Chapter 6
Viruses: At the Threshold of Life

Objectives: After reading Chapter Six, you should understand…
- How viruses were discovered.
- The structures of viruses and how they differ from bacteria.
- Virus replication and how viruses cause disease.
- How to control viruses.

Viruses have been described at being somewhere between chemicals and living objects. Why?

The discovery and structure of viruses

As we have discussed, between 1880 and 1915 discoveries boomed in the field of microbiology.

Tuberculosis, typhoid fever, syphilis and other diseases were becoming understood.

Methods of food preservation and sanitation against bacterial contamination were improving.

Bacteria were being used in industry to benefit society.

However, research into the causes of diseases such as chickenpox, measles, polio and hepatitis was unproductive.

Why?
Two viruses have been key to the development of virology

1. **Smallpox virus** - More than 300 million people died from smallpox from 1900 to 1978.

How many have died from HIV AIDS?

In the 1500s, Europeans came to the “New World” from the West Indies…and unintentionally brought smallpox virus with them.

Shortly thereafter, over one million Aztec Indians died from smallpox (why?).

Later, British soldiers in North America used smallpox as a bioweapon during the French and Indian Wars.

Soldiers gave blankets that had been used by smallpox patients, as “gifts”, to American Indians with the intent of infecting them with smallpox.

Smallpox epidemics wiped out more than 50% of the affected tribes.
The cause and the first cure

Smallpox is caused by the variola virus that emerged in human populations thousands of years ago.

Highly contagious and spreads from person to person primarily by:

(i) droplets or aerosols expelled from the throat of infected persons, and

(ii) direct contact.

The infectious dose is unknown, but is believed to be only a few virons.

1796 - Edward Jenner showed that people who were in frequent contact with cows did not get smallpox.

He realized that an infection caused by cowpox, a relative of smallpox, provided protection against smallpox.

Pus from cowpox blisters became the first smallpox vaccination…why did this work?

Cowpox vaccine was first isolated by Jenner from a cow named Blossom, whose hide hangs on the wall of the library at St George's medical school in England.

In the 1960’s and 1970’s in Europe, during a very limited smallpox outbreak, individuals with the infection transmitted smallpox to as many as 10 to 20 other people, called second-generation infections.

These are the worrisome infections. Why?
Except for laboratory stockpiles, smallpox virus has been eliminated due to a massive worldwide eradication program throughout the 20th century.

The last case of smallpox in the United States was in 1949. The last naturally occurring case in the world was in Somalia in 1977.

To this day, smallpox is the only human infectious disease to have been completely eradicated.

In the aftermath of the terrorist events of September, 2001, there is heightened concern that the variola virus might be used as an agent of bioterrorism.

If smallpox was eradicated over 30 years ago due to effective vaccination, then why is the potential devastation of smallpox re-introduction so great?
2. Tobacco mosaic virus (TMV)

Dmitri Iwanowski (1892) researched tobacco mosaic virus, which causes tobacco leaves to shrivel and die.

Iwanowski attempted to determine the nature of the infectious agent. He crushed diseased leaves and filtered the “mush” to separate the solids from liquids.

The filter was designed to trap bacteria, so presumably, the liquid portion was not infective.

**BUT**

The liquid portion was still infectious. What could be happening?

Martinus Beijerinck (1898) repeated Iwanowski’s work and found that even when very dilute, the fluid was still infective.

Clearly the agent that caused TMV persisted despite filtration and was infectious at very low concentrations.

1930s – viruses were finally crystallized.

Once the electron microscope was invented in the 1940s, the tobacco mosaic virus was visualized.

*How does this timeline compare with that of bacteriology?*
The structure of viruses

Many people can name some popular viruses (influenza, HIV) but cannot tell you much about them.

Virus sizes and configurations vary

Viruses are quite different from typical animal, plant, or bacteria cells.

Viruses contain a core of nucleic acid (genome) enclosed in a coat of protein (capsid).
The viral genome is made up of either DNA or RNA, but not both,

BUT

It is not a genome as we think of one, because it is **not a complete set of genes**…which is why viruses need the help of a **host** to function.

The genes contained in the viral genome are limited to those used to build more viruses (structural genes).

The outer coat is called a **capsid** – made up of protein subunits called **capsomeres**.

The capsid protects the virus from:

1. **Physical damage** - Shearing by mechanical forces.

2. **Chemical damage** - UV irradiation (from sunlight).

3. **Enzymatic damage** - Nucleases derived from dead or leaky cells or deliberately secreted by vertebrates as defense against infection.
A **viral envelope** (lipids, proteins and carbohydrates) is *sometimes* present and is similar to a cell membrane.

1. Contains proteins called **recognition factors** that identify it as a viral structure and determine what type of cells the virus will infect.

   **Why might this be important to the host cell?**

2. Protects the virus

3. Aids with host cell penetration.

In order for a virus to infect a host cell, it must be able to **attach**.

Attachment is achieved in some viruses with the help of **spikes** on the envelope.

HIV, influenza are examples of viruses that have spikes.

Smallpox and TMV do not have spikes

Because spikes are so important for infectivity, they are often a target of antiviral chemotherapy.

The mode of action of the **flu drugs** Relenza, Zanamivir and Tamiflu is based on inactivating the viral protein **neuraminidase**, which is a component of the spikes.
Viruses can exhibit various shapes.

1. **Helical** viruses (Ebola virus, TMV) resemble long rods and their capsids are hollow cylinders surrounding the nucleic acid.

   In helical viruses, the capsid is bound to the helical nucleic acid (usually RNA).

   1. Maintains the spiral shape of the virus
   2. Allows flexibility, which allows the virus to withstand shear forces.
2. **Polyhedral** viruses (chickenpox, adenovirus, herpesvirus, poliovirus) are many-sided.

   Usually the capsid is an **icosahedron**.

   In icosahedral viruses, **20 equilateral triangles** of protein are patched together.

   **Why such an odd shape?**
Viral structures are built of repeated identical protein subunits and the icosahedron is the easiest shape to assemble using these subunits.

This saves information space in the viral genome.

3. Complex viruses have complicated structures. For example, many bacteriophages have a polyhedral capsid with a helical tail attached.

Bacteriophages are complex viruses that infect bacteria.

Colored TEM of bacteriophage viruses (red) attacking an *E. coli* bacterium (X64,000).

It is thought that bacteriophages represent one of the most numerous biological entities on Earth. Why?
Creating artificial viruses

Because viruses are relatively simple compared to living organisms, the creation of artificial viruses have been attempted.

In 2002, a molecular geneticist named Eckard Wimmer created the first live, fully artificial virus in the lab (polio virus).

The RNA sequence of polio virus is converted to a DNA sequence.

DNA is synthesized in short fragments and then assembled in the correct order.

DNA is converted with enzymes into RNA, yielding synthetic poliovirus RNA.

The RNA is mixed with “host” cell components that act as if they are infected by reproducing the virus.
Viruses: Replication and Disease

A virus contains only a strand (or a few strands) of nucleic acid surrounded by a protein coat, and possibly an envelope. Therefore, no metabolism can take place inside the virus.

Therefore, outside of the host cell, viruses are largely inert = obligate parasite.

However, once a virus infects a host cell, it causes problems for the host by making multiple copies of itself.

Stages of replication for an enveloped virus

Figure 4.7
1. Replication is dependent on **attachment**.

   Occurs with high specificity...for example;

   **Hepatitis viruses** – only infect liver cells
   **HIV** – only infect immune system cells

   Specificity is largely due to proteins embedded in the **envelope** or **capsid** AND in the cell membrane of host cells.

   In the host = receptor sites, like a lock for the viral key.

   **Spikes** can contain molecules that help the virus to bind.

   Attachment mechanisms are the focus of many **antiviral therapy** efforts.
2. Virus **penetration** – virus (or virus nucleic acid) enters the cell.

Upon attachment, the viral envelope “blends” with the cell membrane.

**Why does this happen so readily?**

Opens the cell membrane so the virus can enter.

---

**Enveloped virus**

**Host cell membrane**

**Envelope “blending” with the host cell membrane**

---

**What about non-enveloped viruses?** How do they integrate into the host?

i. **Phagocytosis** = engulfment by the host cell

OR

ii. Loss of the capsid and **transfer of only the nucleic acid**
3. **Synthesis and assembly** stage - new viruses are built.

If the virus still contains a capsid when it enters the cell, host cell enzymes will break it down (called “uncoating”), releasing the viral nucleic acid.

Then the host cell enzymes are used to build viral enzymes (proteins) encoded by the viral DNA/RNA.

These viral enzymes will, for example:

i. synthesize new viral nucleic acids,
ii. work with host ribosomes to put amino acids together to make more capsids,
iii. break down host cell parts to liberate building blocks to make more viruses.

Other viral genes will direct the host cell to put the viral parts together to make the new viruses.

4. **Release** – viruses exit the host cell (lytic stage).

All of the viral activities in the host cell cause considerable drain on the host.

Eventually, the cell breaks down and the newly-formed viruses are released.

The damage from the breakdown by non-enveloped viruses can often be repaired, but **enveloped** viruses take a greater toll on the host. Why?

As hundreds of viruses are released, the cell can’t keep up with repair of damage.

The time that passes from virus attachment until release is called the **burst time**.

Can be as short as **20 – 40 minutes**, resulting in hundreds to thousands of new viruses per infected cell.
This burst destroys the cell…Why would a virus destroy its host? That doesn’t make sense, does it?

Virus release strategies can be roughly classified as:

(i) **Lytic** - viruses accumulate inside the host cell and exit in a burst, killing the cell, and;

(ii) **Budding** - viruses are produced and released from the host cell gradually.

If all the parameters, such as the rate of virus production, cell life-span and the neutralizing capacity of the antibodies, were the same for lytic and budding viruses, the budding life-strategy would have a large evolutionary advantage. **Why?**

So why would some viruses be lytic, subsequently killing their host?

When lytic viruses exit the cell in a large burst, the hosts antibodies are “flooded” and a larger proportion of virions can escape the immune system and spread to new cells and new hosts, i.e., **safety in numbers**.
How do viruses cause disease…two examples:

1. **Viral hepatitis** – impacts the liver (what is the liver’s function?)

   Viral replication and release cause liver cells to die.

   Liver tissue degenerates

   Chemistry of the liver is greatly compromised.

   i. it cannot process amino acids
   ii. it cannot metabolize proteins, carbohydrates
   iii. bile production decreases
   iv. fat digestion is effected, etc.

Chronic hepatitis B virus (HBV) infection remains a major health burden and the main risk factor for the development of hepatocellular carcinoma worldwide. However, HBV is not directly cytopathic and liver injury appears to be mostly caused by repeated attempts of the host’s immune responses to control the infection.

<table>
<thead>
<tr>
<th>Hepatitis</th>
<th>Route of transmission</th>
<th>Cause</th>
<th>Damage</th>
<th>Viral type</th>
</tr>
</thead>
<tbody>
<tr>
<td>A</td>
<td>Fecal-oral</td>
<td>Poor hygiene</td>
<td>Not permanent</td>
<td>Picornavirus</td>
</tr>
<tr>
<td>B</td>
<td>Body fluids</td>
<td>Unprotected sex, needle sharing</td>
<td>Acute but not permanent</td>
<td>Hepadnavirus</td>
</tr>
</tbody>
</table>
2. Human immunodeficiency virus (HIV)

HIV is a lentivirus that can lead to acquired immunodeficiency syndrome (AIDS), a condition in humans in which the immune system begins to fail, leading to life-threatening opportunistic infections.

It is estimated that AIDS has killed more than 25 million people since it was first recognized on December 1, 1981, making it one of the most destructive pandemics in recorded history.

How does the number of deaths from HIV compare with that of smallpox?

HIV works by destroying T-cells (used to identify and destroy invading antigens).

T-cells also help to limit the number of bacteria that are normally present in/on your body.

As T-cells are destroyed by HIV, the host becomes more and more susceptible to bacterial and viral infections (lungs, brain, intestine and blood) that are normally not harmful.
Natural History of HIV-1 Infection

The natural history of an HIV infection. Opportunistic infections during the course of the disease.
From: http://pathmicro.med.sc.edu/lecture/HIV3.htm
**Why is HIV so successful?**

**Evolution**

The human immunodeficiency virus is one of the fastest evolving entities known. Two factors drive this:

1. It reproduces sloppily, resulting in the accumulation of many mutations during DNA replication.

2. HIV reproduces quickly, as billions of HIV copies can do produced following the infection of a single HIV virion in a single day.

This has resulted in significant evolutionary changes in the virus.

To fight HIV, we must understand its evolution within the human body and then ultimately find a way to control its evolution.

HIV is closely related to other viruses.

SIVs (simian immunodeficiency viruses) that infect primates, and FIVs (the feline strains), which infect cats.

Primates with SIV and wild cats with FIV don't seem to be harmed by the HIV viruses they carry. **Why?**
If scientists can figure out how non-human primates and wild cats are able to live with these viruses, they may learn how to better treat HIV infections or prevent them altogether.

Does HIV evolve during an infection?

Yes – this is one reason why it can be a deadly virus.

Defense against viruses

Why don’t we die from every viral infection?

**Antibodies** – made by your body to attack invading antigens. (what structures of the viruses might they target?)

**T-cells** – destroy infected cells unless the T-cells themselves are infected (e.g. HIV).
Drugs can also help.

Acyclovir (Zovirax) – *inhibits replication of DNA* in the herpes simplex and chickenpox viruses.

Amantadine – *prevents attachment* of influenza viruses to the respiratory track.

Azidothymidine (AZT) – *inhibits replication* of HIV RNA.

Docosanol – (Abreva) - *inhibits attachment* of the herpes simplex virus (HSV) envelope to the host cell, thereby preventing viral entry.

**Virus vaccines**

If antibodies are one of the host’s best defenses against infection, it makes sense that the presence of antibodies before the virus attempts to infect the host would benefit the host.

This is the underlying principle of **vaccines**.

i.e., expose the person to a chemically inert form of the virus and allow the immune system to produce antibodies that circulate throughout the body, ready to defend when the real virus tries to infect.
Three types of viral vaccines exist:

1. **Inactivated viruses** – the viral genome is destroyed, so the virus cannot replicate in the host, but the capsid remains intact.

   Why keep the capsid intact?

   e.g. Polio (Salk vaccine)

2. **Attenuated viruses** – The virus is still active, but at a very low level, so the full symptoms of the disease are not produced.

   e.g. chickenpox, measles, mumps, rubella, nasal flu mist (swine flu), polio (Sabin vaccine).

   Must infect to protect.

   What might be the risk associated with using an attenuated virus?

3. **Genetically engineered vaccines** – Viral proteins are produced in yeast cells and used in the vaccine.

   Low risk of side effects because there is no live virus.

   Used for hepatitis B (50 to 100 times more infectious than HIV)
Viruses as experimental medical tools

Modern biotechnology uses viruses to carry genes into cells, where the genes become activated to serve specific functions.

**Cystic fibrosis** (~30,000 people in the US) – a genetic disorder in which:

(i) glands produce excessively sticky mucus, and

(ii) reduced airway surface hydration leads to decreased mucus clearance.

The mucus clogs the lungs, liver, pancreas, and intestines, makes it difficult to breathe and to digest food properly, and facilitates bacterial infection and progressive obstructive lung disease.

In 1989 scientists identified the gene mutation that causes cystic fibrosis, which led to the hope that CF lung disease could be “cured” by delivering a corrected version of the gene into affected tissues.

Weakened (attenuated) cold viruses (adenoviruses) or influenza viruses are used to carry normal genes into the respiratory track, infect the abnormal cells and convert them to the normal phenotype by delivering the corrected gene.