about the phi X174?
31. Write an essay about the RNA viruses.
32. Explain in short about the viral separation methods.
33. Write in detail about growth of virology.

34. Give a detailed note on viral genome and viral nomenclature.
35. How the viruses are get replicated? Give a note with clear diagram
36. Give an account of the classification of phages and add a note on Hershey – Chase experiment
37. Explain the replication mechanism of T4 Phage.
38. Explain in detail about the plant virus - TMV
39. Give the brief note on animal viruses.
40. Explain the structural composition, replication and transmission of hepatitis virus
41. Describe the antigenic structure, replication, pathogenisis, prevention and treatment of picorna.
42. Write an essay about the SARS and swine flu viral outbreak.
43. Explain in short about the general properties of viruses and its multiplication process..
44. Write in detail the virus family descriptors used in virus taxonomy.
45. Give a detailed note on Capsid, Prion, Virion, Viriod and Envelope
46. What is the genome organization and replication of viruses?
47. What is the one step growth curve experiment of both bacteriophages?
48. Write about the T even bacteriophages.
49. What are the modes of transmission of plant viruses and the symptoms?
50. Give the brief note on antigenic drift and antigenic shift
51. Explain the interferon and its mode of action
52. Describe the gene therapy
53. Write an essay about the bacteriophages and phage therapy
54. Explain in short about the isolation and purification methods of viruses.
55. Write in detail about serological diagnosis of viruses.
56. Give a detailed note on viral mutation and the viral symmetry.
57. Describe in detail about the replication cycle of viruses with clear diagram
58. Give an account of the reproduction of TMV
59. Explain the replication mechanism of phi Phage.
60. Explain in detail about the viruses which affect the human immune system
61. Give the brief note on T4 phage.
62. Explain in general about Viral vaccination and immunization schedule.
63. Write in detail about the antiviral chemotherapy and viral vaccines.
64. Write an essay about the Hepatitis viruses