VIRUSES - Introduction, structure and classification

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History

- **Watson and Crick, 1956**

  easier to force cell to make large number of a few different small proteins rather than a few copies of a very large protein (information storage by RNA/DNA) stated: nucleic acid in virions was insufficient to code for more than a few protein molecules of limited size

  The only way to build a protein shell was to use the same type of molecule over and over again -hence their theory of identical subunits
The introduction of NEGATIVE STAINING (Brenner and Horne, 1959) revolutionized the field of electron microscopy of viruses.

Potassium phosphotungstate—electron dense salt
Information about the architecture

First high resolution micrographs of negatively stained icosahedral viruses were:
Horne et al., 1959 - adenovirus
Huxley and Zubay, 1960 - turnip yellow mosaic virus
Herpesvirus Particle

HSV-2

All herpesviruses identical morphology

(Linda Stannard, University of Cape Town, S.A.)
OUTLINE

1. What are they?
2. What do they look like?
3. How are they classified?
4. How important are they?
Viruses

- Virus comes from the Latin word for poison

- In two decades HIV has killed >18 million people
Viruses are cellular organisms whose genomes consist of nucleic acid, and which must replicate inside host cells using host metabolic machinery to form a pool of components which assemble into particles called VIRIONS, which serve to protect the genome and to transfer it to other cells.
What is a virus?

- “DNA is just DNA. The only thing that distinguishes viral DNA from host DNA is its expected method of passing into future generations. "Legitimate" host DNA is just DNA that aspires to pass into the next generation via the orthodox route of sperm or egg. "Outlaw" or parasitic DNA is just DNA that looks to a quicker, less cooperative route to the future.” Richard Dawkins, The selfish gene.
Is a virus living?

• viruses do not respire
• they do not move
• and nor do they grow
• however, they do most certainly reproduce

• By older, more zoologically biased criteria, then, viruses are not living. However, this results from a "top down" sort of definition, which has been modified over years to take account of smaller and smaller things.
• If one defines life from the bottom up - that is, from the simplest forms capable of displaying the most essential attributes of a living thing - one very quickly realises that the only real criterion for life is:
  - The ability to replicate
  - and that only systems that contain nucleic acids - in the natural world, at least - are capable of this phenomenon
Virus replication
What is a virion?

- **VIRIONS** are virus particles:
  - INERT CARRIERS of the genome
  - ASSEMBLED inside cells, from virus-specified components:
  - They do not GROW, and do not form by DIVISION.
  - They may be regarded as the **EXTRACELLULAR PHASE** of the virus:
    - they are exactly analogous to "spacecraft"
    - in that they take viral genomes from cell to cell
    - & they protect the genome in inhospitable environments.
OUTLINE

1. What are they?
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smartie
thickness of a 5 cent coin
plant cell
animal cell
bacterium
virus
protein
small molecule
atom
Definitions in viral structure

- **The CAPSID** denotes the protein shell that encloses the nucleic acid. It is built of structural units.
- **STRUCTURE UNITS** are the smallest functional equivalent building units of the capsid.
- **CAPSOMERS** are morphological units seen on the surface of particles and represent clusters of structure units.
- The capsid together with its enclosed nucleic acid is called the **NUCLEOCAPSID**.
- The nucleocapsid may be invested in an **ENVELOPE** which may contain material of host cell as well as viral origin.
- The **VIRION** is the complete infective virus particle
  - **INERT CARRIERS** of the genome
    - Simplest form: Virion structure = nucleic acid contained within a protective protein coat
Virion Architecture

Architecture of virions regardless of host is based on two simple themes:

**Sphere** - normally in the form icosahedron (cubical)
- Best way of producing a shell of equivalently bonded identical structures
- Minimum free energy state
- Strong structure that can enclose a maximal volume

**Helix** - cylindrical shape (spiral staircase)
Helical viral structure II

- Plant virus with this structure are rod shaped and non-enveloped, while animal viruses tend to be long and flexible.

- TMV, 2130 capsomers assemble into a helix, 6kb of RNA genome fits into a groove formed on assembly.

Examples:
- Ebola
- Lassa
- Mumps/Measles
- Rabies
- SARS
An ICOSAHEDRON is composed of 20 facets, each an equilateral triangle, and 12 vertices (corners).

Because of the axes of rotational symmetry is said to have 5:3:2 symmetry.
Consider $T=4$: The minimum free energy solution is to divide each triangle into 4 further triangles and place a subunit at each corner. There will be $12 \times 20$ subunits, i.e. 240 with 12 pentamers and 30 hexamers. They are obviously not equivalent, but 180 are and the remaining 60 are making similar contacts. They are said to be quasi equivalent.
Examples of virus structure

- Herpes simplex
- Adenovirus
- 500 Å
- Rotavirus
- Reovirus
- Bacteriophage PRD1
- Semliki Forest virus
- Bacteriophage lambda
- Human papilloma
- Phi6 nucleocapsid
- Polyoma
- Bacteriophage phiX174
- Human rhinovirus
- Poliovirus
- Cowpea chlorotic mottle
- Parvovirus B19
- IgG
Some animal viruses have special proteins in the penton position

Different to those at the hexon position.

These are frequently used as attachment points for long projections called spikes

e.g. adenovirus
• All animal viruses with helical symmetry have a lipid envelope

• Virions acquire an envelope during maturation through a process termed budding - from cellular membranes

• Proteins in the envelope are viral encoded. Two main types:
  • Glycoproteins that form the projections known as spikes
  • Matrix protein - layer on inside envelope - added rigidity

• No envelope = naked virus, hydrophilic - so are protected from organic solvents
Bacteriophages

T2

T4

bacteriophage T2 by H.-W. Ackermann
Properties of Viruses

- **Small**
  - can pass through 0.22\(\mu\)m filters
  - 20 - 350nm in size

- Totally dependent on living cells for replication & existence
- Possess only one species of nucleic acid, either DNA or RNA. (Can be single stranded or double stranded)

- Have a component for attaching or docking to cells

- Able to take over the host cell to propagate themselves
OUTLINE

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Classification

How are viruses named:

• A universal system for classifying viruses, and a unified taxonomy, has been established by the International Committee on Taxonomy of Viruses (ICTV) since 1966.

• Predictions about replication, pathogenesis

• More than 1,550 virus species belonging to 3 orders, 56 families, 9 subfamilies and 233 genera are recognized by the ICTV
Virus classification

- Hierarchical
- Order

- Family
  - viridae
- Genus
  - virus
- Strain
- Quasispecies
Main criteria used for classification of Viruses

• The type of nucleic acid
• Number of strands of nucleic acid and their physical construction: ss or ds, linear, circular, circular with breaks, segmented.

• Polarity of the viral genome – viral genome acts as mRNA are termed “positive-stranded”. Those where a transcript is made first are termed “negative-stranded”

• Replication strategy
• The symmetry of the nucleocapsid
• The presence or absence of a lipid envelope
The system makes use of a series of ranked taxons, with the:
- **Order** (-virales) being the highest currently recognised.
- then **Family** (-viridae)
  - **Subfamily** (-virinae)
  - **Genus** (-virus)
  - **Species** (eg: tobacco mosaic virus)

**Order:** suffix - virales  
**Families:** suffix - viridae  
**Subfamilies:** suffix - virinae  
**Genera:** suffix- virus  
**Type species:** eg  
Mononegavirales
Paramyxoviridae
Paramyxovirinae
Rubulavirus
Mumps virus
Other examples

Family: Flaviviridae (Flaviviruses)
Genus: Flavivirus eg yellow fever virus
Genus: Hepacivirus - hepatitis C virus

Family: Paramyxoviridae (Paramyxoviruses)
Subfamily: Paramyxovirinae
Genus: Paramyxovirus (parainfluenzaviruses)
Genus: Morbillivirus (measleslike viruses)
Genus: Rubulavirus (mumps virus)
Subfamily: Pneumovirinae
Genus: Pneumovirus (respiratory syncytial virus)
All viruses regardless of the nature of their genomes must use mRNA as the template for the synthesis of proteins.

Baltimore system is based on the relationship between the viral genome and the mRNA used for translation.

- mRNA is defined as the positive strand.
- Other molecules with the same sequence are also designated as positive strands (e.g., DNA).
- Nucleic acid molecules that are the complement of mRNA are designated as negative strands.
The six classes of the Baltimore classification are organised as follows:

- **Class I**: ±DNA
- **Class IIa**: +DNA
- **Class IIb**: -DNA
- **Class III**: ±RNA
- **Class IV**: +RNA
- **Class V**: -RNA
- **Class VI**: +RNA
Baltimore classification system

- Poxviridae. Class I ±DNA: mRNA synthesised in the normal fashion; -DNA as the template
- Parvoviridae. Class IIa +DNA: synthesis involves a dsDNA intermediate
- Class IIb -DNA: synthesis involves a dsDNA intermediate
- Reoviridae. Class III ±RNA: one strand acts as mRNA
- Flaviviridae. Class IV +RNA: can serve as mRNA directly (IVa/b)
- Orthomyxoviridae. Class V -RNA: acts as template for synthesis of mRNA (Va/b)
- Retroviridae. Class VI +RNA: dsDNA molecule needed for replication and expression
Antiviral evasion mechanisms

- Escape interaction (VZV – CNS)
- Latency + restricted gene expression (HSV-LATs)
- Become invisible – immunosuppression (HBV - ↓ transcription)
  - ↓ Ag presentation (Vaccinia)
  - Down regulate host antiviral – associated genes (CMV – MHC Class II)
  - Continuous changing appearance (HIV quasispecies)
- Destroy immune mechanisms (HIV – CD4)
Some Emerging Issues

- New respiratory virus – SARS
  - hMPV
  - Associations with chronic conditions
- Transfusion – HCV
  - Non A non B non C
  - New viruses (GBV, TTV, Sen V)
- Old viruses re-emerging
  - Smallpox
- Zoonoses
  - SARS
  - Rabies
  - Arenaviruses
  - Hantaviruses
Old viruses reemerging

SMALLPOX
Human metapneumovirus (hMPV)

Paramyxoviridae
  Paramyxovirinae
  Pneumovirinae
    Rubulavirus (mumps)
    Paramyxovirus (PIV 1,2,3)
    Morbillivirus (measles)
    Pneumovirus
      (hRSV)
      (bRSV)
      (oRSV)
      (PVM)
    Metapneumovirus (APV)
      (hMPV)
Phylogenetic relationship of hMPV isolates, based on N-gene sequence analysis

Prepared using BioEdit3 software suite in combination with TREEVIEW4
ENTEROVIRUSES & DM
New viruses - Zoonoses

SARS CoV
Rabies
Arenaviruses
Hantaviruses
QuickTime™ and a TIFF (LZW) decompressor are needed to see this picture.
Probable cases of SARS by date of onset (n=5,923*)
Worldwide, 1 November 2002 - 16 June 2003

* This graph does not include 2537 probable cases (2522 from Beijing), for whom no dates of onset are currently available.
Source: Ministry of Health, China, WHO
Rabies Virus

Structure of rabies virus (Source: CDC)

Rabies virus particles
Epidemiology

Rabies is a zoonosis which is prevalent in wildlife. The main animals involved differs from continent to continent.

<table>
<thead>
<tr>
<th>Continent</th>
<th>Animals</th>
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<tbody>
<tr>
<td>Europe</td>
<td>fox, bats</td>
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<tr>
<td>Middle East</td>
<td>wolf, dog</td>
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<tr>
<td>Asia</td>
<td>dog</td>
</tr>
<tr>
<td>Africa</td>
<td>dog, mongoose, antelope</td>
</tr>
<tr>
<td>N America</td>
<td>foxes, skunks, raccoons, insectivorous bats</td>
</tr>
<tr>
<td>S America</td>
<td>dog, vampire bats</td>
</tr>
</tbody>
</table>
Management and Prevention

- **Pre-exposure prophylaxis** - Inactivated rabies vaccine may be administered to persons at increased risk of being exposed to rabies e.g. vets, animal handlers, laboratory workers etc.

- **Post-exposure prophylaxis** - In cases of animal bites, dogs and cats in a rabies endemic area should be held for 10 days for observation. If signs develop, they should be killed and their tissue.

- Wild animals are not observed but if captured, the animal should be killed and examined. The essential components of postexposure prophylaxis are the local treatment of wounds and active and passive immunization.

- Once rabies is established, there is nothing much that could be done except intensive supportive care. To date, only 2 persons with proven rabies have survived.
Arenaviruses

- Enveloped ssRNA viruses
- Virions 80-150nm in diameter
- Genome consists of 2 pieces of ambisense ssRNA.
- 7-8 nm spikes protrude from the envelope.
- Host cell ribosomes are usually seen inside the outer membrane but play no part in replication.
- Members of arenaviruses include Lassa fever, Junin and Macupo viruses.

Lassa fever virus particles budding from the surface of an infected cell. (Source: CDC)
Lassa Fever

- Found predominantly in West Africa, in particular Nigeria, Sierra Leone and Liberia.
- The natural reservoir is multimammate rat (*Mastomys*)
- Man may get infected through contact with infected urine and faeces.
- Man to man transmission can occur through infected bodily fluids and Lassa fever had caused well-documented nosocomial outbreaks.
Clinical Manifestations

- Incubation period of 3-5 days.
- Insidious onset of non-specific symptoms such as fever, malaise, myalgia and a sore throat.
- Typical patchy or ulcerative pharyngeal lesions may be seen.
- Severe cases may develop the following:
  - Myocarditis
  - Pneumonia
  - Encephalopathy
  - Haemorrhagic manifestations
  - Shock
- The reported mortality rate for hospitalized cases of Lassa fever is 25%. It carries a higher mortality in pregnant women.
Rodent Carriers of Hantaviruses

Striped field mouse (*Apodemus agrarius*)

Bank vole (*Clethrionomys glareolus*)

Deer Mouse (*Peromyscus maniculatus*)

Rat (*Rattus*)
Clinical Features of Hantavirus Disease

- The multisystem pathology of HVD is characterized by damage to capillaries and small vessel walls, resulting in vasodilation and congestion with hemorrhages.
- Classically, hantavirus disease consists of 5 distinct phases. These phases may be blurred in moderate or mild cases.
  - **Febrile phase** - abrupt onset of a severe flu-like illness with a erythematous rash after an incubation period of 2-3 days.
  - **Hypotensive phase** - begins at day 5 of illness
  - **Oliguric phase** - begins at day 9 of illness. The patient may develop acute renal failure and shock. Haemorrhages are usually confined to petechiae. The majority of deaths occur during the hypotensive and oliguric phases
  - **Diuretic phase** - this occurs between days 12-14.
  - **Convalescent phase** - this may require up to 4 months.
Major Public Health Issues

Hepatitis C virus

HIV
Hepatitis viruses

- Very different viruses, similar presentations
- Different therapies
- Many other non viral causes
- Diagnosis highly focussed and closed
- Continually emerging agents (HCV SEN-V)
QuickTime™ and a TIFF (LZW) decompressor are needed to see this picture.
<table>
<thead>
<tr>
<th>HEPATITIS VIRUS</th>
<th>ACUTE HEPATITIS</th>
<th>CHRONIC HEPATITIS</th>
<th>FULMINANT HEPATITIS</th>
<th>CIRRHOSIS</th>
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Hepatitis - one syndrome many causes

<table>
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<th>Incubation period (wks)</th>
<th>Chronic infection</th>
<th>Genus</th>
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The incubation period is dose dependent, a larger dose results in a reduced incubation period.
HCV genotypes in Australia

PCR products amplified from 296 HCV infected patients were sequenced and analysed to determine their genotype.

Distribution of genotypes in Australian patients:

- 1a: 40%
- 3a: 35%
- 2b: 5%
- 4c/d: 1%
- Co-infected: 3%
Major Public Health Issues

SCREENING FOR BLOOD BORNE VIRUSES
Brief history of transfusion

1660  Sir Christopher Wren invents hypodermic needle

1832  Saline transfusions for cholera

1834  First human-human BTF

1847  Handwashing [Semmelweiss]
      Germ theory [Lister]
      Widespread use of vaccines

1910  Anticoagulant allows long term storage BTF

1940  Blood banking

1950  <20% pts receive IV therapy

Widespread transfusion
      plasmapheresis
      transplantation
COUGH

The Sult of Clinical Experience Deposes Glyco-Heroin (Smith)
as a Respiratory Sedative Superior to All Presents to the Prognos-
secs of Opium, Morphine, Codiorin and Other Narcotics and which
freed of the toxic or depressing effects which characterize the
latter when given in doses sufficient to reduce the reflex irrita-
tibility of the bronchi, trachea and laryngeal mucous membranes.

THE PROBLEM
of administering drugs to promote sleep in such wise as will give the
onset of deep sleep but will prevent the occurrence of an attack of
trouble at night and with which to combat the most severe cough or the most agitated with

HAS BEEN SOLVED BY
the pharmaceutical compound known as

GLYCO-HEROIN (Smith)

The results attained with Glyco-Heroin (Smith) in the allevia-
tion and cure of cough are attested by numerous clinical studies
that have appeared in the medical journals within the past two years.

Scientifically Compounded. Scientifically Conceived.
GLYCO-HEROIN (SMITH) simply stands upon its merits
before the profession, ready to prove its efficacy to all who
are interested in the advances in the art of