Viruses and Virus Genetics

Viruses are unique substances. They are composed of a genetic molecule surrounded by a protein coating, and sometimes a membranous "envelope". Viruses cannot replicate themselves, but when they enter a host cell, they are capable of turning the host genetic molecules into virus making "machines".

Adolf Meyer first detected what was ultimately identified as a virus in 1883 when he was studying a disease of tobacco that caused the plants to be stunted and their leaves to have a mottled or mosaic appearance. The disease could be transmitted by a sap extract, but whatever was in the sap extract that caused the disease was too small to be seen in a microscope.

It was first thought that the organism was either a bacterium so tiny that it passed through bacterial-trapping filters or else it was a toxin produced by the bacterium. Since it was demonstrated that the substance could reproduce, it could not be a toxin.

However, the infectious agent could not reproduce outside of the tobacco plant on any known growth medium, nor did it succumb to toxins that normally destroyed bacteria. Beijerinck postulated that the substance was a particle smaller and simpler than bacteria. Stanley confirmed Beijerinck's hypothesis when he crystallized the substance in 1935.

The infectious agent in the sap extract was ultimately called the **Tobacco Mosaic Virus** (proving that biologists sometimes give things sensible names). Even so, the first virus particle was not seen until electron microscopes. Viruses may be as small as 20 nm, which is smaller than a ribosome. Eukaryotic cells are about 1000 times bigger than a typical virus.

Virus Structure – Genetic Molecule and Protein Coating

Genetic Molecule

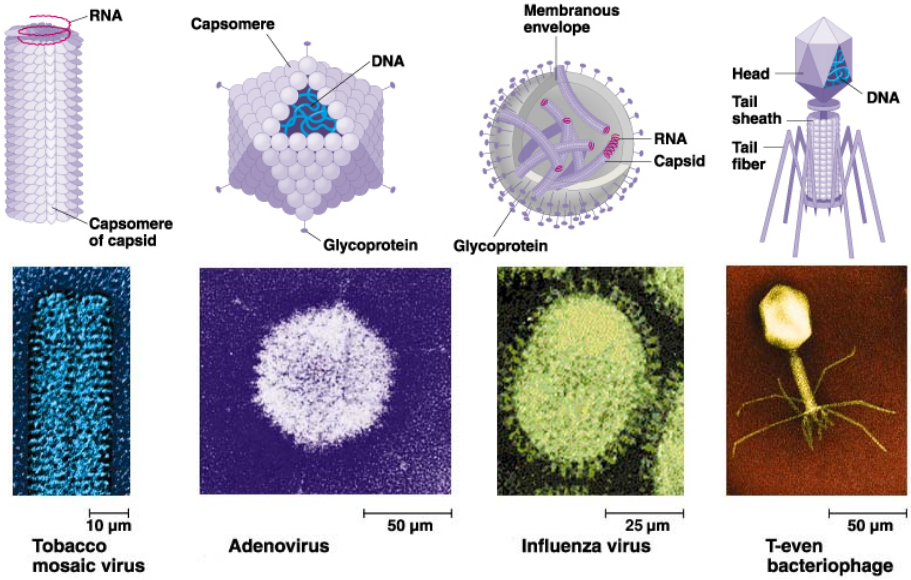
Virus genetic material is more variable than that of living organisms. The virus genetic molecule has from 4 to a few hundred genes and is either a single linear nucleic acid or a circular nucleic acid molecule. It can be:

- Double stranded DNA
- Single stranded DNA
- Double stranded RNA
- Single stranded RNA

Protein Coating

The genetic molecule of the virus is enclosed in a protein shell or coating called a **capsid**. The capsid, composed of protein molecules called **capsomeres**, can be:

- Helical (forming a rod-shaped virus)
- Polyhedral
- Complex
    - With a tail
    - With a membranous coating or **viral envelope** that is derived from host cell membrane with viral proteins and glycoproteins added. Such viral envelopes may enclose a number of individual virus particles. The human flu virus is an enveloped virus that has 8 RNA molecules, each with its own capsids.



Viruses that invade bacteria have historically been called **bacteriophages** or **phages**, and are the most studied. The bacterial host most frequently studied has been *Escherichia coli* (*E. coli*) and the first phages studied with *E. coli* were simply numbered Type 1 -> Type 7 (or T1 -> T7).

Virus Reproduction

Viruses are not considered living organisms because they cannot reproduce by themselves. They are obligate intracellular parasites. Viruses cannot synthesize their own proteins and have no metabolic enzymes. An isolated virus can do nothing except invade its target cell if it, by chance, comes into contact with the appropriate host cell.

Viruses have a narrow range of hosts, generally just one or a few types of cells (or tissues) within the host. Rarely can viruses invade more than one type of host. A virus can identify appropriate host cells just like a substrate fits an enzyme. The virus "key" fits into its host cell's membrane receptor "lock".

Once inside of the host cell, the virus reprograms the host cell into a virus-making cell. All raw materials for making new viruses are provided by the host cell. However, there are a number of variations on this general theme:

- Most DNA viruses enter the host cell, use the host cell's DNA polymerases to replicate the viral DNA, and then use the host's ribosomes for making protein coatings and viral assembly enzymes. After sufficient viral copies are made and new viruses assembled, the host cell is lysed and newly made viruses are freed to infect new cells.

- RNA viruses use special virus encoded polymerases that use the viral RNA as a template for the host cell to make new virus RNA molecules, some of which are mRNA needed to provide directions for making viral coatings, and some are the RNA genetic molecules. Many RNA viruses leave the host cell surrounded by host plasma membrane, and need not lyse the host cell. Many plant viruses are RNA viruses, as are the viruses that cause colds and flu in humans.

- Some RNA viruses are known as **retroviruses.** They use a process called reverse transcription, in which their RNA is used as a template for the host cell to make a DNA strand. That DNA strand functions as a template to make a complement DNA strand forming a double helix that is incorporated into the host cell DNA as a **provirus**. The provirus can get transcribed and is used to make more virus RNA, which can then form new viruses that leave the cell to infect new cells. HIV is a retrovirus.

Some viruses that infect eukaryotes are fully replicated in the host's nucleus, and use nuclear envelope membrane as their coating material. Such viruses may also be incorporated within the host's chromosomes as latent DNA, (a **provirus**), and reactivate during stress situations. The Herpes virus is one example of this kind of virus.

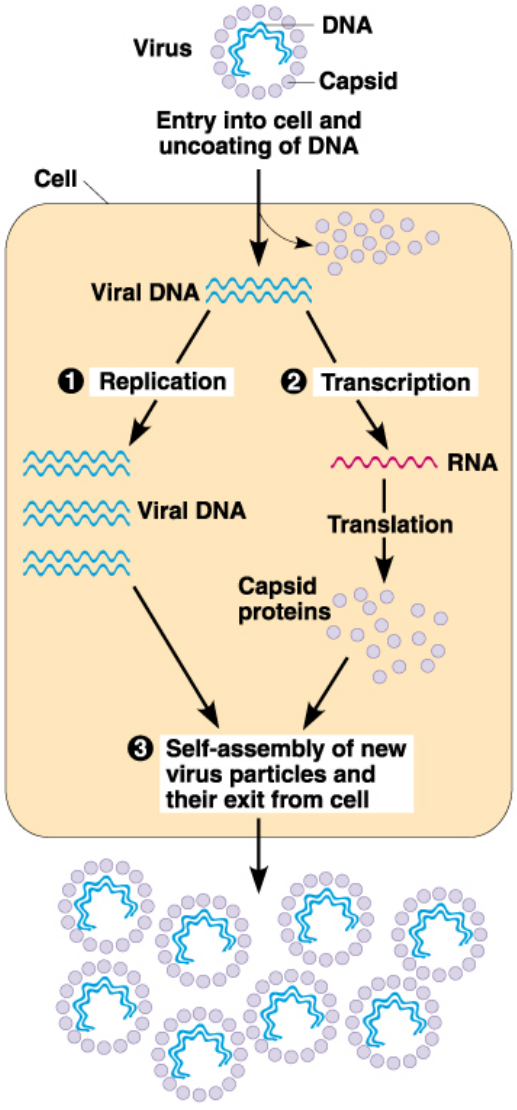
As an aside, it is believed that the origin of the restriction endonucleases (**restriction enzymes**) so important to DNA technology evolved as mechanisms to degrade invading virus DNA molecules.

Virus Cycles – or how something that is not living goes from "generation to generation"

Virus Cycles in Bacteria

Lytic cycle

Many, if not most, viruses destroy the host cell after replication and new virus assembly. This virus cycle is known as a **lytic cycle** and such viruses are lytic viruses. Lytic viruses are **virulent** viruses because they cause cell death.



Lytic Virus Cycle

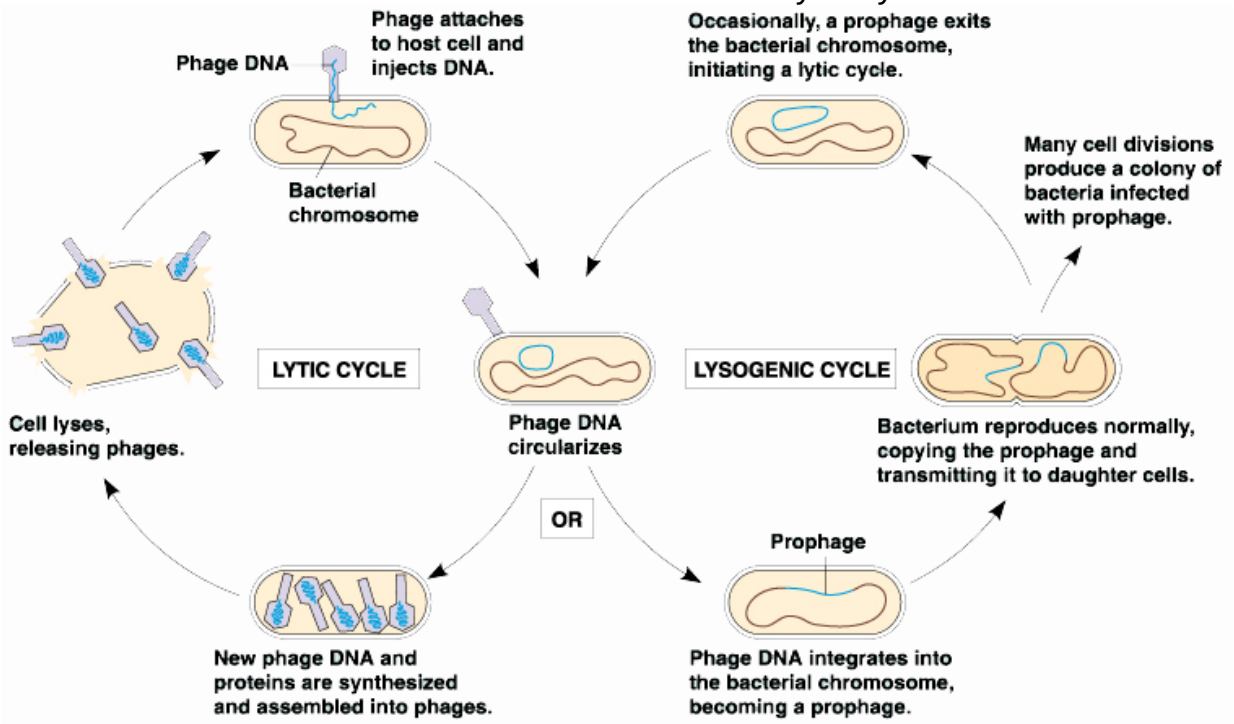
Lysogenic Cycle of Bacterial Viruses

Some viruses have what is called a **lysogenic cycle** rather than a lytic cycle. Lysogenic viruses enter a host cell and join their viral DNA with the host cell's DNA for an unlimited period of time. The virus in this phase is called a **prophage.** (If the virus was doing this in a eukaryotic cell, it would be called a **provirus**.)

The host bacterium will transcribe one gene of the viral DNA that represses the remainder of the prophage's genes from activity. In some cases, the host may transcribe some additional viral DNA, with resulting biochemical and phenotypic changes to the host cell. (Three of our more deadly diseases are caused by toxins coded by prophages within the infectious bacterium - diphtheria, botulism and scarlet fever.) The host cell will also replicate the viral DNA when the host cell divides passing the viral instructions along to new cells.

Temperate Viruses

A **temperate virus** can have both lytic and lysogenic phases. The *E. coli* lambda (l) phage is a temperate phage. Temperate viruses can convert the lysogenic stage into a virulent lytic stage when an appropriate signal is provided. The signal causes the viral DNA to exit the host cell's DNA and activate the lytic cycle.

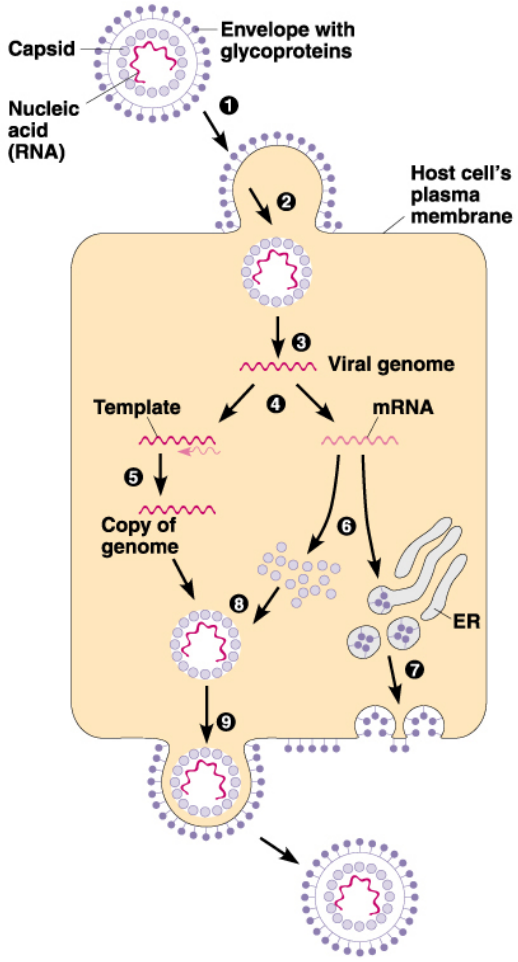


Viruses That Infect Animals

Animal viruses are classified on a number of variables, including the type of genetic molecule (RNA or DNA) and presence or absence of a viral envelope.

Virus Envelopes

Enveloped viruses facilitate the entry of the virus into the host cell. The phospholipid viral membrane can merge with the host cell membrane. Once within the cytosol, the capsid and genetic molecule are dissembled and the viral genetic material directs the host cell to manufacture new viral components. The host ER is used to make new viral glycoproteins that are transported to the plasma membrane in Golgi vesicles. These vesicles merge with the plasma membrane that facilitates the exit of new viruses, complete with a bit of the host plasma membrane as its new viral envelope.



If a double-stranded DNA virus, such as the Herpes virus, replicates within the nucleus, its viral envelope is comprised of nuclear membrane rather than plasma membrane.

Nucleic Acid Component of Animal Viruses

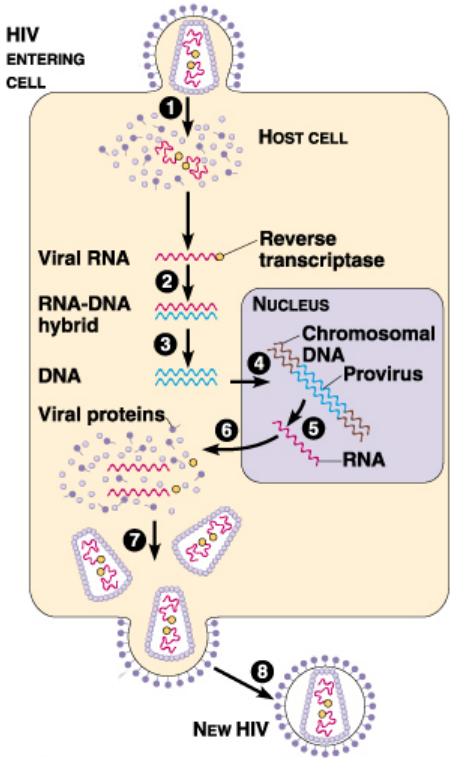
Animal virus nucleic acid can be:

- Double-stranded DNA
- Single-stranded DNA
- Double-stranded RNA
- Single-stranded RNA that functions as mRNA
- Single-stranded RNA that functions as a mRNA template
- Single-stranded RNA retroviruses that function as a DNA template

RNA viruses of Animals

Many animal viruses are RNA viruses. They are classified by how the RNA is active in the host cell.

- Some mRNA viruses can be directly translated at ribosomes.
- Some RNA viruses serve as a template to manufacture mRNA in the host cell, followed by translation. Such viruses have an **incorporated enzyme that catalyzes the RNA -> RNA transcription** process. These RNA enzymes transcribe both new viral RNA as well as mRNA that will be used to synthesize the proteins needed for the new viruses.
- The RNA retroviruses have an enzyme called **reverse transcriptase** that catalyzes the formation of a DNA strand from the RNA of the virus. The DNA formed becomes a **provirus** and then serves as the template to make mRNA, which is then translated to make new virus as well as making new viral genome particles. HIV is a retrovirus



Plant Viruses

Most known plant viruses are RNA viruses. They are responsible for a number of plant diseases and are important in agriculture. Plant viruses can be transmitted in a number of ways:

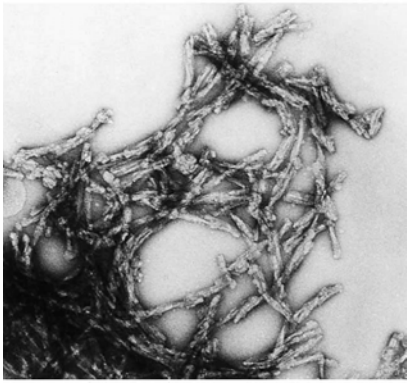
- By direct contact with a virus from the environment, which is easier if the plant is damaged
- Through a vector (most often an insect)
- Transmitted from generation to generation as a lysogenic virus. Some prized tulip variations are caused by lysogenic viruses.

Genetic transmission is called **vertical transmission**. Environmental infection is called **horizontal transmission**. Plant viruses readily pass from cell to cell via plasmodesmata. Cures for plant viruses are virtually unknown. Most efforts are expended on breeding resistant varieties of plants. Some research is focused on trying to minimize the transmission of viruses from plant to plant.

Non Viruses

Viroids are a group of naked circular RNA molecules that infect plants. Viroids cause the infected plant cell to make viroid RNA. The host plant's metabolism that directs plant growth and development is affected and the plant may be stunted and die. A viroid is an **infectious molecule**.

There are **infectious proteins**, called **prions**, notorious for being the proteins that are responsible for "mad cow" disease, human Creutzfeldt-Jakob disease and "mad sheep" disease or scrappie. All are degenerative brain diseases. Stanley Prusiner won the Nobel prize for his work on how prions probably work. A prion is a variant of a normal brain protein that has a different tertiary structure. When it contacts the normal protein, it somehow converts the normally shaped protein into a prion, increasing the number of prion forms rapidly in a chain reaction. However, this hypothesis for prions is not proven. It is possible that prions are coded for by unknown and undetected viral nucleic acids.



Prions

Where Viruses Fit

It is clear that viruses do not have all of the characteristics that we humans have declared are essential to be called a living organism. They can not reproduce themselves, they have no means of providing the energy needed to stay alive (that is they can not synthesize ATP) and outside of the appropriate host they are inert. But viruses have a set of genetic instructions that uses the universal genetic code and even share some of the same genes that living organisms have. Biologists believe that viruses post-date cells because viruses need to invade cells to increase in number.

The favored hypothesis today is that viruses are renegade pieces of DNA (or RNA) that were able to move from cell to cell, much as plasmids can move from bacterium to bacterium and transposons can move from chromosome to chromosome. Support for this hypothesis comes from the fact that any one virus shares more in common with its host than it does with other types of viruses.

What all of these types of particles (viruses, plasmids, transposons) have in common is that they are **mobile genetic elements**. We have interest in viruses because when we study how viral replication can be controlled we gain models for the control of transcription and translation that we can apply to our cells. We also have interest in viruses because they impact our health, and with DNA technology, viruses are often the vector of choice for carrying desired DNA into an eukaryotic host.

Viruses and Human Disease

The ways in which viruses cause the symptoms of disease are as varied as the viruses themselves.

- Some viruses cause lysosomes to release their hydrolytic enzymes, which then destroy the host cell.
- Some induce the host cell to synthesize toxins that cause a disease
- Some have toxic proteins in their envelope

The intensity of a viral infection is related to the host's ability to regenerate and replace the damaged and infected cells as well as the host's immune system to be able to detect the virus and destroy it.

- **Human cold viruses** invade the upper respiratory cells. They are lytic viruses. The symptoms are caused by our immune system's response to the detected foreign substance. Symptoms include fever, inflammation and histamine reactions (the congestion, for example) -- Colds are "cured" because our epithelial tissues replace themselves about every 10 days or so, which coincides with the response time of our immune system to the invading viruses.

- **Chicken pox virus** is the equivalent of a temperate phage. It can invade nerve cells as a lysogenic virus and reside for years. It's original lytic stage concentrates in skin cells where it causes acne-like symptoms. The immune system response takes about 10 days. Chicken pox has few lasting effects. However, if activated in the nerve cells, it causes shingles, rumored to be excruciatingly painful**.**
- **Polio viruses** invade nerve cells that can not be repaired or replaced, so the damage caused prior to our immune system's means of destroying the foreign invader is permanent.
- **HIV** invades critical Helper T-cells of the immune system, which are essential for the body to mobilize the assorted armies needed to ward off any number of foreign invaders. HIV does not kill, per se, but weakens our immune system's ability to do its job so that we succumb eventually to any number of infectious diseases. Some diseases are more common in HIV-compromised individuals.
- As discussed, there are also **cancer-causing viruses**. Liver cancer is related to the Hepatitis B virus, and Burkitt's lymphoma is related to the Epstein-Barr virus. One form of leukemia is linked to a virus as is cervical cancer. There is good evidence that some viruses activate cancer-causing genes (oncogenes). Similar genes exist in non-cancer cells but are not active. These genes are called proto-oncogenes, and some viruses can activate proto-oncogenes. Oncogenes work collaboratively with other carcinogens. They, by themselves, cannot produce cancers.

Treating and Preventing Viral Infections - Vaccines

Viral infections cannot be treated with antibiotics or medications used for infections by bacteria or eukaryotic pathogens. In fact, the only "treatment" for most viral infections is the action of our immune system. We can however, augment the response of our immune system with vaccines. A vaccine triggers the immune system to develop memory cells so that exposure to that virus activates the immune system faster.

A **vaccine** is a deliberate introduction of the antigen to promote development of memory lymphocytes. A vaccine can be a weakened (non-lethal) form of the virus or a weakened toxic by-product of the virus. In either case it will trigger an immune system response without activating symptoms of the viral disease. It usually works. A tiny proportion of the time, individuals will have mild symptoms of the viral infection and with polio, a very few will have more serious symptoms. Boosters are often required to reactivate and ensure a faster response of the memory cells. How often one needs a booster depends on the particular vaccine and virus.

Since HIV, humans have been producing more classes of drugs that inhibit the virus from successfully invading its target cells by enhancing the immune system's methods of locating, targeting and de-activating the virus. There are a few drugs that attack viral genetic function, such as AZT for AIDS.

New Viruses

It seems as if new, lethal viruses have emerged in the world at an unprecedented rate during the past few decades. Whether this is true or not, viruses can evolve just like living organisms.

- RNA viruses in particular can **mutate frequently** because RNA polymerase lacks proof-reading capabilities. Viral mutations can lead to **more deadly forms** of a virus, or mean the virus can **increase its host range** to spread to new species.
- In some cases loss of territory means that organisms that might carry a virus can now be in **more frequent contact** with humans than previously when they were not forced share their habitat with humans.
- **Population growth patterns** (exponential growth) also apply to viruses. A disease that was very, very rare slowly increases in occurrence and then goes around the proverbial J curve and "explodes".
- **Changing environments** can also affect the rate of virus spread.

A Mini-Lecture on the Immune System related to Helper T-cells just in case one is curious:

Specific Immunity)

- Responsible for maintaining tissue environment
- Responsible for destroying specific invaders
- Specific immunity is the function of two groups of wbc (**Lymphocytes**) which "patrol" body and reside in lymph tissues (about two trillion of them)
  - T-cells (T-lymphocytes)
    - Made in bone marrow - mature in thymus (with specific antigen recognition)
  - B-cells (B-lymphocytes)
    - Made and mature in bone marrow (with specific antigen recognition)

How do specific immune cells work?

Primary Immunity

1. The non-specific defense system triggers immune cells via the macrophages , which circulate through the body. These macrophages are responsible for programming specific lymphocytes in the body

2. As circulating macrophages "digest" invaders, the antigen (the substance recognized as foreign, or the "trigger') of the invading substance is transferred to the macrophage surface as a unique **antigen marker**.

   Virgin B-cells may also contact the antigen in while in circulation, and process the antigen. Once a B-cell processes an antigen it becomes a specific B-cell.

3. As antigen-marked macrophages circulate, they come into contact with Helper T-cells.
    - The macrophage secretes interleukin -l
    - Interleukin -l stimulates Helper T-cells to secrete chemicals (B-cell growth factor and interleukin 2) which stimulate:
        - Rapid division of B-cells (sensitized or non-virgin B-cells)
        - Rapid division of Cytotoxic (Killer) T-cells

Specific Immunity Response options

- Anti-body mediated response (extracellular targets)
    - Sensitized B-cells (with the antigen marker) divide rapidly and differentiate into **plasma cells**
        - Plasma cells secrete antibodies
        - Antibodies act in the following ways
            - Neutralizes toxins by coating molecule so it becomes inactive
            - Causes agglutination of foreign cells, so macrophages can consume the clump
            - Can cause antigens to precipitate so macrophages can consume
            - Can activate the Protein Complement system to destroy
    - Some B-cells divide and form memory cells for subsequent attacks. These memory cells reside in lymph nodes until needed.

- Cell-mediated immune response (T-cell activity)
    - Cytotoxic T-cells (or Killer T-cells)

      A few sensitized (marked) T-cells can be stimulated by a helper T-cell to divide rapidly and form an army of **Cytotoxic T-cells.** Killer T-cells work by "punching holes" into target cells (those infected with a virus, damaged, diseased, or a foreign substance) and therefore causing cells to lyse and be destroyed
    - Helper T-cells (60 to 70% of all circulating T-cells)

      Enhance the immune response when activated by antigens by secreting the chemicals noted above.
    - Supressor T cells

      Appear to secrete chemicals that diminish the division of B and T cells as the amount of antigen diminishes (when the job is done)
    - Some T-cells become memory cells needed for a secondary response.....

A first exposure (primary immunity) requires about 6 - 10 (or more) days to be effective. After the first exposure memory cells can activate responses in 1-2 days!