NAME OF THE COURSE WORK
VIROLOGY

UNIT-V
OTHER VIRUSES & ANTIVIRAL AGENTS

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Cyanophages

- Blue-green algae are widespread in the aquatic environment and often occur in mass blooms related to eutrophication of water bodies.
- In 1963, Safferman and Morris first isolated the virus known to attack and destroy cyanobacteria.
- These agents, now known to attack a wide range of blue-green algae, have been variously called phycoviruses, algophages, blue-green algal viruses, and Cyanophage.
- Cyanophages are very similar to bacteriophages both in structure and infection cycle so, it is suggested that they should be considered a subgroup of the bacteriophages.
- The economic and nuisance effects of blue-green algae on water quality and in causing fish intoxications.
- The first cyanophage was isolated named as strain LPP-1,and it attacks filamentous blue green algae *Lyngbya*, *Phormidium*, and *Plectonema* (Non heterocyst).
- Several serological strains of LPP were isolated from different parts of world and named LPP-I, LPP-2, LPP-3, LPP-4 and LPP-5.
- Cyanophages have a complex pattern of host ranges are widely distributed and can be readily isolated from marine and fresh waters.

Morphology

- Cyanophages intact particles and on separate head and tail preparations.
- The viral head capsid is a polyhedron, appearing hexagonal in projection.
- Head is 58.6 ± 2.1 nm. icosahedral form and models for its construction from the Capsomers.
- A short tail 20nm long and 15nm wide, is attached to one of the vertices of the head.

Physiological properties

Nucleic acids

- Linear double stranded DNA. Head-tail bacteriophages contain only this type of nucleic acid.
- No homology was evident when the DNA of LPP-1 was reacted with the DNAs of A, 434 hybrid, 080, P22, and T7.

Protein

- The major head proteins are the 39,000 and 13,000 molecular weight species.
- The major tail protein has a molecular weight of 80,000. No internal proteins were detected. The sum of molecular weights accounts for about 35% of the coding capacity of LPP-1 DNA.
- The sum of the molecular weights of SM-1 structural proteins requires a coding capacity of 16% of the entire chromosome, a considerably smaller fraction than is found in viruses such as LPP-1 and T7

Physiological properties

- Compared with bacterial viruses, which are generally stable from pH 5 to 8.

Physiological parameter	**Cyanophage Group**			
	LPP-1	**N-1**	**SM-1**	**AS-1a**
Sedimentation co efficient	555-548	539	1.021, 1.029	-
Buoyant Density in CsCl (g/cm3)	1.48	-	1.48	-
Mg2+ requirement (M)	0.001	-	Not required	Not required
Temperature of Inactivation (C)	55	-	55	60
Temperature range of great stability	4-40	-	4-40	-
pH range of great stability	5-11	-	5-11	4-10

Classification

- Cyanophages are have the diverse morphology and it classified into three families based on the tail size and contractility.
  - Cyanomyovirus
  - Cyanostylovirus
  - Cyanopodovirus

Cyanomyovirus
Family- *Myoviridae*
Host- *Anacystis, Synechococcus.*
***Ex*-** Cyanophage AS-1

Cyanostylovirus
Family- *Siphoviridae*
Host- *Synechococcus***.**
Ex- *Synechococcus* WH7803

Cyanopodovirus
Family- *Podoviridae*
Host- *Lyngbya, Plectonema, Phormidium.*
***Ex*-** Cyanophage LPP-1

Host range

LPP GROUP

- The original cyanophage isolates [LPP-1] and a group of related phages were isolated from waste stabilization ponds.
- LPP cyanophages have short non contractile tails similar to that of bacteriophage T7.
- Family- Podovirus.
- The host range of cyanophages was restricted to ***Lyngbya***, ***Plectonema***, and ***Phormidium*** subgroup of cyanobacteria.
- Different LPP isolates varied in host range and that caution should be used when assigning cyanobacteria to specific taxa based on phage susceptibility.

A, AN, N and NP Groups

- Cyanophages also infect and cause lysis of filamentous cyanobacteria assigned to Anabaena and Nostoc spp.
- It includes both *Podoviruses* and *Myoviruses*.
- Although the viruses represent two different families, with few exceptions they posses similar host ranges and infect both *Anabaena* and *Nostoc* species.
- N-1 virus may not attack heterocystous cyanobacteria. This virus successfully infects the dense cultures of *Nostoc* in the exponential growth.

AS and SM groups

- These groups as they have wide host range.
- *Podovirus* SM-1 was described as infecting *Synechococcus elongatus* and *Microcystis aeruginosa* while *Myovirus* AS-1 was described as infecting *Microcystis aeruginosa* as well as *Synechococcus cedrorum*.
- SM-1 virus appears to be polyhedron but without an obvious tail. The average diameter of the polyhedron is 880 A.
- the virus contains DNA and unlike LPP-1 does not appear to resemble the basic morphology of the phage types.
- The pH and thermal stability ranges are similar to those of LPP-1 virus.

Distribution

- Cyanophages are first discovered in freshwater and widely distributed in marine and they are probably ubiquitous in aquatic systems.
  - Fresh water
    - Marine water

Fresh Water

- LPP cyanophages were found in waste stabilization ponds in the United States, India, Scotland as well as fishponds of Israel.
- LPP cyanophages have good evidence for strong seasonal variations in their abundance.
- Viruses that infect cyanobacteria in the *Anabaena -Nostoc* group are also widespread and were found in lakes, reservoirs and sewage settling ponds.

Marine water

- The first report of cyanophages in the marine environment was for Romanian coastal waters of the Black Sea.
- Most of the cyanophages isolated that were isolated were *Myoviridae*, although *Styloviridae* and *Podoviridae* were also isolated.
- The abundance of infectious cyanophages was related first and most strongly to the abundance of *Synechococcus* spp.
- Infectious cyanophages can occur at the considerable depth in marine sediments.
- Viruses probably attached to particles either directly or via infected cells and sink to the sediment surface where they are subsequently buried.

- Sediments are the important reservoir of infectious cyanophages.
- Unlike freshwater environments where viruses that infect filamentous cyanobacteria are commonly isolated.
- viruses in marine waters which infect unicellular cyanobacteria appear to be by far most abundant.
- Example of filamentous cyanobacterium that was demonstrated to be infected by a virus was *Phormidium persicinum.*
- *Podoviruses* that have short non contractile tails and infect other filamentous bacteria the virus which infected *P. persicium* possessed a long flexible tail and are morphologically similar to *Siphoviruses*.

Phycophages

- Phycophages or Phycodnavirus (members of the family Phyvodnaviridae) are large (160 to 560 thousand base pairs), double stranded DNA viruses that infect marine or freshwater eukaryotic algae.

  GROUP: ds DNA

  FAMILY: Phycodnaviridae

  GENUS: Cholorovirus, Coccolithovirus, Prasinovirus, Phaeovirus, Prymnesiovirus.

- Phycodnaviruses have icosahedral morphology, an internal lipid membrane and replicate, completely or partly, in the cytoplasm of their host cells. They belong to a super-group of large viruses known as Nucleocytoplasmic large DNA viruses (NCLDVs).
- So far, the genomes of two phycodnaviruses have been completely sequenced, a Paramecium bursaria Chlorella virus (PCBV-1) and an Ectocarpus siliculosus virus (EsV).

Virion Structure

Morphology

- The virions of phycodnaviridae consist of a non-enveloped capsid it and exhibits polyhedral symmetry.
- The isometric capsid has a diameter of 130-200 nm. The capsid shells of virions are composed of multiple layers and are referred to as multilaminated capsids. The capsids appear hexagonal in outline.

Nucleic Acid

- Non segmented, single molecule of linear double-stranded DNA.
- The MW of the genome constitutes 21-25% of the virion by weight. Complete genome is 250000-350000 nucleotides long. G+C content 40-52 %.
- The genome contains unusual bases (varying from 0.1-47 %), they are 5-methyl deoxy-cytosine residues and N6-methyl deoxy-adenosine residues (in some DNAs).

Protein

- Proteins constitute about 64% of the particle weight. The viral genome encodes structural proteins and non-structural proteins.

Lipid

- Lipids are present and located in the internal component (located inside the glycoprotein shell).
- Virions are composed of 5-10% lipids by weight (beneath the outer glycoprotein shell). Lipids are essential for infectivity.

Phycodnaviridea Family-an out line

Chlorovirus

- **Virion** - Non-enveloped, spherical capsid with an icosahedral symmetry (T=169), 100-220 nm in diameter.
- **Genome** - Linear, dsDNA genome of about 330 kb with cross linked hairpin ends. PBCV-1 genome encode for approximately 700 ORFs.

Coccolithovirus

- **Virion** - Coccolithovirus is a giant double-stranded DNA virus that infects Emiliania huxleyi, a species of coccolithophore.
- **Genome** - Its genome is 407,339 base pairs long with a G+C content of 41.1%, and contains 472 predicted coding sequences.
- *Coccolithoviruses* genome, a sequence of genes responsible for production of ceramide was discovered .
- Ceramide is a controlling factor in cell death, and it is currently thought that *Coccolithovirus* uses this to prolong the life of *Emiliania huxleyi* while it uses the host cell to replicate.
- This is a unique ability unseen in any other viral genome to date.

Phaeovirus

- **Virion** - Non-enveloped, spherical capsid with an icosahedral symmetry (T=169), 120-150 nm in diameter.
- **Genome** - Linear, dsDNA genome of 150-350 kb with cross linked hairpin ends.
- Worldwide Distribution.
- Transmission by passive diffusion through water.
- No antiviral drugs against this *Phaeovirus*.

Phaeovirus

- Non-enveloped, spherical capsid with an icosahedral symmetry (T=169), 104-118 nm in diameter.
- Worldwide Distribution.
- Transmission by passive diffusion through water.
- No antiviral drugs.

Raphidovirus

- **Virion** - Non-enveloped, spherical capsid with an icosahedral symmetry (T=169), 200 nm in diameter.
- **Genome** - Linear, dsDNA genome of about 295 kb with crosslinked hairpin ends.
- Worldwide Distribution.
- Transmission by passive diffusion through water.
- No antiviral drugs.

Mycophages

- Hypoviridae is a fungal virus family that infects fungi. They have been identified in all major fungal families.
- Spherical in shape, double stranded RNA genomes or more than one dsRNA present per virus particle.
- The virus never leaves its host and probably replicates in the host derived lipid pleomorphic vesicles.
- Transmission is probably dependent on host hyphal anastomosis.

La France Disease

- An example of a true mycovirus is the causal agent of La France disease.
- That affects the edible mushroom, *Agaricus bisporus*.
- It is also known as X disease, watery stripe, dieback and brown disease.
- Symptoms include:
  - Slow and aberrant mycelial growth
  - Malformation
  - Premature maturation
  - Increased post-harvest deterioration (reduced shelf life).
- Linear dsRNA genome of 9-13kb.
- Poorly understood cytoplasm.
- **Geography**-Originated from East Asia. CHV-2, CHV-3 and CHV-4 are present in North America. CHV-1 is specifically found in Europe.
- **Transmission**-The virus never leaves its fungal host. Virus movement is completely dependent on the movement of the fungus.

GENERAL PROPERTIES OF FUNGAL VIRUSES

Host Range

- Mycoviruses is thought to be limited to the same or closely related vegetative compatibility groups that allow lateral transmission.
- Experimental host ranges for fungal viruses are essentially nonexistent because of lack of suitable infectivity assays.
- Recent improvement of infectivity/transfection assays allowed the extension of experimental host ranges of some mycoviruses to different vegetative compatibility groups and even to different genera.
- Many of the viruses that infect phytopathogenic fungi do not induce macroscopic symptoms.
- Mycoviruses that attenuate the virulence of plant pathogenic fungi provide excellent model systems for basic studies on development of novel biological control measures and for dissecting the mechanisms underlying fungal pathogenesis.

Transmission

- Extracellular transmission of mycoviruses is not known to occur in any regular fashion and there are no known specific natural vectors .
- Instead, these viruses are transmitted Macroscopic symptoms caused by fungal viruses.
- Host factors involved in symptom expression are not well studied except for host genes involved in the killer phenotype in yeast infected with the totivirus L-A and associated satellite dsRNAs .

INSECT VIRUSES

Introduction

- Insect viruses may be dsDNA and ssDNA, respectively or dsRNA and ssRNA, respectively, enveloped or non-enveloped, and occluded in a protective protein matrix or non-occluded.
- Viruses that are primarily or exclusively found in insects.
- Currently placed in 12 families and one unclassified group viruses in families that have been isolated but are not common in insects are not included here.
- Viral diseases have been found in 13 insect orders and most likely occur in all orders.
- Viruses are the simplest "**Life Forms**" so far recovered from insects, and consist of a nucleic acid core and a protein shell or capsid.

Insect Virus Taxonomy

A. **DNA viruses** - Isometrics, Without Envelope.

1. Family: ***Parvoviridae*** (ssDNA, mo-nopartite genome)

   Genus: Denso Virus

2. Family: ***Iridoviridae*** (dsDNA, ino-nopartite)

   Genus: Iridovirus

   Genus: Chloriridovirus

B. **DNA viruses -** Helicoidal, Enveloped.

3. Family: ***Baculoviridae*** (dsDNA, nonopartite)

   Genus: Baculovirus Nuclear polyhedrosis; Granulosis; non-occluded.

4. Family: ***PolyDNAviridae*** (dsDNA, Multipartite)

   Genus: Poly DNA Virus Fusiform nucleocapsid; rodshaed nucleocapsid

C. **DNA viruses** - Complex, Enveloped.

5. Family: ***Poxviridae*** (dsDNA, Monopartite)

   Genus: Entomopdx Subgenus A. B, C.

   Genus: Ascovirus (dsDNA, mono-partite)

D. **RNA viruses** - Isometric, Non-enveloped

6. Family: ***Picornaviridae*** (ssRNA, Monopartite) Genus: Enterovirus
7. Family: ***Caliciviridae*** (ssRNA, mo-nopartite) Genus: Cahumus
8. Family: ***Nodaviridae*** (ssRNA, bipar-tite) Genus: Noda Virus

DNA Viruses

Family: *Parvoviridae*

- ssDNA, Non enveloped virus.
- The nuclei become strongly Eosinophilic.
- Very virulent and infectious, and the type species GmDNV, has caused serious problems for the wax worm bait industry.
- Despite their **high virulence**, Densoviruses do **not replicate** in **vertebrates**.
- **Densovirus:**
- ssDNA viruses, Non-enveloped.
- Isometric (spherical) Nucleocapsid with a diameter of either 18-22 nm or 20-26 nm.
- Sixty **capsomers** are present in a capsid.
- 5000 nucleotides in length and could either be negative-sense or positive-sense.

Iridovirus

Family: *Iridoviridae*

- First detection of an iridovirus when Claude Rivers, in March of 1954, discovered crane fly larvae (*Tipula spp*) glowing with patches of Blue coloration.
- Large (120 to 300 nm in diameter) non-occluded viruses with icosahedral symmetry.
- dsDNA genome that ranges in size from 150 to 280 kbp
- Hosts - Both invertebrate and non-mammalian vertebrate.
- Hosts is the aquatic or moist environment in which they are found.
- Iridoviruses from the blackfly, Simulium spp., are found in two states, covert (inapparent) and patent (lethal).
- Ratios of each dependent on environmental conditions and host densities.
    - Insects become flaccid and iridescent 7-10 days post-infection although death may take 3 weeks or longer.

Baculovirus

Family: *Baculoviridae*

- Non-segmented and contains a molecule of circular, dsDNA.
- Complete genome sequence is 80000-180000 nucleotides long.
- Baculovirus virions have a complex structure which consists of an envelope and a rod-shaped nucleocapsid.
- The capsid has helical symmetry.
- The capsid is 200-450nm in length, and 30-100nm in diameter.
- Baculoviruses infect **insects**. Most infections occur in a closely related species of insects, such as **Lepidoptera.**
- Dispersal by passively interacting with other insects.
- Soil is another reservoir for the virus.
- They can remain in the soil for a very long time.

- Not all baculoviruses are lethal. Some baculoviruses set up persistent or even latent infections in healthy hosts.
- Transmission of such baculoviruses is vertical between hosts.
- Most obvious interaction is the dissolution of the protective proteinaceous matrix in the midgut of the host larvae.

Polydnavirus

Family: *Polydnaviridae*

- Two genera: **Ichnoviruses** (IV) and **Bracoviruses** (BV).
- The **ichnoviruses o**ccur in ichneumonid wasps (**parasitoid**) species and **bracoviruses** in braconid wasps.
- Double-stranded, super helical DNA packaged in capsid proteins and a double layer (IV) or single layer (BV) envelope.
- The genome of each is integrated into the host wasp genome.
- The virus particles are only replicated (produced) in specific cell types in the female wasp reproductive organs.
- Its infection does not lead to replication of new viruses; rather it affects the caterpillar's immune system.
- The virus and wasp have a symbiotic (**Mutualistic**) relationship.

Entomopoxvirus

Family: *Entomopoxvirinae*

- Envelope, a surface membrane, a core, and lateral bodies, **or** a surface membrane, a core, and lateral bodies.
- Ovoid, or brick-shaped, or pleomorphic.
- Virions measure 170-250 nm in diameter; 300-400 nm in length displaying tubular units.
- The genome is not segmented and contains a single molecule of linear double-stranded DNA.
- The complete genome is 130000-300000 nucleotides long. **G + C** content 17-27 %.
- The viral genome encodes structural proteins and non-structural proteins.
- Virions are composed of 4% lipids by weight.
- The lipids are host derived and synthesized ***de novo*** (during the early phase of virus replication) and are derived from plasma membranes.
- Viral membranes include glycolipids.

Replication

Virus bind to a Glycosaminoglycans (GAGs) receptor on the host cell surface

↓

virus enters the cell where it uncoats (Uncoating of the virus)

↓

Virus particle (without the outer membrane) is uncoated further to release the core into the cytoplasm

↓

Expression of pox viral genes (genes encode the non-structural protein, including proteins necessary for replication of the viral genome)

↓

Genome has been replicated and encode the structural proteins to make the virus particle

↓

The assembly of the virus particle occurs in the cytoskeleton of the cell

↓

Release of Viral particles.

RNA virus

Family: *Picornaviridae*

- Non-enveloped, spherical, about 30 nm in diameter.
- Monopartite, linear, ssRNA(+) genome of 7.1-8.9 kb, polyadenylated
- The capsid consists of a densely-packed icosahedral arrangement of 60 protomers, each consisting of 4 polypeptides, VP1, VP2, VP3 and VP4. VP4 is located on the internal side of the capsid.
- Number of proteins that modify the host cell, ultimately leading to cell lysis.

Replication

Virus attaches to host receptors, formation of a coated vesicle

↓

Uncoating, and release of the viral genomic RNA into the cytoplasm

↓

VPg is removed from the viral RNA, which is then translated into a processed Polyprotein

↓

Translation through the cleavage of the translation initiation factor eIF4G by viral protease

↓

Replication of viral RNA takes place on membrane

↓

New genomic RNA synthesized using the negative-sense RNA as a template is believed to be packaged into preformed procapsids

↓

Cell lysis and virus release

↓

Maturation of provirions by an unknown host protease

Biological Control

- Picorna-like viruses are often chronic, and because of their apparent homologies to mammalian picacornaviruses, little effort has been made to incorporate them into biological control programs.
- Some, however, are good pathogens and cause epizootics in their hosts.
- Some of these viruses have potential for use in biological control programs.

VIROIDS

- A viroid is a virus (VIR) like (OID) particle.
- Viroid's are "sub-viruses" composed exclusively of a single circular strand of nucleic acid (RNA) that codes for a single protein
- Small circular molecules, a few hundred nucleotides long, with a high degree of secondary structure.
- They do not code for any polypeptides plant virus.
- Smallest known agents of infectious disease.
- The most studied viroid's is potato spindle tuber viroid (PSTVd).
- Names are abbreviated with 'd' to distinguish them from abbreviations for virus names.

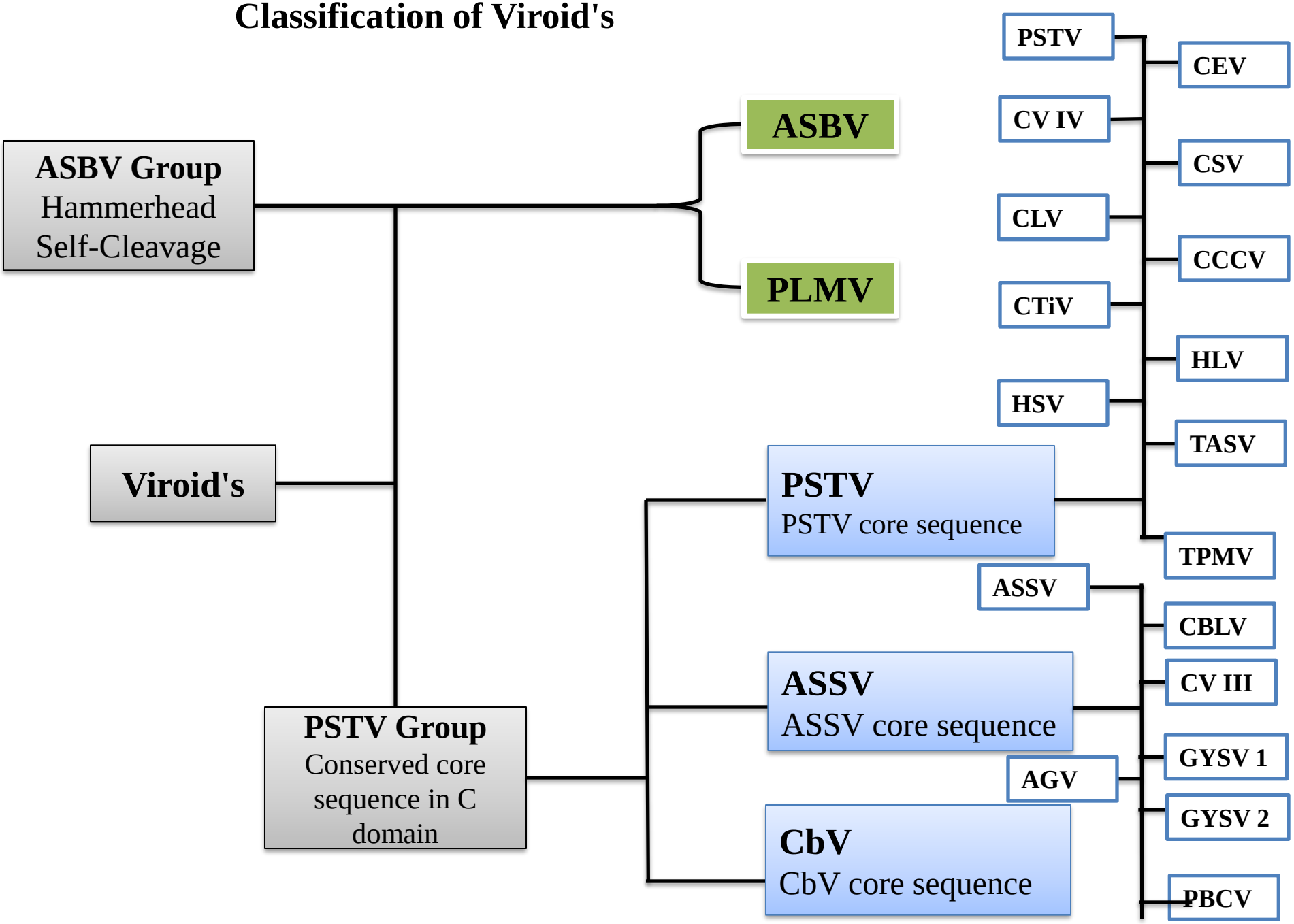
Properties

- Obligate parasite
- Infectious
- Low molecular weight
- Non encapsidated
- Replicate autonomously
- Single viroid capable of infecting a cell
- Resistant to nuclease (proteases) digestion

Viroid Structure

- Covalently closed circular RNAs
- Fold to tightly base-paired structure
- Self-cleaving - replicate in nucleus and fold into “dog bone” or rod-like structure.
- Non-self-cleaving - Five domains (Left hand terminal Domain, Pathogenic domain, Central Domain, Variable domain, Right hand terminal domain)are identifiable in non-self-cleaving.

Classification of Viroid's



Structure

- Infectious agents composed of a single piece of circular single stranded RNA which has some double-stranded regions.
- Both prions and viroid's are sometimes called Subviral particles - Because of their simplified structures.
- Mainly cause plant diseases but have recently been reported to cause a human disease.
- The molecules appear as small rods with an axial ratio of about 20:1, and for PSTVd an average length of about 37nm.
- Under denaturing conditions, the molecules can be seen to be covalently closed circles of about 100 nm.

- 27 member of the viroid group and those of numerous variants are now known.
- Range in size from 246 to 375 nucleotides.
- All viroids have some degree of overall sequence similarity.
- Most viroids have a relatively high G+C content (53-60%), but ASBVd is rich in A+U (62%).
- The RNAs of members of the *Pospiviriodae* are predicted to form rod-like molecules with base-paired region interspersed with unpaired loops, whereas those of the *Avsunviroidae* are less structured.

Replication

- Three enzymatic activities are required - an RNA polymerase, an RNAse and an RNA ligase.
- *Avsunviroids* - Symmetric Rolling Circle Mechanism.
- *Pospiviroids* – Asymmetric Rolling Circle mechanism.

+ve circular RNA strand of a viroid – As a template to make negative Strand.

↓

RNA pol II - make +ve RNA from this long linear molecule.

↓

A host RNAse activity cleaves the +ve strand into unit viroid lengths.

Pathogenesis

- More than one mechanism responsible for viroid pathogenesis.
- Recent evidence suggests that one pathway is due to viroid RNA activating a plant RNA activated protein kinase, or PKR(analogous to the PKR enzyme activated by viral RNAs in mammalian cells).
- Protein synthesis is reduced and this causes pathogenic effects.
- In the case of potato spindle tuber viroid, there is a good correlation between a strains pathogenicity and its ability to activate PKR *in vitro.*

Transmission

- By mechanical breaks-Tools, breaks, insects.
- Biologiquement - Germs, Co-infection

Diseases

- Potato Spindle Tuber (PSTVd)- **1922**
- Avocado Sunblotch (ASBVd)- **1928**
- Tomato Apical Stunt (TASVd)- **1931**
- Coconut CADANG-CADANG (CCCVd)- **1937**
- Chrysanthemum Stunt (CSVd)- **1947**
- Citrus Exocortis (CEVd)- **1948**
- Chrysanthemum Chlorotic Mottle (CChMVd)- **1969**
- Hop Stunt (HSVd)- **1970**

Potato Spindle Tuber Viroid (PSTVd)

Classification:
Unranked: Sub viral agents
Unranked: Viroid
Family: *Pospiviroidae*
Genus: *Pospiviroid*
Species: PSTV*d*

- PSTVd was the **first** viroid to be identified.
- Small, circular RNA molecule closely related to the Chrysanthemum stunt viroid.
- The natural hosts are potatoes (*Solanum tuberosum*) and tomatoes (*Lycopersicon esculentum*).
- All potatoes and tomatoes are susceptible to PSTVd and there is no form of natural resistance.
- The PSTVd viroid has **359 nucleotides.**

Distribution

- Poland and Russia. Also North and South America and parts of north Africa and Asia.

Symptoms

- Leaves of infected plants may have small leaflets,
- Upright spindly growth and a darker, Dull green color;
- Plants can be severely stunted and may show a proliferation of axillary buds.
- Infected tubers are small, elongated, characteristically spindle or dumb-bell shaped, often with prominent bud scales (‘eyebrows’).

Sources

- Infected seed potatoes.
- Through breeding and propagation programs using infected true seed or micro plants.
- Also be introduced by infected tomato seed.

Development

- Highly contagious and is readily transmitted mechanically.
- It may also be spread by chewing insects and by the peach-potato aphid (*Myzus persicae*).
- It is also spread by infected pollen.
- Tuber yields may be greatly reduced, depending on the cultivar, the viroid strain.

Avocado Sunblotch (ASBVd) Viroid

- Family : *Avsunviroidae*
- Genus *: Avsunviroid*
- A Viroid is known to be the second smallest genome to a Prion that can cause a plant disease.
- Pest Avocado sunblotch viriod (ASBVd) is not a virus but is a graft transmissible viral-like pathogen now described as a Viroid.
- In the 1970s The National Agricultural Research Centre in Maracay, Venezuela determined that the causal agent of ASBVd.

Morphology

- single stranded, small, circular RNA molecule.
- Does not encode proteins itself.
- Avocado sunblotch viroid lacks a central conserved region (CCR) and possesses a ribozyme activity

Distribution

- USA – California, Florida; Venezuela; Australia, Spain, Peru, South Africa, Israel.

Symptoms

- Yellow spots appear on mature leaves along with consistent and longitudinal pale bands on young leaves.
- Deformity and often white blotching of fruits and leaves are outstanding symptoms of the disease.
- Stems can have necrotic streaks.
- Twigs have a slight yellow, sunken streak that follows the length of the twig.
- Affected trees exhibit unthrifty growth, low production and poor vigour.

Transmission

- Infected bud wood, wounds caused by contaminated tools and infected pollen.
- Avocado fruit can become infected with ASBVd by pollen transmission.
- There is no evidence of insect transmission.
- ASBVd is transmitted by root graphs and is seedborne.

Management

- Application of general cultural practices on a routine basis to maintain sanitation of the orchard will assist to prevent spread of the disease. When pruning trees it is important that pruning tools be disinfected before moving from one tree to another.

Citrus Exocortis (CEVd) viroid

- Causes stunting of plants, shelling of bark
- May be useful to promote dwarfing for agronomic advantage
- Transmitted though stock, graft
- Control by removal of infected plants, detection, clean stock

Chrysanthemum Chlorotic Mottle (CCHMVd) Viroid

- First identified in Akita Prefecture, Japan, from chrysanthemums (Dendranthema grandiflorum)
- Cause yellow leaf mottling and necrosis.
- No noticeable symptoms
- Dected based on their UUUC sequence positions 82–85 in the CChMVd tetra loop.

Hop Stunt (HSVd) Viroid

- Mainly infects hop gardens especially in the northern part of Japan
- Causes a serious disease of hop (Humulus lupulus) and cucumber plant results dwarfing of the plant, shortening of the internodes of the main and lateral bines, yellowing and curling of the upper leaves.
- Transmitted mechanically sensitive to RNase, but not to DNase

Peach Latent Mosaic (PLMVd) Viroid

- Infects peach (*Prunus persica*) .
- The first signs of disorder become apparent 2 years after planting.
- Cause foliation, flowering and ripening; irregularly shaped, flattened, Colorless fruits with cracked sutures and swollen stones.
- Results pink broken lines on the rose-white petals in warm temperatures bud necrosis; induce furrows in the wood(stem pitting).
- Transmitted by grafting, budding and experimentally by razor-slashing.

Prions

- Prions are infectious agents composed exclusively of a single sialoglycoprotein called PrP 27-30.
- They contain no nucleic acid.
- PrP 27-30 has a mass of 27,000 - 30,000 Dalton's and is composed of 145 amino acids with glycosylation at or near amino acids 181 and 197.

Isoforms

- Different forms of the same **Protein – Isoforms.**
- Prions are made of (PrP) protein is found throughout the body, even in healthy people and animals.
- **PrP** found in infectious material has a different structure and is resistant to proteases.
- The normal form of the protein is called **PrP^{C}.** The infectious form is called **PrP^{Sc}** .
- **C** refers to '**cellular**' or 'common' PrP, while the **Sc** refers to '**scrapie**‘.

PrP^{C}

- Normal protein found on the membranes of cells.
- A transmembrane glycoprotein normally found at the surface of certain cells (e.g., neural and hematopoietic stem cells)
- Its secondary structure dominated by alpha helices (probably 3 of them)
- Easily soluble, Easily digested by proteases
- Encoded by a gene designated (in humans) PRNP located on our chromosome 20.

PrP^{sc}

- The abnormal, disease-producing protein.
- The same amino acid sequence as the normal protein; that is, their primary structures are identical but its secondary structure is dominated by beta conformation.
- Insoluble in all but the strongest solvents, Highly resistant to digestion by proteases.
- These molecules bind to each other forming aggregates.

Prion Diseases

- Amyloid - optically homogenous, waxy, translucent glycoprotein; it is deposited intercellularly and/or intracellularly in many human diseases. Such as

Human Prion Disease

- Creutzfeldt-Jakob Disease (CJD)
- Variant Creutzfeldt-Jakob Disease (vCJD)
- Gerstmann-Straussler-Scheinker Syndrome
- Fatal Familial Insomnia
- Kuru

Animal Prion Disease

- Bovine Spongiform Encephalopathy (BSE)
- Chronic Wasting Disease (CWD)
- Scrapie
- Transmissible mink encephalopathy
- Feline spongiform encephalopathy
- Ungulate spongiform encephalopathy

Human Prion Disease

Creutzfeldt-Jakob Disease (CJD)

- 10–15% of the cases of CJD are inherited.
- The disease is inherited as an autosomal dominant.
- The patients have inherited at least one copy of a mutated PRNP gene -“prion protein” gene.
- Most common mutations are
- Change in codon 200 converting glutamic acid (E) at that position to lysine (K) (thus designated "E200K").
- Change from aspartic acid (D) at position 178 in the protein to asparagine (D178N) when it is accompanied by a polymorphism in both PRNP genes that encodes valine at position 129.
- Change from valine (V) at position at position 210 to isoleucine (V210I)

Creutzfeldt-Jakob Disease (CJD)

- CJD - another spongiform encephalopathy that occurs in humans.
- Grafts of Dura mater taken from patients with inherited CJD have transmitted the disease to more than 100 recipients.
- Corneal transplants have also inadvertently transmitted CJD.
- Instruments used in brain surgery on patients with CJD have transmitted the disease to other patients.
- Over 100 people have acquired CJD from injections of human growth hormone (HGH) or human gonadotropins.
- Available through recombinant DNA technology, such disastrous accidents need never recur.

Variant Creutzfeldt-Jakob Disease (vCJD)

- Very rare, Appeared some years after the epidemic of BSE (Mad Cow Disease).
- The cow and human PRNP genes differ at 30 codons.
- The disease acquired from eating contaminated beef.
- Possible sources of person-to-person transmission include growth hormone and blood transfusions.
- All the patients are homozygous for the susceptibility polymorphism of methionine at position 129.
- This change from normal to abnormal prion protein spreads in the brain, where the misshapen protein aggregates in the spaces between brain cells and may produce disease.
- Another human type of TSE, CJD, is similar to vCJD but progresses much more quickly and affects older people.

Gerstmann-Sträussler-Scheinker disease (GSS)

- This prion disease is caused by the inheritance of a PRNP gene with a mutations
    - Leucine instead of proline at position 102 (P102L) or
        - Valine instead of alanine at position 117 (A117V).
- Brain extracts from patients with GSS can transmit the disease to
    - Monkeys and apes
    - Transgenic mice containing a portion of the human PRNP gene.
- Transgenic mice expressing the P102L gene develop the disease spontaneously.

Fatal Familial Insomnia (FFI)

- People with this rare disorder have inherited
    - a PRNP gene with asparagine instead of aspartic acid encoded at position 178 (D178N)
    - the susceptibility polymorphism of methionine at position 129 of the PRNP genes.
- Extracts from autopsied brains of FFI victims can transmit the disease to transgenic mice.

Kuru

- Once found among the Fore tribe in Papua New Guinea whose rituals included eating the brain tissue of recently deceased members of the tribe.
- Since this practice was halted, the disease has disappeared.
- The disease was studied by transmitting it to chimpanzees using injections of autopsied brain tissue from human victims.

Bovine Spongiform Encephalopathy (BSE) or "Mad Cow Disease "

- Epidemic of this disease began in Great Britain in 1985 and before it was controlled.
- Its origin appears to have been cattle feed that
    - Contained brain tissue from sheep infected with scrapie and
    - Had been treated in a new way that no longer destroyed the infectiousness of the scrapie prions.
- The use of such food was banned in 1988 and after peaking in 1992, the epidemic declined quickly.

Scrapie

- This disease of sheep (and goats) was the first TSE to be studied.
- It seems to be transmitted from animal to animal in feed contaminated with nerve tissue.
- It can also be transmitted by injection of brain tissue

Miscellaneous Infectious Prion Diseases

- A number of TSEs have been found in other animals.
    - Cats are susceptible to Feline Spongiform Encephalopathy (FSE)
    - Mink are also susceptible to a TSE.
    - Even though mad cow disease has not been seen in North America, a similar disease is found in elk and mule deer in the Rocky Mountains of the U.S.

Sporadic Prion Diseases

- CJD and FFI occasionally occur in people who have no family history of the disease and no known exposure to infectious prions. The cause of their disease is uncertain,
    - Perhaps a spontaneous somatic mutation has occurred in one of the PRNP genes in a cell.
    - Perhaps their normal PrPC protein has spontaneously converted into the PrPSc form or perhaps the victims were simply unknowingly exposed to infectious prions, and sporadic prion diseases do not exist!

Other Pathogenic Prion-like Proteins

- Deposits of PrPScin the brain are called amyloid.
- Amyloid deposits are also found in other diseases involving the brain.
- Alzheimer's disease is characterized by amyloid deposits of the peptide amyloid-beta (Aβ) the protein tau in the brain.
- The brains of Parkinson's disease patients have deposits of α-synuclein.
- Deposits of the protein hunting tin are found in the brains of victims of Huntington's disease.
- Most cells, including neurons in the brain, contain proteasomes that are responsible for degrading Misfolded or aggregated proteins.

Satellite viruses

- A **Satellite** is a sub viral agent composed of nucleic acid that depends on the co infection of a host cell with a helper or master virus for their multiplication.
- Satellite encodes the coat protein in which its nucleic acid is encapsidated it is referred to as a **Satellite Virus.**
- A satellite virus of mimivirus that inhibits the replication of its host has been termed a **Virophage**.
- Cannot multiply itself.
- **Satellitism -** Virus depends on the helper virus for replication, So this virus is called **Incomplete virus.**
- **Virusoids** are circular single-stranded RNAs dependent on plant viruses for replication and encapsidation.
- 220-338 nucleotides long, circular, single stranded and possess a ribozyme activity.

Physicochemical and Physical Properties

- The infectivity is retained when deproteinized with proteases, phenol or detergent.
- Proteins constitute about 75% of the particle weight.
- Viral genome encodes structural proteins and non-structural proteins.
- Virions consist of 1 structural protein(s) located in the capsid.
- **Satellite Tobacco Necrosis**
- The helper virus for Satellite Tobacco Necrosis Virus (STNV) is Tobacco Necrosis Virus (TNV).
- TNV size is 30nm.It is icosahedral plant virus, STNV is obligatory parasite.
- It contains 1239 nucleotides and appear to as a monocistronic mRNA for the synthesis of 22kda coat protein.
- Prior in 1962 certain TNV isolates were known to contain substantial amounts of a smaller virus like particle

- **Bamboo mosaic virus**
    - Family:Flexiviridae
    - Genus:Potexvirus
    - Species:Bamboo mosaic virus
    - SynonymsBoMV

- The virus occurs in the United States of America
- BaMV is a filamentous, flexuous rod, 490 nm in length and 15 nm in width.
- 6366 nucleotides long.
- It is easily mechanically transmitted on contaminated tools used for propagation or harvesting.
- The natural host of this virus is bamboo. It is know to infect at least 12 different species in 7 different genera.
- Symptoms in bamboo include chlorotic mottling/mosaic patterns on the leaves which run parallel with the veins.
- However not all susceptible plants show recognizable symptoms.

Replication

- Replicate via a rolling circle pathway- **Symmetric** and **Asymmetric** models of rolling circle replication are involved.
- **Symmetric**
- The multimeric positive sense RNA first cleaved to unit length and Circularized resulting circular negative sense RNA is formed.
- Then the negative sense RNA act as a template for synthesize new multimeric positive sense precursor.
- **Asymmetric**
- Multimeric negative sense RNA is copied in a multimeric positive sense precursor is formed and then it is cleaved & circularized.
- Satellite RNAs usually have little or no effect in either helper virus accumulation or expression.

Virusoids

- Satellite RNAs exhibit only limited sequence homology with the genomes of their respective helper virus.
- The larger virus contain greater than 1000nt molecules.
- The smaller satellite virus contain less than 400nt molecule.
- A virusoid genome does not code for any proteins, but instead serves only to replicate itself.

Genome Structure

- Satellite RNAs exhibit only limited sequence homology with the genomes of their respective helper virus.
- The larger virus contain greater than 1000nt molecules.
- The smaller satellite virus contain less than 400nt molecule.

- Diseases and Virusoids:
- Barley yellow dwarf virus: Helper virus-luteo virus.
- Tobacco ring spot virus: Helper virus-nepovirus.
- Subterranean clover mottle virus: Helper- sobemo virus.

- **Hepatitis Delta Virus (HDV)**
- HDV was first identified in the 1970s in Australia as a nuclear antigen, the delta antigen.
- This virus has a circular single stranded RNA genome of about 1700 nucleotides.
- It was found to be the cause of a particularly virulent form of hepatitis known as type D hepatitis.
- In the West, transmission is associated with drug abuse and transfusion of blood products.
- No specific treatment.
- The delta antigen is associated with a defective pathogen which is obligatorily associated with Hepatitis B helper virus.

- The genomic strand is 70% Watson Crick base paired and rod like in gross structure.
- The delta antigen is a 22kd nuclear phosphoprotein essential for replication and particle formation.
- Two forms are made differing by 19 amino acids at the C-terminus.
- Dominant inhibitor of genome regulation and directs genome packaging into HBV virus particles.
- In fact sequence similarities suggest it is a cellular homologue of the delta antigen.
- This suggests that it may be able to modulate viral replication.

The genomic strand transferred onto the mRNA for the cell homologue of the delta antigen.

↓

This was copied into the antigenomic strand and stabilize as part of the genome.

↓

They replicate, spread, and if appropriate, form particles

Summery of Differences among Sub viral Particles

- **Satellite viruses** are by nature defective, that is they do not have a related replication competent virus.
- These viruses are present in cells infected by another unrelated virus, which acts as helper.
- Good example of this would be AAV which requires adenovirus as ahelper, but the virus codes for its own unrelated coat protein.

Summery of Differences among Sub viral Particles

- **Virusoids** are satellite of certain plant viruses and are encapsidatedwith their helper RNAsin the virions, Structurally resemble viroids.
- Non-infectious and only replicate with viral helper.
- Sequence domains similar to viroids and introns.
- **Satellite RNA** needs helper virus for replication and are encapsidatedin coat of helper virus.
- May code for two or three proteins enhance pathogenicity of the helper virus.

Antiviral Agents

Introduction

- Antiviral drugs (Like antibiotics for bacteria) are a class of medication used specifically for treating viral infections..
- It do not destroy their target pathogen; instead they inhibit their development.

Classes of Antiviral Agents

- Non-nucleoside reverse transcriptase inhibitors (NNRTI)
- Nucleoside reverse transcriptase inhibitors (nucleoside analogs) (NRTI)
- Protease inhibitors (PIs)
- Nucleotide analog reverse transcriptase inhibitors (NtARTIs or NtRTIs)

- Each class of drug attacks a different phase in the life cycle of the virus.

NNRTI - NNRTIs bind to reverse transcriptase, blocking DNA polymerase required to convert viral RNA to DNA.

- Ex. Nevirapine [Viramune®], Delavirdine [Rescriptor®], Efavirenz [Sustiva® and Stocrin®]

NRTI - NRTIs interrupt the formation of viral DNA from RNA by substituting a look-alike compound (analog) that resembles nucleosides (building blocks) used by the virus to synthesize DNA. DNA cannot be synthesized from the look-alike compound and the virus is blocked from replicating**.**

- Ex. Zidovudine, Didanosine, Zalcitabine, Stavudine, Lamivudine, Abacavir, Emtricitabine

PIs – The enzyme that cuts viral protein into segments and assembles the segments around viral RNA to form infectious viral particles. Inhibition of protease results in non-infectious virus particles.

Ex: Amprenavir, Fosamprenavir, Indinavir, Ritonavir, Saquinavir, Nelfinavir.

NtARTIs or NtRTIs - Whereas nucleoside analogs are converted into nucleotide analogs during cellular metabolism, NtARTIs do not require cellular activation. NtARTIs result in less toxicity than NRTIs.

Ex: Tenofovir, Adefovir.

ANTIVIRAL TARGETTING

- The targets should also be common across many strains of a virus, or even among different species of virus in the same family, so a single drug will have broad effectiveness.
- Once targets are identified, candidate drugs can be selected, either from drugs already known to have appropriate effects, or by actually designing the candidate at the molecular level with a computer-aided design program.
- The target proteins can be manufactured in the lab for testing with candidate treatments by inserting the gene that synthesizes the target protein into bacteria or other kinds of cells.
- The cells are then cultured for mass production of the protein, which can then be exposed to various treatment candidates and evaluated with "rapid screening" technologies.

Drug	Viruses	Chemical Type	Target
Vidarabine	Herpesviruses	Nucleoside analogue	Virus polymerase
Acyclovir	Herpes simplex (HSV)	Nucleoside analogue	Virus polymerase
Gancyclovir and Valcyte ™ (valganciclovir)	Cytomegalovirus (CMV)	Nucleoside analogue	Virus polymerase (needs virus UL98 kinase for activation)
Nucleoside-analog reverse transcriptase inhibitors (NRTI): AZT (Zidovudine), ddI (Didanosine), ddC (Zalcitabine), d4T (Stavudine), 3TC (Lamivudine)	Retroviruses (HIV)	Nucleoside analogue	Reverse transcriptase
Non-nucleoside reverse transcriptase inhibitors (NNRTI): Nevirapine, Delavirdine	Retroviruses (HIV)	Nucleoside analogue	Reverse transcriptase
Protease Inhibitors: Saquinavir, Ritonavir, Indinavir, Nelfinavir	HIV	Peptide analogue	HIV protease
Ribavirin	Broad spectrum: HCV, HSV, measles, mumps, Lassa fever	Triazole carboxamide	RNA mutagen
Amantadine / Rimantadine	Influenza A strains	Tricyclic amine	Matrix protein / haemagglutinin
Relenza and Tamiflu	Influenza strains A and B	Neuraminic acid mimetic	Neuraminidase Inhibitor
Pleconaril	Picornaviruses	Small cyclic	Blocks attachment and uncoating
Interferons	Hepatitis B and C	Protein	Cell defense proteins activated

ANTIVIRAL CHEMOTHERAPY

- Most anti-viral agents have proved of little use therapeutically since the virus uses host-cell metabolic reactions and thus, for the most part, anti-viral agents will also be anti-cell agents.
- Viruses that have large genomes and code for their own replication enzymes. Even so, unfortunately, many anti-virals that are apparently effective in vitro are ineffective in vivo.

Anti-viral drug

- Interfere with a virus-specific function (either because the function is unique to the virus or the similar host function is much less susceptible to the drug)

or

- Interfere with a cellular function so that the virus cannot replicate. To be specific, the anti-viral drug must only kill virus-infected cells. This could be done by restricting drug activation to virus-infected cells.

Anti-viral drug should be

- Water-soluble
- Stable in the blood stream
- Easily taken up by cells

ANTI-VIRAL DRUG SHOULD NOT BE

- Toxic
- Carcinogenic
- Allergenic
- Mutagenic
- Teratogenic

ACYCLOVIR (ACV)

- Synthetic guanine nucleoside analogue.
- ACV triphosphate is the pharmacologically active form of the drug.
- It inhibits herpes DNA polymerase with little effect on the host cell DNA polymerase. It also has some chain termination activity and thereby it behaves as a "suicide inhibitor"

ABACAVIR

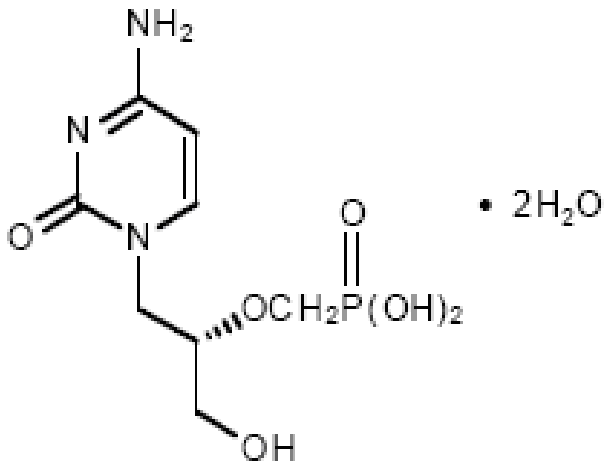
- Abacavir (ABC) is a nucleoside analog reverse transcriptase inhibitor (NRTI) used to treat HIV and AIDS.
- Trade name Ziagen and combination with Trizivir and Kivexa/Epzicom.
- The main side effect is hypersensitivity, which can be severe, and in rare cases, fatal. Genetic testing can indicate whether an individual will be hypersensitive; over 90% of patients can safely take abacavir.

AMANTADINE

- Organic compound, formally as 1-aminoadamantane. The molecule consists of adamantane backbone that is substituted at one of the four methyne positions with an amino group.
- Amantidine is only effective against influenza A.
- Amantidine can occasionally induce mild neurological symptoms such as insomnia, loss of concentration and mental disorientation.
- Rimantadine is not as effective as amantadine but is less toxic. One factor that limits the usefulness of amantidine and rimantidine is the rapid development of resistance of these molecules in 30% of patients.
- Trade name "Symmetrel" for use both as an antiviral and an antiparkinsonian drug.

CIDOFOVIR

- Injectable antiviral medication for the treatment of cytomegalovirus (CMV) retinitis in patients with AIDS.
- It suppresses CMV replication by selective inhibition of viral DNA polymerase and therefore prevention of viral replication and transcription.
- It is an acyclic nucleoside phosphonate, and is therefore independent of phosphorylation by viral enzymes, in contrast to, for instance, acyclovir.



DIDANOSINE

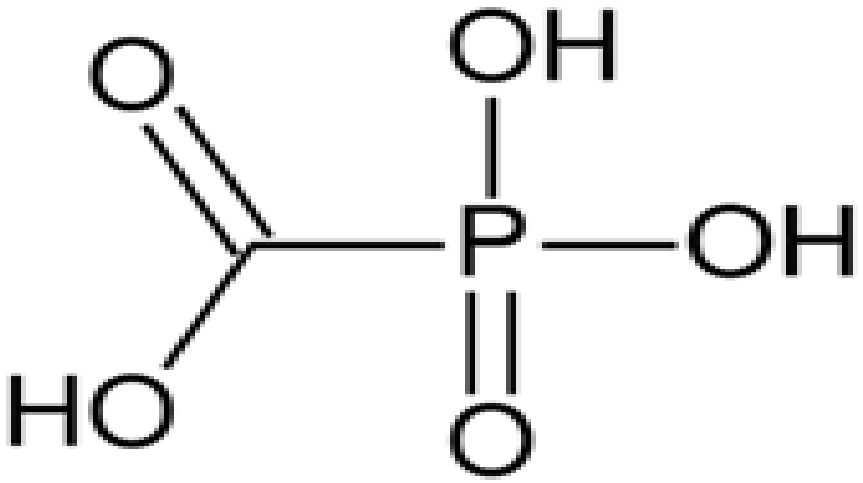
- Didanosine (2',3'-dideoxyinosine, ddI, DDI). Trade names Videx and Videx EC.
- It is a reverse transcriptase inhibitor, effective against HIV and used in combination with other antiretroviral drug therapy as part of highly active antiretroviral therapy (HAART).

RIBAVIRIN

- Not a pyrimidine or a purine.
- Inhibits influenza RNA polymerase non-competitively in vitro but poorly in vivo.
- Act as a guanosine analog and inhibit 5' cap formation on mRNA. The cap normally contains methyl guanosine.
- Inhibit the production of infectious polio virus and this virus does not have a methyl guanosine cap; so there must be alternative mechanisms for ribavirin action.
- It is likely that this drug introduces multiple mutations into viral RNA rendering it incapable of a new round of cell infection

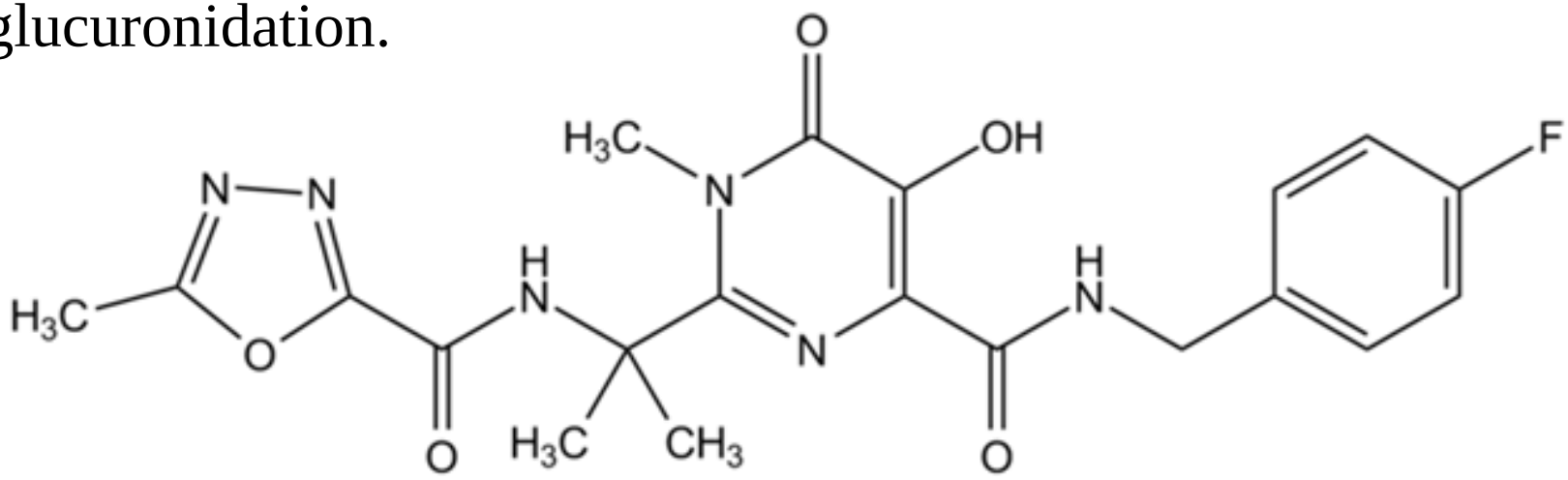
FOSCARNET

- Forscarnet binds directly to the pyrophoshate- binding sites of RNA or DNA polymerases.
- Forscarnet is difficult to use as it must be given continuously intravenously via an infusion pump.
- It is used for the treatment of CMV retinitis in AIDS patients receiving AZT therapy, as it does not have overlapping toxicity with AZT.
- It is also used in the treatment of AZT resistant HSV infections.
- Its major adverse effect is on renal function



RALTEGRAVIR

- Antiretroviral drug produced by Merck & Co., used to treat HIV infection.
- Approved by the U.S. Food and Drug Administration (FDA) in October 2007, the first of a new class of HIV drugs, the integrase inhibitors.
- Raltegravir targets integrase, an HIV enzyme that integrates the viral genetic material into human chromosomes, a critical step in the pathogenesis of HIV. The drug is metabolized away via glucuronidation.

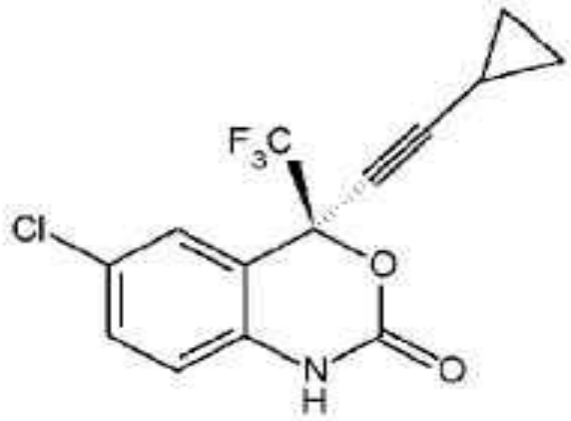


TRIFLURIDINE

- Mode of action to BVDU and IDU. It also is activated by viral thymidine kinase.
- TFT is used as a topical cre.am or in eye drops for HSV keratitis.

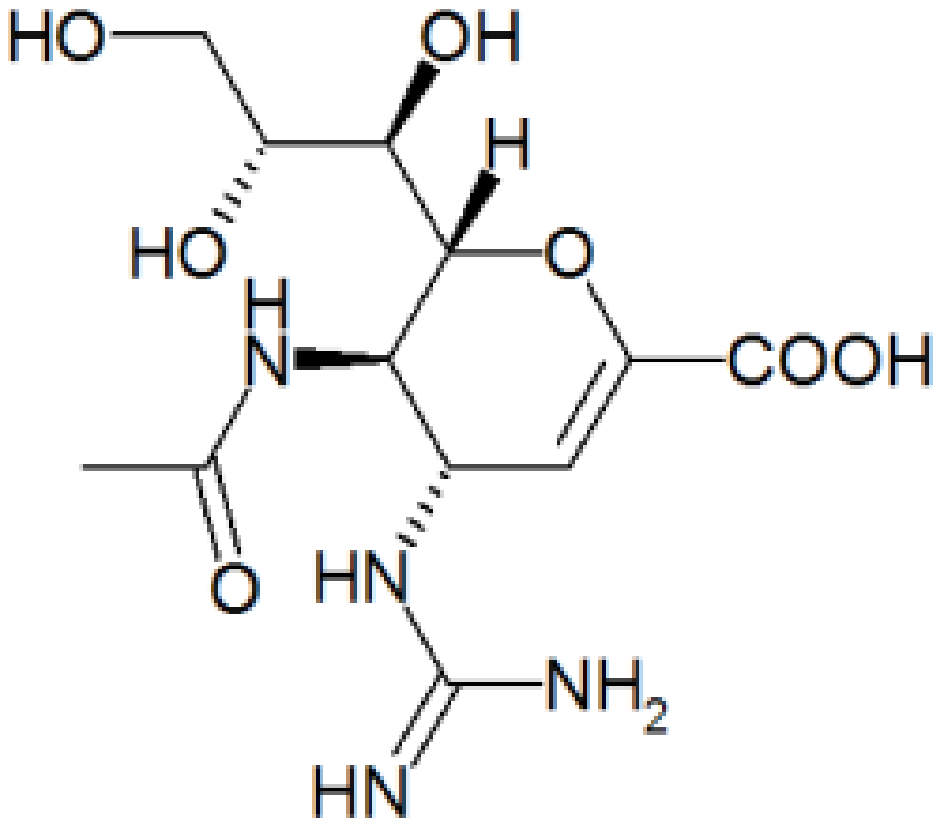
EFAVIRENZ

- Used in combination with other drugs, can suppress viral load at least as well as the protease inhibitor Indinavir and combination with nucleoside reverse transcriptase inhibitors.



ZANAMIVIR

- A neuraminidase inhibitor used in the treatment and prophylaxis of Influenza virus A and Influenza virus B.
- Zanamivir was the first neuraminidase inhibitor commercially developed. It is currently marketed by GlaxoSmithKline under the trade name Relenza.



ZIDOVUDINE

- Zidovudine (INN) or azidothymidine (AZT) (also called ZDV) is a nucleoside analog reverse transcriptase inhibitor (NRTI), a type of antiretroviral drug. It is an analog of thymidine.
- AZT was the first approved treatment for HIV, sold under the names Retrovir and Retrovis.
- AZT use was a major breakthrough in AIDS therapy in the 1990s that significantly altered the course of the illness and helped destroy the notion that HIV/AIDS.
- AZT slows HIV spread significantly, but does not stop it entirely.
- This allows HIV to become AZT resistant over time, and for this reason AZT is usually used in conjunction with other NRTI's and anti-viral drugs.
- In this form, AZT is used as an ingredient in Combivir and Trizivir, among others.

Vaccines
Introduction

- Vaccine is a biological preparation that improves immunity to a particular disease.
- A vaccine is a suspension of whole (live or inactivated) or fractionated bacteria or viruses that have been rendered nonpathogenic, and is given to induce an immune response and prevent disease.
- The term **“Vaccine”** was coined by Louis Pasteur to commemorate first successful immunization against small pox by Edward Jenner

Properties of a Ideal Vaccine

- Provide long lasting immunity.
- Should induce both humoral and cellular immunity.
- Should not induce autoimmunity or hypersensitivity.
- Should be inexpensive to produce, easy to store and administer.
- Vaccines must also be perceived to be safe.

Types of Vaccine

- Killed Vaccines
- Subunit Vaccines
- Recombinant Vaccines
- anti- Idiotypic Vaccine
- Live Attenuated Vaccines
- Conjugate Vaccines
- DNA Vaccines

Killed Vaccines

- When it is unsafe to use live microorganisms to prepare vaccines, they are killed or inactivated.
- Preparations of the normal (wild type) infectious, pathogenic microorganisms that have been rendered nonpathogenic, usually by treatment with using heat, formaldehyde or gamma irradiation so that they cannot replicate at all.
- Such killed vaccines vary greatly in their efficacy.

Micro organism	Vaccine	Method	Route
Salmonella typhi	TAB	Heat, Phenol, Acetone	SC
Yersinia pestis	Haffine	Formalin	SC
Poliomyelitis	Salk	Foramlin	IM
Hepatitis A	HM 175	Formalin	IM

Advantages

- Safe to use and can be given to immuno deficient and pregnant individuals.
- Cheaper than live attenuated vaccine
- Storage not as critical as live vaccine

Disadvantages

- Since the microorganisms cannot multiply, a large number are required to stimulate immunity.
- Periodic boosters must be given to maintain immunity.
- Only humoral immunity can be induced.
- Most killed vaccines have to be injected.
- Some vaccines such as *Bordetella pertussis* induce ill effects like postvaccinial encephalomyelitis.

Disadvantages

- Anaphylactic reaction to neomycin or streptomycin may occur in (Inactivated Polio Vaccine) recipients.
- Anaphylactic hypersensitivity to eggs may occur in recipients of influenza vaccine.
- Inactivation, such as by formaldehyde in the case of the Salk vaccine, may alter antigenicity.
- Presence of some un-inactivated microbes can lead to vaccine-associated disease.

Live Attenuated Vaccines

- These vaccines are composed of live, attenuated microorganisms that cause a limited infection in their hosts sufficient to induce an immune response, but insufficient to cause disease.
- The strains are altered to a non-pathogenic form. for example, its tropism has been altered so that it no longer grows at a site that can cause disease.
- These vaccines may be given by injection or by the oral route.
- A major advantage of live virus vaccines is that because they cause infection, the vaccine very closely reproduces the natural stimulus to the immune system

Micro organism	Vaccine	Method	Route
Salmonella	Ty21A	Genetically Modified	Oral
Mycobacterium	BCG	Prolonged Subculture	ID
Yellow Fever	17D	Passage in Chick embryo Cells	SC
Chicken Pox	Oka/merck	Human Diploid cell Cultures	SC

Advantages

- Infectious microbes can stimulate generation of memory cellular as well as humoral immune responses.
- Since these can multiply in the host, fewer quantities must be injected to induce protection.
- A single administration of vaccine often has a high efficacy in producing long-lived immunity.
- Multiple booster doses may not be required.
- Whole microbes stimulate response to antigens in their natural conformation. They raise immune response to all protective antigens.
- Some live vaccines can be given orally; such vaccines induce mucosal immunity and IgA synthesis, which gives more protection at the normal site of entry.
- Oral preparations are less expensive than giving injections.
- They can lead to elimination of wild type virus from the community.

Disadvantages

- May very rarely revert to its virulent form and cause disease.
- Live vaccines cannot be given safely to immunosuppressed individuals. Administration of live attenuated
- Vaccines to people with impaired immune function can cause serious illness or death in the vaccine recipient.
- Since they are live and because their activity depends on their viability, proper storage is critical.
- Spread to contacts of vaccinee who have not consented to be vaccinated. In some cases, it turns out be an advantage.

Subunit Vaccines

- Subunit vaccines contain purified antigens instead of whole organisms.
- Such a preparation consists of only those antigens that elicit protective immunity.
- Subunit vaccines are composed of toxoids, subcellular fragments, or surface antigens.
- The effectiveness of subunit vaccines in increased by giving them in adjuvants.
- Adjuvants slow antigen release for a more sustained immune stimulation.

Antigen	Vaccine	Microorganism	Route
Cell wall Polysaccharide	Hib	*Haemophilus influenza b*	IM
Toxoid	Tetanus	*Clostridium tetani*	IM
Microbial proteins	Acellular DTP	*Bordetella pertussis*	IM
Membrane proteins	HbsAg	Hepatitis B	IM

Advantages

- They can safely be given to immunosuppressed people
- They are less likely to induce side effects.

Disadvantages

- Antigens may not retain their native conformation, so that antibodies produced against the subunit may not recognize the same protein on the pathogen surface.
- Isolated protein does not stimulate the immune system as well as a whole organism vaccine

Peptide Vaccine

- Peptide vaccine consists of those peptides from the microbial antigen that stimulates protective immunity.
- Synthetic peptides are produced by automated machines rather than by microorganisms.
- Injected peptides, which are much smaller than the original virus protein, induce an IgG response

Advantages

- If the peptide that induces protective immunity is identified, it can be synthesized easily on a large scale.
- It is safe and can be administered to immuno deficient and pregnant individuals IgG response.

Disadvantages

- Poor antigenicity. Peptide fragments do not stimulate the immune system as well as a whole organism vaccine.
- Since peptides are closely associated with HLA alleles, some peptides may not be universally effective at inducing protective immunity

Conjugate Vaccines

- Conjugate vaccines are primarily developed against capsulated bacteria.
- While the purified capsular antigen can act as subunit vaccine, they stimulate only humoral immunity.
- Polysaccharide antigens are T independent, they generate short-lived immunity.
- Infants cannot mount good T-independent responses to polysaccharide antigens.

Examples

- *Haemophilus influenzae* HiB polysaccharide is complexed with diphtheria toxoid.
- Tetramune vaccine, which combines the tetanus and diphtheria toxoids, whole-cell pertussis vaccine, and *H. influenzae* type b conjugate vaccine.

Recombinant Vaccines

- The vaccines are produced using recombinant DNA technology or genetic engineering.
- Recombinant vaccines are those in which genes for desired antigens of a microbe are inserted into a vector.
- **Different strategies are:**
- Using the engineered vector (e.g., Vaccinia virus) that is expressing desired antigen as a vaccine.
- The engineered vector (e.g., yeast) is made to express the antigen, such is vector is grown and the antigen is purified and injected as a subunit vaccine. Other expression vectors include the bacteria *Escherichia coli*, mutant *Salmonella spp*., and BCG.
- Introduction of a mutation by deleting a portion of DNA such that they are unlikely to revert can create an attenuated live vaccine.
- Live attenuated vaccines can also be produced by reassortment of genomes of virulent and a virulent strains.

Advantages

- Those vectors that are not only safe but also easy to grow and store can be chosen.
- Antigens which do not elicit protective immunity or which elicit damaging responses can be eliminated from the vaccine. Example Cholera toxin A can be safely removed from cholera toxin.

Disadvantages

- Since the genes for the desired antigens must be located, cloned, and expressed efficiently in the new vector, the cost of production is high.
- When engineered vaccinia virus is used to vaccinate, care must be taken to spare immunodeficient individuals.

Example:

- *Salmonella typhimurium* engineered to express antigens of *Vibrio cholerae*.
- Bacille Calmette-Guérin vaccine strain engineered to express genes of HIV-1.

DNA Vaccines

- These vaccines are still in experimental stage. Like recombinant vaccines, genes for the desired antigens are located and cloned.
- DNA can be introduced into tissues by bombarding the skin with DNA-coated gold particles.
- Some muscle cells express the pathogen DNA to stimulate the immune system. DNA vaccines have induced both humoral and cellular immunity.

Advantages

- Very stable, resists extreme temperature and hence storage and transport are easy.
- A DNA sequence can be changed easily in the laboratory.
- The inserted DNA does not replicate and encodes only the proteins of interest.
- Because of the way the antigen is presented, there is a cell-mediated response that may be directed against any antigen in the pathogen.

Disadvantages

- Potential integration of DNA into host genome leading to insertional mutagenesis.
- Induction of autoimmune responses: anti-DNA antibodies may be produced against introduced DNA.
- Induction of immunologic tolerance: The expression of the antigen in the host may lead to specific non responsiveness to that antigen.

Anti- Idiotypic Vaccine

- An antigen binding site in an antibody – **Paratope**
- Reflection of the three-dimensional structure of part of the antigen **– Epitope.**
- This unique amino acid structure in the antibody is known as the **idiotype** - considered as a mirror of the epitope in the antigen.
- Antibodies can be raised against the idiotype by injecting the antibody into another animal.
- When the anti-idiotype antibody is injected into a vaccinee, antibodies (antianti-idiotype antiobodies) are formed that recognize a structure similar to part of the virus and might potentially neutralize the virus.

Advantages

- Antibodies against potentially significant antigen can be produced.

Disadvantages

- Only humoral immunity is produced. There is no cellular immunity and poor memory. Identification and preparation of idiotypes is labor intensive and difficult