MOVIE SCRIPTS

Microbiology: a clinical approach

SECOND EDITION

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This document contains the transcripts of the voice-over narration for the movies that accompany *Microbiology: A Clinical Approach,* Second Edition.

The movies can be found at http://www.garlandscience.com/micro2

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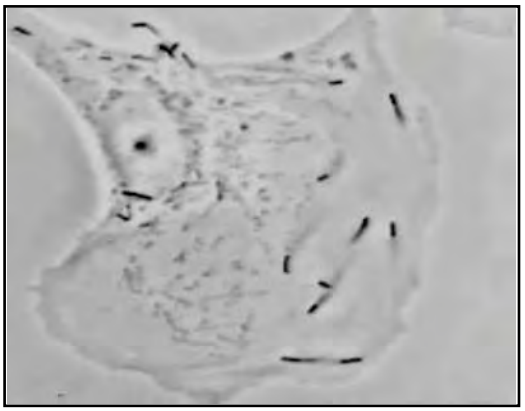
4.1 *Listeria* Infection

This mammalian cell has been infected with pathogenic *Listeria monocytogenes*. These bacteria move throughout the cytosol by recruiting host cell actin which polymerizes and pushes them forward, producing a comet's tail in their wake.

Whenever a bacterium is pushed into the plasma membrane, it creates a temporary protrusion and is then bounced back to continue its random path. If we look closely, we can see a bacterium divide inside the host cell. Immediately after separation, the two daughter cells assemble their own actin tails and start moving about.

These bacteria can also form actin comet tails and move in cell extracts. Here, the bacteria are expressing the green fluorescent protein, and actin is labeled red with a fluorescent dye.

The dynamics of the actin tails, that propel the bacteria through the cytosol, can be modeled, based on known biochemical and physical properties of actin and actin filaments.



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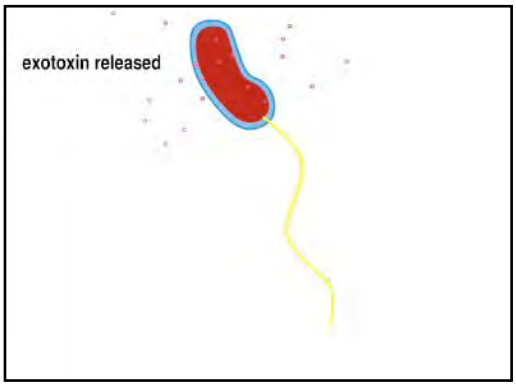
5.1 Cholera Toxin

Vibrio Cholerae is a Gram-negative bacterium that produces a powerful exotoxin. Once in the host's intestine, the bacteria secretes the exotoxin into the surrounding environment.

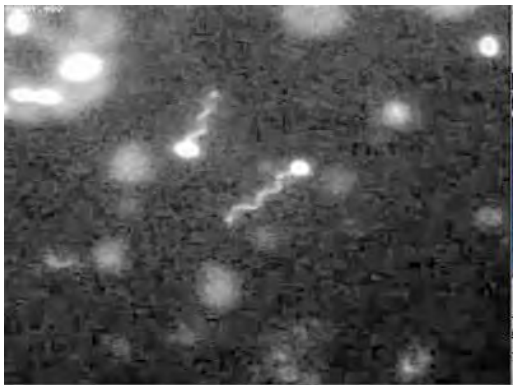
Each exotoxin molecule consists of two parts: an A subunit and a B subunit. The A subunit is made of two parts: A1 and A2. The B subunit allows the exotoxin to bind to membrane proteins on intestinal epithelial cells. Binding of the B subunit stimulates the host cell to engulf the exotoxin by endocytosis.

The vesicle containing the exotoxin fuses with the Golgi apparatus and is transported to the endoplasmic reticulum (the, ER). Inside the ER, the active part of the A subunit (A1) is released into the cytoplasm, while the B and A2 subunits return to the cell membrane where they are released by exocytosis. The A1 subunit activates a host protein, which opens a channel in the cell membrane, causing water and electrolytes to leave the cell, and enter the intestine. This results in severe diarrhea, which is the primary symptom of a cholera infection.



9.1 Bacterial Flagellum

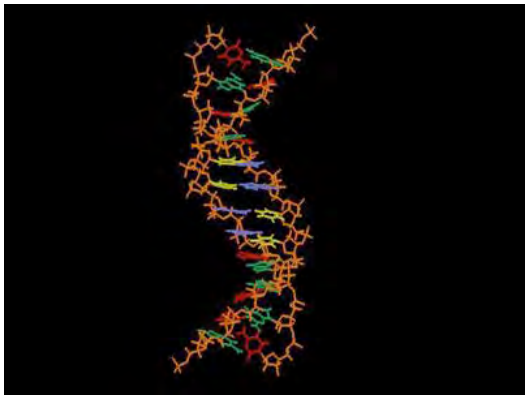
Many species of bacteria propel themselves through their environment by spinning helical motorized flagella. *Rhodobacter* cells have one flagellum each, whereas *E. coli* cells have multiple flagella that rotate in bundles. Each flagellum consists of a helical filament that is 20 nanometers wide and up to 15 microns long and spins on the order of 100 times per second.



Howard C. Berg, Harvard University

11.1 DNA Structure

Two DNA strands intertwine to form a double helix. Each strand has a backbone composed of phosphates and sugars to which the bases are attached. The bases form the core of the double helix, while the sugar–phosphate backbones are on the outside. The two grooves between the backbones are called the major and minor groove based on their sizes. Most protein–DNA contacts are made in the major grove, because the minor groove is too narrow.



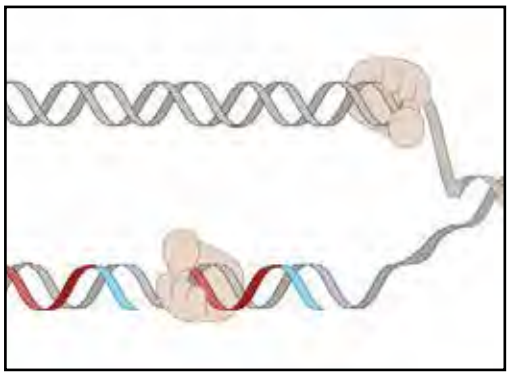
The DNA backbone is assembled from repeating deoxyribose sugar units that are linked through phosphate groups. Each phosphate carries a negative charge, making the entire DNA backbone highly charged and polar. A cyclic base is attached to each sugar. The bases are planar and extend out perpendicular to the path of the backbone. Pyrimidine bases are composed of one ring and purine bases of two rings.

Adjacent bases are aligned so that their planar rings stack on top of one another. Base stacking contributes significantly to the stability of the double helix.

In a double helix, each base on one strand is paired to a base on the other strand that lies in the same plane. In these base pairing interactions, guanine always pairs with cytosine, and thymine with adenine. A GC pair is stabilized by three hydrogen bonds formed between amino and carbonyl groups that project from the bases.

In contrast, an AT pair is stabilized by two hydrogen bonds. The specificity of base pairing—that is, C always pairing with G, and A always pairing with T—ensures that the two strands are complementary. This is important for DNA replication and transcription.

11.2 DNA Replication I



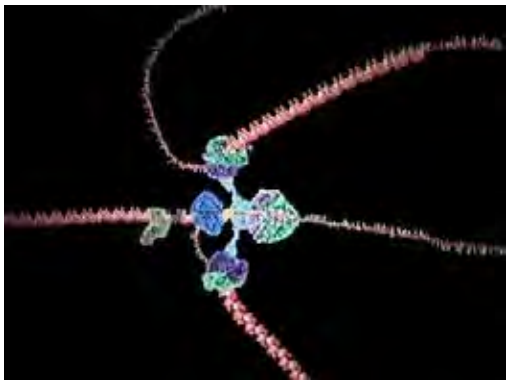
Replication begins when enzymes uncoil the double-stranded DNA molecule and separate the strands. These strands serve as templates for synthesizing new DNA molecules. The area where the strands separate is called the replication fork.

The two template strands are anti-parallel; that is, they are oriented in opposite directions. One strand is oriented in the 5′ to 3′ direction, and called the leading strand template; the other strand is oriented in the 3′ to 5′ direction, and called the lagging strand template.

On the leading strand template, DNA polymerase attaches at an area where a small piece of RNA, called a primer, has been attached to the DNA. As the DNA polymerase moves down the leading strand in the 5′ to 3′ direction, it synthesizes a complimentary strand. This synthesis of a new DNA strand, called replication, proceeds continuously toward the opening replication fork.

Replication of the lagging strand is more complicated because DNA polymerase only works in the 5′ to 3′ direction. Thus, the lagging strand must be completed in segments using a backstitching mechanism. The DNA polymerase begins replicating at an RNA primer attached to the DNA and continues until it reaches the end of the fragment. Each segment of DNA replicated on the lagging strand is called an Okazaki fragment. As the lagging strand is being made, the enzyme DNA ligase will connect the Okazaki fragments together. Though the leading strand is made continuously and the lagging strand is made in Okazaki fragments, the process is simultaneous and continuous and replication of each strand keeps up with the uncoiling of the parental DNA at the replication fork.

11.3 DNA Replication II: Molecular Detail

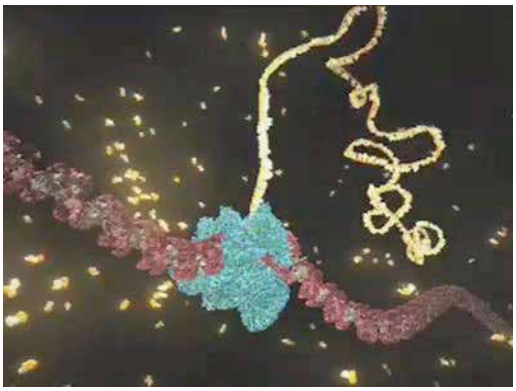


During DNA replication both strands of the double helix act as templates for the formation of new DNA molecules. Copying occurs at a localized region, called the replication fork, which is a y-shaped structure where new DNA strands are synthesized by a multi enzyme complex. Here the DNA to be copied enters the complex from the left. One new strand is leaving at the top of frame and the other strand is leaving at the bottom.

The first step in DNA replication is the separation of the two strands by an enzyme called helicase. This spins the incoming DNA to unravel it at 10,000 rpm in the case of bacterial systems. The separated strands are called 3′ and 5′, distinguished by the direction in which their component nucleotides join up. The 3′ DNA strand, also known as the leading strand, is diverted to a DNA polymerase and is used as a continuous template for the synthesis of the first daughter DNA helix. The other half of the DNA double helix, known as the lagging strand, has the opposite orientation and consequently requires a more complicated copying mechanism. As it emerges from the helicase, the lagging strand is organized into sections called Okazaki fragments. These are then presented to a second DNA polymerase enzyme in the preferred 5′ to 3′ orientation. These sections are then effectively synthesized backwards.

When the copying is complete, the finished section is released and the next loop is drawn back for replication. Intricate as this mechanism appears, numerous components have been deliberately left out to avoid complete confusion. The exposed strands of single DNA are covered by protective binding proteins. And in some systems, multiple Okazaki fragments may be present.

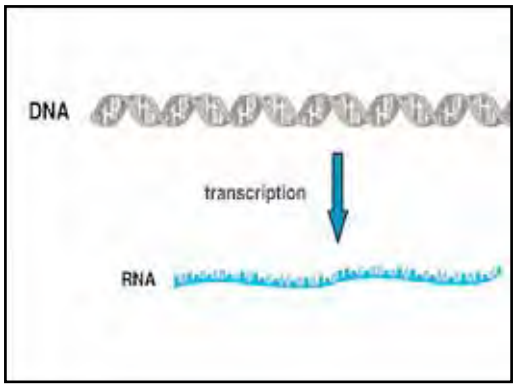
11.4 Transcription



Transcription is the process by which DNA is copied into RNA in the first step of gene expression. It begins with a bundle of factors assembling at the start of a gene, that is, a linear sequence of DNA instructions, here shown stretching away to the left. The assembled factors include an RNA polymerase, the blue molecule.

Suddenly, RNA polymerase is let go, racing along the DNA to read the gene. As it unzips the double helix, it copies one of the two strands. The yellow chain snaking out of the top is the RNA, a copy of the genetic message. The nucleotide building blocks that are used to make the RNA enter through an intake hole in the polymerase. In the active site of the enzyme, they are then matched to the DNA, nucleotide by nucleotide, to copy the As, Cs, Ts and Gs of the gene. The only difference is that in the RNA copy, thymine is replaced with the closely related base uracil, commonly abbreviated "U." You are watching this process, called transcription, in real time.

11.5 Transcription II: Molecular Detail



The genetic information encoded in a gene on a DNA molecule is copied into a single-stranded RNA molecule by the process of transcription.

Transcription is carried out by an enzyme called RNA polymerase. In bacteria, RNA polymerase slides along the DNA until it locates a specific site on the DNA, called the promoter site. At the promoter site, RNA polymerase attaches to the DNA and then separates the two strands of DNA, forming a bubble.

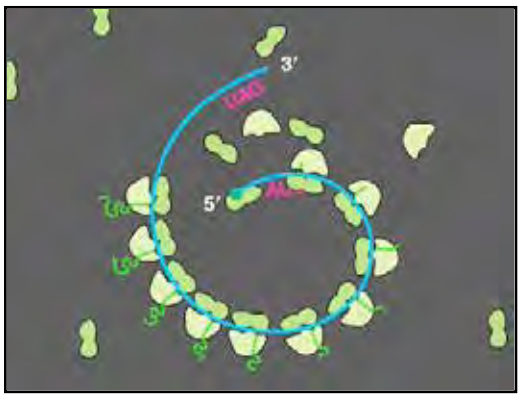
Within the bubble, the polymerase creates a single-stranded RNA molecule by making complementary base pairs to the nucleotides on one strand of the DNA molecule. In this way, the information contained in the DNA is transferred to the RNA being made.

Notice that as the bubble created by the RNA polymerase moves along the DNA, the RNA polymerase separates the DNA strands in front and then reconnects the strands behind. This process continues until the information from the entire gene is transcribed to the RNA.

The transcription process is completed when the RNA polymerase reaches a special terminator site on the DNA. The RNA polymerase disengages from the DNA, releasing the newly made RNA.

11.6 Polyribosome

Ribosomes translate RNA into amino acids. Typically, many ribosomes translate the mRNA simultaneously. Each ribosome begins at the 5′ end of the mRNA and progresses steadily toward the 3′ end. New ribosomes attach to the 5′ end at the same rate as the previous ones move out of the way. These multiple initiations allow the cell to make much more protein from a single message than if one ribosome had to complete the task before another could begin. When a ribosome reaches a stop codon, the ribosome and the new protein dissociate from each other and from the mRNA. This electron micrograph depicts a membrane-bound polyribosome from a eucaryotic cell.



Electron Microscopy:
John Heuser
Washington University in St. Louis

11.7 Translation

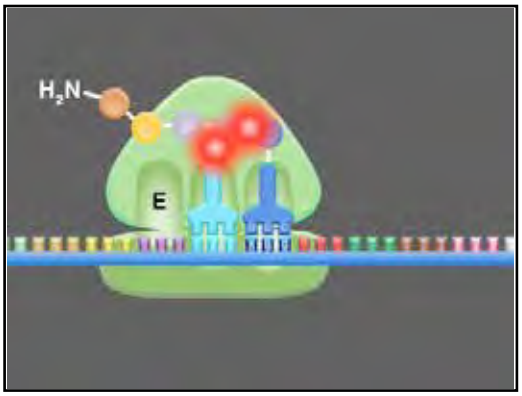
To extend a growing polypeptide chain, the ribosome must select the correct amino acids that are specified by the messenger RNA.

An aminoacyl-tRNA enters the free A site on the ribosome. If the anticodon of the charged tRNA does not match the codon in the messenger RNA, the tRNA is rejected.

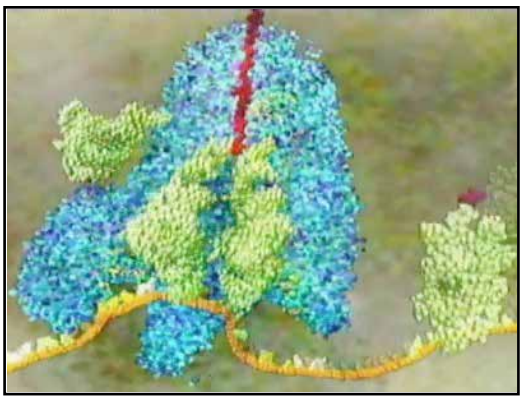
The process of trial and error repeats until the correct tRNA is identified.

If the tRNA is correctly matched and remains bound for a long enough time, it is committed to be used in protein synthesis.

The ribosome catalyzes the formation of the new peptide bond and undergoes a dramatic conformational change. This switches the ribosome back to the state in which it can accept the next incoming tRNA.



11.8 Translation II: Molecular Detail

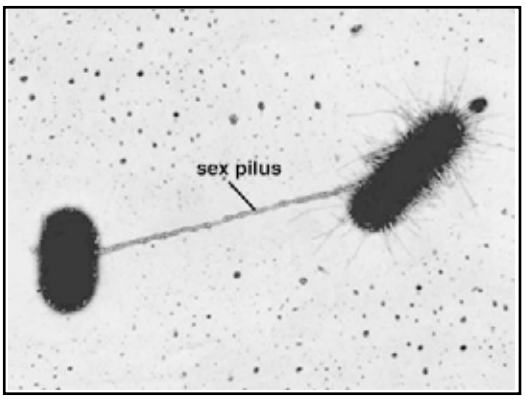


The job of this mRNA is to carry the gene's message from the DNA out of the nucleus to a ribosome for production of the particular protein that this gene codes for. There can be several million ribosomes in a typical eukaryotic cell. These complex catalytic machines use the mRNA copy of the genetic information to assemble amino acid building blocks into the three dimensional proteins that are essential for life. Let's see how it works.

The ribosome is composed of one large and one small subunit that assemble around the messenger RNA, which then passes through the ribosome like a computer tape. The amino acid building blocks, that's the small, glowing, red molecules, are carried into the ribosome attached to specific transfer RNA's, that's the larger green molecules also referred to as tRNA. The small subunit of the ribosome positions the mRNA, so that it can be read in groups of three letters known as a codon. Each codon on the mRNA matches a corresponding anti-codon on the base of the transfer RNA molecule. The larger subunit of the ribosome removes each amino acid and joins it onto the growing protein chain. As the mRNA is ratcheted through the ribosome, the mRNA sequence is translated into an amino acid sequence.

There are three locations inside the ribosome designated the A site, the P site, and the E site. The addition of each amino acid is a three-step cycle. First, the tRNA enters the ribosome at the A site and is tested for a codon, anit-codon match with the mRNA. Next, provided there is a correct match, the tRNA is shifted to the P site and the amino acid it carries is added to the end of the amino acid chain. The mRNA is also ratcheted on three nucleotides, or one codon. Thirdly, the spent tRNA is moved to the E site and then ejected from the ribosome to be recycled. As the protein synthesis proceeds, the finished chain emerges from the ribosome. It folds up into a precise shape, determined by the exact order of amino acids. Thus, the central dogma explains how the four-letter DNA code is quite literally turned into flesh and blood.

11.9 Conjugation



Electron Micrography:
Charles C. Brinton, Jr. and Judith Carnahan

In this electron micrograph, one bacterium is seen contacting another through a protein filament known as a sex pilus. Such contact initiates a type of bacterial mating in which one bacterium—the donor with the sex pili—transfers DNA to a recipient, which lacks sex pili.

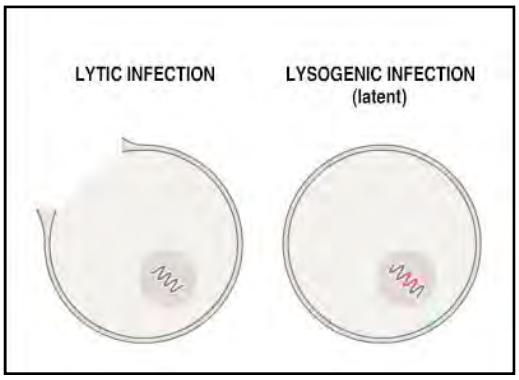
After the initial contact, the sex pilus retracts, reeling in the recipient cell. Thus brought into intimate contact, the two cells form a cytoplasmic bridge to consummate the encounter.

The donor cell contains a circular piece of DNA, called an F plasmid, that is transferred to the recipient. The recipient acquires the F plasmid through a process of DNA nicking and DNA synthesis.

Because the F plasmid contains all the genes required for making sex pili and for transferring the DNA, both resulting bacteria are now covered with the pili and both are potential DNA donors.

The F plasmid can also bring along other genes from the donor's chromosome, thereby allowing for a potentially extensive genetic transfer between the two cells.

12.1 Lytic and Latent Cycles



Viruses can cause two types of infections: lytic and latent. In a lytic infection large numbers of virions are produced and burst out of the host cell. In a latent infection little or no virus is produced but the viral genome is inserted into the host chromosome.

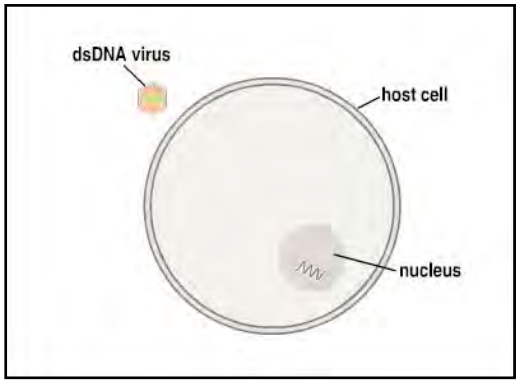
Now we will look at the steps of both infection cycles in greater detail. The lytic cycle begins when a virus binds to the surface of a host cell in a process known as attachment. After attachment, the virus penetrates the host cell and uncoats its viral genome. Through the process of biosynthesis both the viral genome and all the other parts of the virus are reproduced in large numbers. Then all the pieces come together during maturation. When the host cell fills with virions it eventually bursts, releasing large numbers of virions, thus completing the lytic cycle of viral infection. The newly released virions will go on to infect new host cells, and the cycle will continue.

Now we will look at the cycle of a latent infection. In a latent infection, viruses follow the same initial steps as the lytic cycle: that is … attachment … penetration … and uncoating. After uncoating, the viral genome moves into the host cell nucleus, and then integrates into the host cell chromosome. Once integrated, it is referred to as a provirus. The provirus remains integrated in the host cell chromosome. During this time, the virus is said to be latent. That is, inactive but capable of becoming active again.

While the virus is latent, the host cell can continue to reproduce itself through cell division, without making new virions. Although no virions are created, the daughter cells become new hosts with viral genomes integrated into their DNA.

… At some point in the future, when the conditions are right, the viral genome eventually leaves the host chromosome in a process called excision. After excision, the viral genome will enter the remaining lytic cycle of infection: that is … biosynthesis … maturation … and release.

12.2 Double-Stranded DNA (dsDNA Virus Biosynthesis)

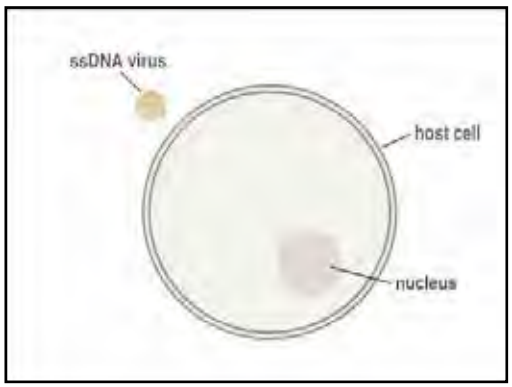


The biosynthesis of double-stranded DNA viruses takes place in the nucleus of the host cell and uses the host cell's machinery. After entering the host cell, the virion uncoats in the cytoplasm, releasing the double-stranded DNA. The double-stranded DNA then moves into the nucleus, where transcription begins. RNA polymerase uses one strand of the viral DNA to transcribe some of the viral genes (the early genes) into messenger RNA, which leaves the nucleus and is translated into viral proteins, known as early proteins.

These early proteins move into the nucleus and interact with the host-cell machinery and viral DNA. The host cell's DNA polymerase replicates the double-stranded viral DNA, producing multiple copies of the original genome. After replication of the viral genome, additional genes of the viral DNA are transcribed (the late genes). This mRNA enters the cytoplasm where it is translated into the capsomere proteins used to assemble new viral capsids. After replication, the newly synthesized double-stranded DNA viral genomes migrate to the cytoplasm, where they are moved into the new capsids, forming complete, intact virions.

12.3 Single-Stranded DNA (ssDNA Virus Biosynthesis)

The biosynthesis of single-stranded DNA viruses takes place in the nucleus of the host cell and involves the formation of double-stranded DNA. After entering the host cell, the virion uncoats in the cytoplasm, releasing the single-stranded DNA, which then moves into the nucleus.

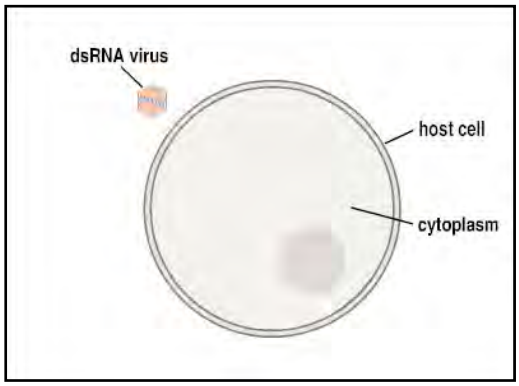


Inside the nucleus, a host cell DNA polymerase synthesizes a complementary DNA strand to form double-stranded DNA. The newly synthesized strand acts as a template for host cell RNA polymerase, which transcribes some viral genes (the early genes) into messenger RNA. This messenger RNA leaves the nucleus and is translated into viral proteins, known as early proteins.

These early proteins move back into the nucleus and interact with the host cell machinery and viral DNA. The viral DNA is further transcribed and the resulting messenger RNA moves into the cytoplasm where it is translated into the capsomere proteins used to assemble new viral capsids. As new capsids are being constructed in the cytoplasm, the newly synthesized strand of viral DNA is used as a template to make new single-stranded DNA viral genomes in the nucleus. After replication, the newly synthesized single-stranded DNA viral genomes migrate to the cytoplasm, where they are moved into the new capsids, forming complete, intact virions.

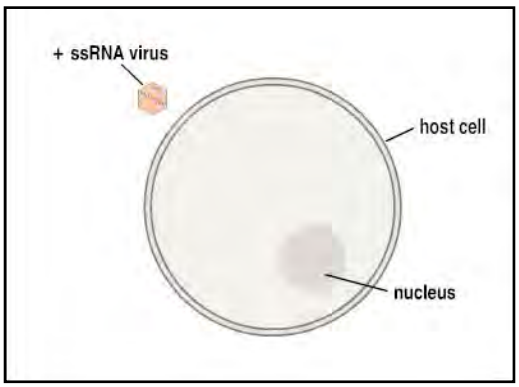
12.4 Double-Stranded RNA (dsRNA Virus Biosynthesis)

Biosynthesis of double-stranded RNA viruses takes place in the cytoplasm of the host cell. After entering the host cell, the virion uncoats in the cytoplasm, releasing the double-stranded RNA and a viral RNA polymerase. The negative strand is used as a template by a viral RNA polymerase to make messenger RNA. The virus must use its own RNA polymerase since host RNA polymerases cannot use RNA as a template. The messenger RNA produced is translated into capsomere proteins used to construct the viral capsid. The newly synthesized mRNA is also used as a template to synthesize a complementary RNA strand, thus making a new double stranded genome, which is incorporated into the capsid to produce complete, intact virions.



12.5 (+) Single-Stranded RNA (+ssRNA Virus Biosynthesis)

Single-stranded RNA viruses contain either a negative RNA strand or a positive RNA strand. In this animation, we will look at the biosynthesis of a positive RNA strand.

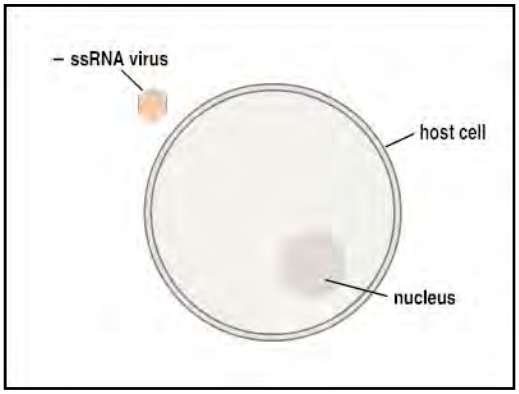


After entering the host cell, the virion uncoats in the cytoplasm, releasing the positive, single-stranded RNA. Since the positive RNA strand is already in the form of messenger RNA, it can be translated immediately into capsomere proteins used to assemble new viral capsids. The viral messenger RNA is also translated into an RNA-dependent RNA polymerase that is used to replicate the viral genome. The virus must make its own enzyme to replicate its genome since host cell RNA polymerases cannot use RNA as a template.

Replication of the positive RNA strand is accomplished in two steps. First, the viral RNA polymerase uses the positive strand as a template to synthesize a complementary negative strand. Second, this newly synthesized negative strand acts as a template to make many positive RNA genomes. The newly made positive strands combine with the new capsids to make new virions.

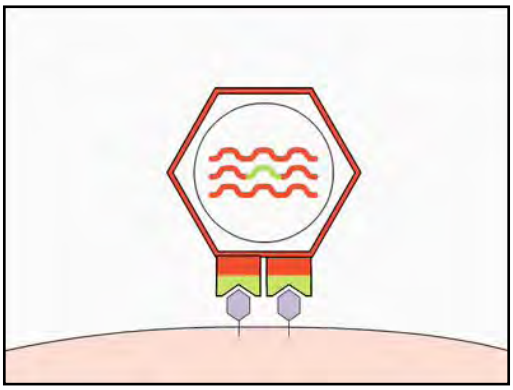
12.6 (-) Single-Stranded RNA (-ssRNA Virus Biosynthesis)

Single-stranded RNA viruses contain either a positive RNA strand or a negative RNA strand. In this animation, we will look at the biosynthesis of a negative RNA strand.



After entering the host cell, the virion uncoats in the cytoplasm, releasing the negative, single-stranded RNA and a viral RNA polymerase. Unlike positive, single-stranded RNA, the negative stranded RNA is not in the form of messenger RNA. So the viral RNA polymerase must first make positive RNA strands. These positive RNA strands are in the form of messenger RNA and can be translated into capsomere proteins used to assemble new viral capsids and other viral proteins. The positive RNA strand is also used as a template to produce new negative RNA strand genomes. The newly made negative RNA strands combine with the capsids and viral RNA polymerase to make new virions.

13.1 Antigenic Drift



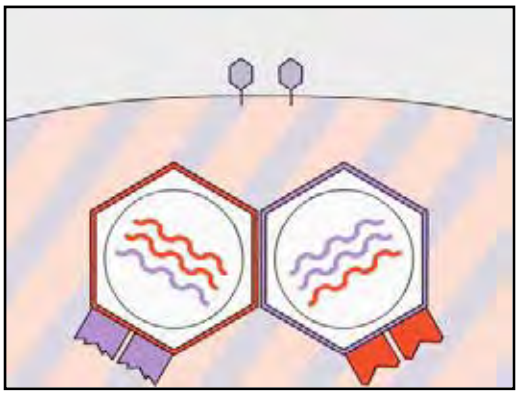
Pathogens, such as the influenza virus, have receptors that enable them to bind to host cell surfaces.

Antibodies to these viral receptors prevent the virus from binding to and infecting cells. These are neutralizing antibodies, since they neutralize the ability of the virus to infect the cell.

However, some viruses will have mutations that alter the receptor in ways that prevent the binding of neutralizing antibodies while leaving the virus able to bind to, and infect, host cells.

In this way the pattern of antigens expressed by a virus can change over time. This process of accumulation of small changes is called antigenic drift, and contributes to our susceptibility to influenza infections year after year.

13.2 Antigenic Shift



Pathogens, such as the influenza virus, have receptors that enable them to bind to host cell surfaces.

Antibodies to these viral receptors prevent the virus from binding to and infecting cells. These are neutralizing antibodies, since they neutralize the ability of the virus to infect the cell.

In some cases, viruses arise that are able to escape the effects of neutralizing antibodies. This can happen when two different strains of influenza virus are able to infect the same host cell.

The progeny viruses produced from such doubly-infected cells can contain segments of genome from either of the two original viruses. Some viruses will acquire a segment of genome from the other strain encoding the receptor for host cell surfaces.

Neutralizing antibodies that block the binding of the original virus will be unable to recognize the receptor from the second strain and will be unable to prevent the virus binding to and infecting host cells. This process, in which large changes in the antigenicity of the virus occur, is known as antigenic shift. These large changes can mean that much of the immunity against the original virus is ineffective, and such antigenic shift mutations are often associated with large-scale virus epidemics. Pathogens, such as the influenza virus, have receptors that enable them to bind to host cell surfaces.

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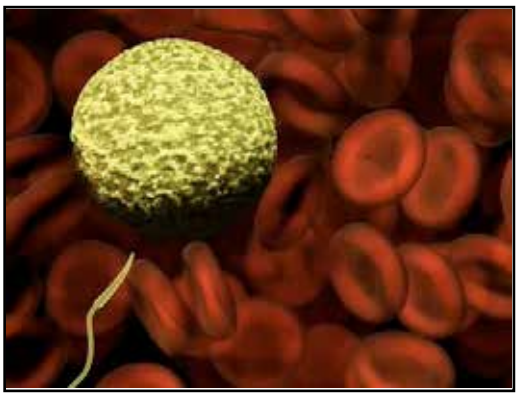
Neutralizing antibodies that block the binding of the original virus will be unable to recognize the receptor from the second strain and will be unable to prevent the virus binding to and infecting host cells. This process, in which large changes in the antigenicity of the virus occur, is known as antigenic shift. These large changes can mean that much of the immunity against the original virus is ineffective, and such antigenic shift mutations are often associated with large-scale virus epidemics.

14.1 Malaria: Mosquito Host

Mosquitos are usually vegetarian, preferring to drink nectar, fruit juices and honey dew. Only a pregnant mosquito will bite humans, seeking nutrients from blood to nourish her developing eggs. If she drinks blood from someone infected with malaria, she too becomes infected with the disease. The tiny drop of blood, filling the insect's stomach is teaming with malaria parasites. The parasite form that is deadly inside humans cannot survive in a mosquito's stomach and is slowly digested with the rest of her blood meal.



Drew Berry
The Walter and Eliza Hall Institute of Medical Research

However, back in the human host a few of the parasites turned into a different type of cell, one that is sexual but remains dormant. Malaria sex is triggered when the warm human blood begins to cool inside the insect's stomach. The female form of the parasite matures into an egg. The male form takes a while longer to mature into sperm. This sperm is from an earlier feed. The fertilized cell can glide, and begins to explore its new environment. It migrates to the outer lining of the mosquito's stomach, before transforming into a cyst. Each cyst produces thousands of thin, tiny parasites, which seek out and infest the mosquito's salivary glands.

The next time this mosquito bites a victim, the malaria parasite will ride in with her saliva and infect another human. This year, 10% of people on earth will be struck down with malaria. Most people who die from the disease will be pregnant women and children under the age of five.

14.2 Malaria: Human Host

The malaria parasite's lifecycle is complex. It moves back and forth between mosquitos and humans, and develops through several stages in each host.

Drew Berry
The Walter and Eliza Hall Institute of Medical Research

This mosquito is infected with the malaria parasite. It has bitten and is feeding on a human host. During the bite, she injects saliva to stop the blood from clotting. Her infected saliva carries malaria sporozoites. The sporozoites ride the bloodstream like a network of roads, seeking their first target: the core of your body's blood filter system, the liver.

Sensing its arrival at the liver, a sporozoite searches for an exit from the blood. It leaves the blood through a sentinel Kuppfer cell, and invades liver cells, killing one or more by necrosis before eventually invading the cell it will infect. Over the next few days, the parasite undergoes hundreds of nuclear divisions, copying its DNA over and over again. This generates tens of thousands of merozoites, which are released from the infected cell and enter the bloodstream.

Merozoites are specialized to infect a new target: red blood cells. Inside a red blood cell, the parasite can hide from the body's immune system. The parasite slowly devours the contents of the infected cell, and creates more merozoites. The infected cell becomes sticky, and grips onto blood vessel walls. Once mature, the infected cell bursts, spreading merozoites through the bloodstream.

Malaria victims suffer fever, loss of blood, convulsions, brain damage and coma. This year, 10% of people on earth will be struck with malaria. Countless millions have been killed by it. Most people who die from the disease are pregnant women, and children under the age of 5.

15.1 Innate Recognition of Pathogens

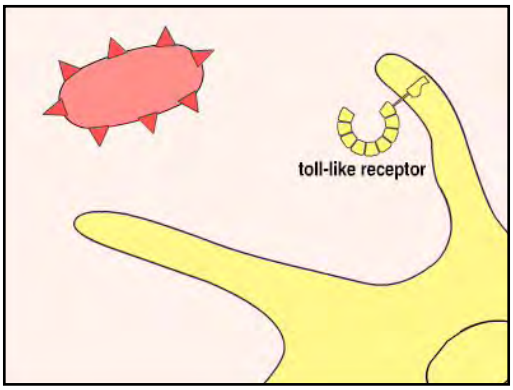
In the initial stages of an immune response, the innate immune system recognizes the presence of pathogens and provides the first line of defense.

Dendritic cells, which are circulating through the tissues, have the ability to recognize the presence of pathogen associated molecular patterns known as PAMPS. PAMPS are conserved features of pathogens, such as the lipopolysaccharides or LPS, which are components of the cell membranes of all Gram-negative bacteria.

Dendritic cells have the ability to recognize PAMPS through the expression of a family of Toll-like receptors called TLRs. In the case of LPS, it is recognized by one member of the TLR family, TLR-4, which is expressed on the surface of the dendritic cell.

LPS is transported by a soluble LPS-binding protein, to the surface of the dendritic cell, and deposited on a cell surface protein. The presence of LPS is detected by TLR-4 through its interaction and recognition of the LPS bound to the surface protein.

The signal delivered by the TLR initiates maturation of the dendritic cell. The dendritic cell can now migrate to regional lymph nodes and activate the adaptive immune response.

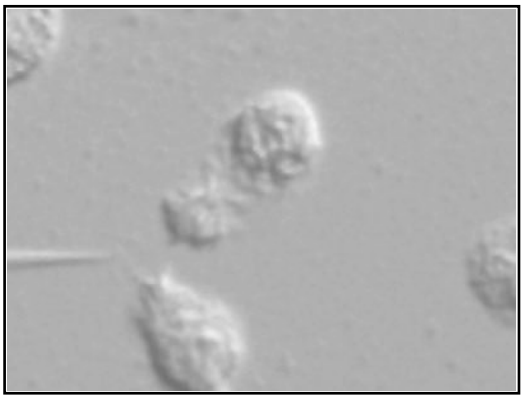


15.2 Chemotaxis of Neutrophils

These human neutrophils, taken from the blood of a graduate student, are mobile cells that will quickly migrate to sites of injury to help fight infection.

They are attracted there by chemical signals that are released by other cells of the immune system or by invading microbes.

In this experiment tiny amounts of chemoattractant are released from a micropipette. When neutrophils sense these compounds they polarize and move towards the source. When the source of the chemoattractant is moved, the neutrophil immediately sends out a new protrusion, and its cell body reorients towards the new location.



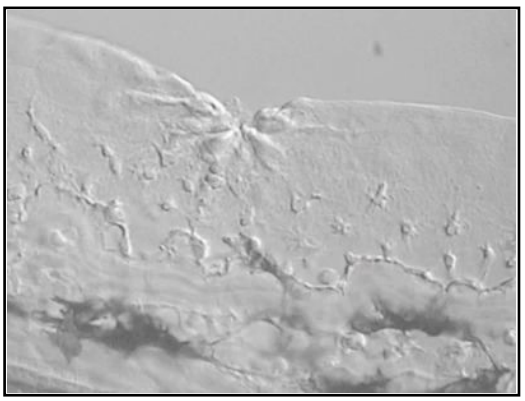
Henry Bourne and John Sedat
University of California, San Francisco

15.3 Leukocyte Homing

To visualize leukocyte homing to a site of injury, a zebrafish larva was anaesthetized and its fin pierced with a needle to introduce a small wound. A vein is seen at the bottom of the frame.

Because the fin is very thin and transparent, we can watch directly as leukocytes crawl out of the blood vessel and migrate towards the wound. They are attracted there by chemicals released from damaged cells, invading bacteria, and other lymphocytes.

In a zoomed out view we can appreciate that leukocyte invasion is restricted to the wounded area. The static cells that are dispersed in the connective tissue are fibroblasts. In these movies, 60 minutes of real time are compressed into 15 seconds.

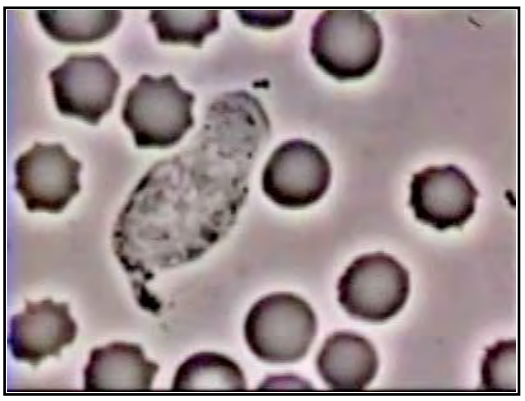


Michael Redd and Paul Martin
University College London

15.4 Neutrophil Chase

Neutrophils are white blood cells that hunt and kill bacteria. In this spread a neutrophil is seen in the midst of red blood cells. *Staphylococcus aureus* bacteria have been added. The small clump of bacteria releases a chemoattractant that is sensed by the neutrophil. The neutrophil becomes polarized, and starts chasing the bacteria. The bacteria, bounced around by thermal energy, move in a random path, seeming to avoid their predator. Eventually, the neutrophil catches up with the bacteria and engulfs them by phagocytosis.

Digital capture: Tom Stossel, Brigham and Women's Hospital, Harvard Medical School
Music: Freudenhaus Audio Productions



David Roger
Vanderbilt University

15.5 Leukocyte Rolling

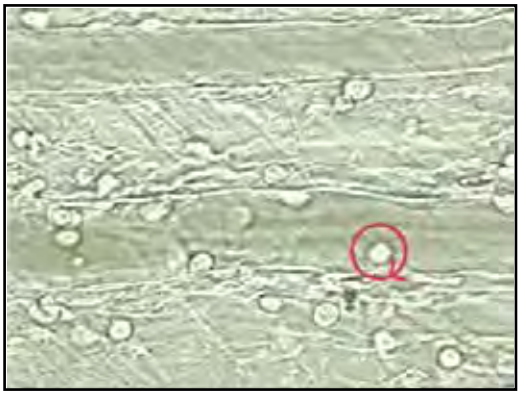
Leucocytes are white blood cells that help fight infection. At sites of injury, infection, or inflammation, cytokines are released and stimulate endothelial cells that line adjacent blood vessels.

The endothelial cells then express surface proteins, called selectins. Selectins bind to carbohydrates displayed on the membrane of the leucocytes, causing them to stick to the walls of the blood vessels. This binding interaction is of sufficiently low affinity that the leucocytes can literally roll along the vessel walls in search for points to exit the vessel. There, they adhere tightly, and squeeze between endothelial cells—without disrupting the vessel walls—then crawl out of the blood vessel into the adjacent connective tissue.

Here, leucocyte rolling is observed directly in an anaesthetized mouse. The up and down movement of the frame is due to the mouse's breathing. Two blood vessels are shown: the upper one is an artery—with blood flowing from right to left. The lower one is a vein—with blood flowing from left to right. Leucocytes only adhere to the surface of veins; they do not crawl out of arteries.

Some leucocytes are firmly attached and are in the process of crawling through the vessel walls, whereas others have already left the vessel and are seen in the surrounding connective tissue.

When the blood flow is stopped temporarily by gently clamping the vessels, we can appreciate how densely both vessels are filled with red blood cells. Red blood cells do not interact with the vessel walls and move so fast under normal flow that we cannot see them. When the blood flow is restored, some of the leucocytes continue rolling, whereas all noninteracting cells are immediately washed away by the shear.



Marko Salmi and Sami Tohka
MediCity Research Laboratory, University of Turku, Finland.

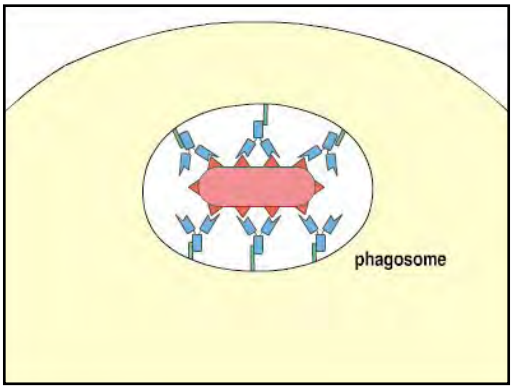
15.6 Phagocytosis – Opsonization

The uptake of bacteria by phagocytes is an active process, which requires the triggering of specific receptors on the phagocyte. Special receptors, which bind antibody-coated bacteria, trigger phagocytosis.

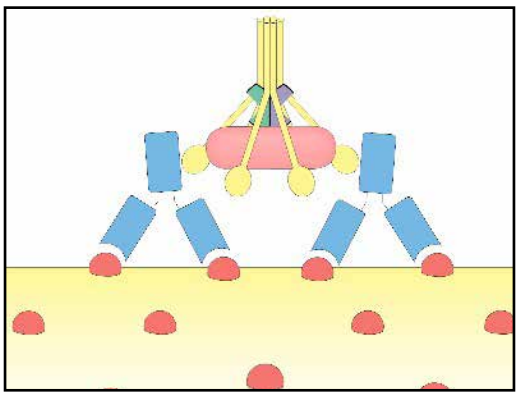
Binding of the aggregated antibody molecules to the receptors on the phagocyte causes the cell to engulf the bacterium.

The phagocyte first produces pseudopods or ruffles that surround the bacterium and then fuse, trapping the bacterium within what is now an intracellular vesicle, the phagosome.

Within the phagocyte, lysosomes fuse with the vesicle, delivering their enzymatic contents to degrade the engulfed bacterium.

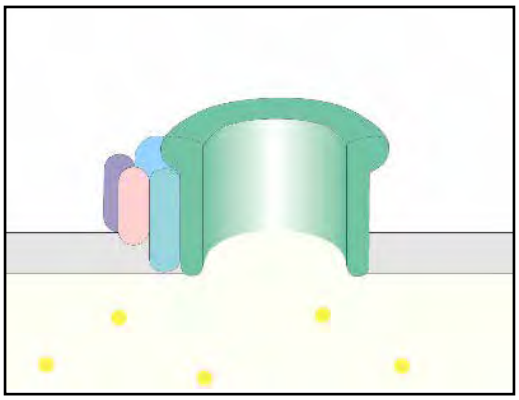


15.7 Complement: Part 1: Activation of Classical Pathway



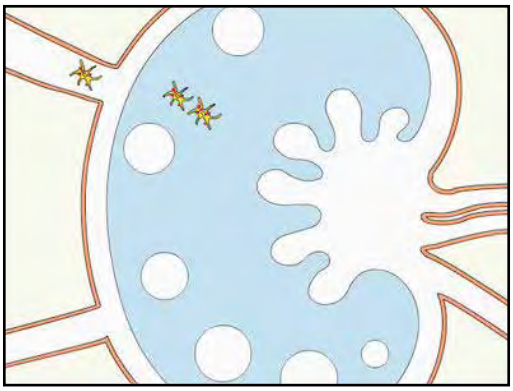
Activation of the complement system occurs on surfaces, such as the surface of a bacterium, as shown here. In this example, the activation of complement is initiated by antibodies that bind to bacterial cell surface antigens. The C1 complement complex made up of one molecule of C1q, two molecules of C1r, and two molecules of C1s, binds to the aggregated antibody molecules on the surface of the bacterium. Binding of the C1 to antibodies results in the activation by cross proteolysis of the C1r and C1s proteases. Active C1s cleaves and activates the complement protein C4, releasing a small peptide fragment, C4a, which acts as an anaphylotoxin. The remaining large fragment, C4b, which contains a labile thioester bond, covalently attaches to the surface of the bacterium through formation of ester or amide linkages. The C2 proenzyme binds to C4b and is then cleaved by the activated C1s, releasing a small peptide, C2b. This leads to the generation of a new protease, C4bC2a, also known as the classical complement pathway C3 convertase. The C4bC2a enzyme binds C3, cleaving it to release another anaphylotoxin peptide C3a, while C3b fragments which also contain a labile thioester, bind to the bacterial surface adjacent to the C4bC2a complex and associate with C4bC2a to create the C4bC2aC3b protease, that is a C3/C5 convertase.

15.8 Complement: Part 2: From C3 to C9



Many molecules of C3 can be cleaved by the C3/C5 convertase, releasing many molecules of C3a. Many of the resulting C3b fragments, are able to covalently attach to the bacterial surface, decorating it with many molecules of C3b that can induce phagocytosis of the bacterium. The C3/C5 convertase also cleaves and activates C5. Again, the small peptide fragment released by this cleavage, C5a, is a potent anaphylotoxin, and is also the most important chemoattractant derived from the complement system. The large fragment, C5b, acts to initiate the formation of the membrane attack complex, the terminal stages of the complement cascade. In this process C5b assembles with C6 and C7. C5b itself is not membrane associated, and the C7 molecule allows this complex to insert itself into the bacterial cell membrane. C8 also binds to the C5bC6C7 complex, and inserts itself into the cell membrane. The C5b,6,7,8 complex catalyzes the assembly of many molecules of C9 to create a cylindrical pore spanning the cell membrane. The pore disrupts the ionic and osmotic balance across the membrane and thus kills the bacterial cell.

16.1 Dendritic Cell Migration



Dendritic cells, the key antigen presenting cells of the immune system, are generated from progenitors in the bone marrow that migrate into peripheral tissues through the blood stream.

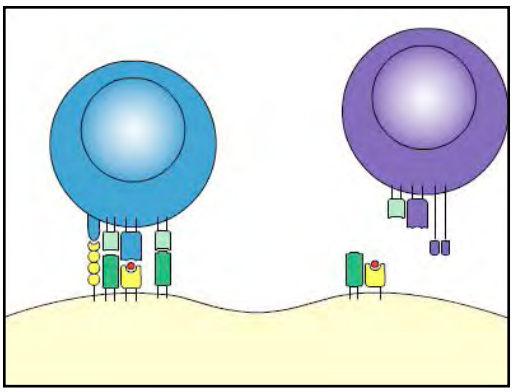
There the immature dendritic cells lie in wait for pathogens entering the body, through sites of injury, for example. Dendritic cells express various pattern recognition receptors that can recognize common features of many bacterial and fungal pathogens. Through these receptors they are able to bind to and phagocytose pathogens.

When these receptors bind pathogens, they activate the dendritic cells, which then start to mature. In this process they migrate from the tissues and change their behavior to stop phagocytosis and to start expressing immune stimulatory molecules.

The activated dendritic cells migrate from the tissues into lymphatic vessels, where lymphatic fluid drains through lymph nodes, carrying the dendritic cells with it.

T cells, migrating through the lymph nodes, inspect the dendritic cells for the presence of antigen. T cells that fail to recognize antigen on one dendritic cell carry on to inspect others and eventually return again to the circulation. T cells that do recognize their specific antigen become activated, and both proliferate and differentiate into effector cells.

16.2 TCR-APC interaction



Antigen presenting cells express both MHC molecules, in this case MHC class II, and co-stimulatory molecules.

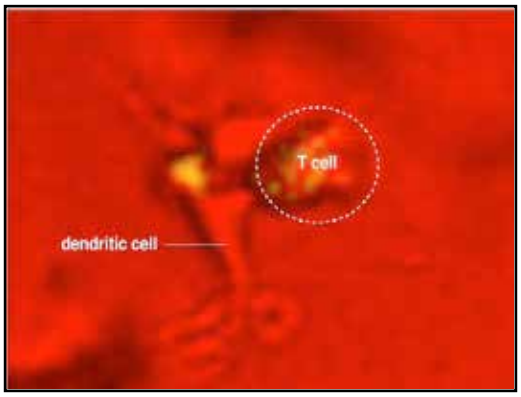
Stimulation of a T cell, in this case a CD4 T cell, by the antigen presenting cell involves the interaction of the T cell receptor and co-receptor molecules with the MHC:peptide complex.

The interaction of the antigen presenting cell with the T cell causes signals to pass in both directions, signaling the antigen presenting cell and the T cell to express additional co-stimulatory molecules.

The interaction between these co-stimulatory molecules results in the full activation of the CD4 T cell.

Activation of CD8 T cells also requires multiple co-stimulatory interactions. The same activation signals that induce the antigen presenting cell to express co-stimulatory molecules are thought to be required for full activation of the CD8 T cells.

16.3 Activated T Cell

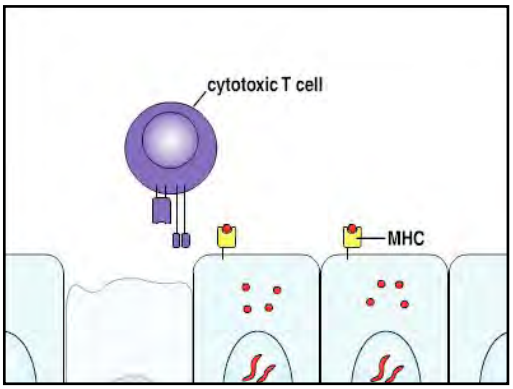


In this video we can see a T cell that becomes activated when it interacts with a dendritic cell. The T cell is labeled with a dye that fluoresces when it binds calcium ions. At the moment the T cell is not activated. Its intracellular calcium concentrations are low, and so little green fluorescence is visible.

As the T cell contacts the surface of the dendritic cell, we can see it suddenly fluoresce bright green as it becomes activated. However, it still continues to move, crawling over the surface of the dendritic cell, perhaps to sample the cell's display of peptide:MHC complexes.

Eventually the T cell loses interest. While it is still contacting the dendritic cell you can see the fluoresence start to fade. The T cell then migrates away from the dendritic cell.

16.4 T Cell Killing



Viruses are intracellular pathogens that infect cells of the body, in this example epithelial cells, usurping their biosynthetic machinery to produce new viral proteins.

Degradation of virus proteins within the cell allows viral peptides to be displayed at the cell surface bound to MHC molecules. Cytotoxic T cells that recognize these MHC:antigen complexes are activated and kill the infected cell. Having killed one cell, the T cell can move to a new target, kill that cell, and move on again.

The killing process is initiated when the T cell receptor binds the MHC molecule bearing a viral antigen, producing signals that activate the T cell.

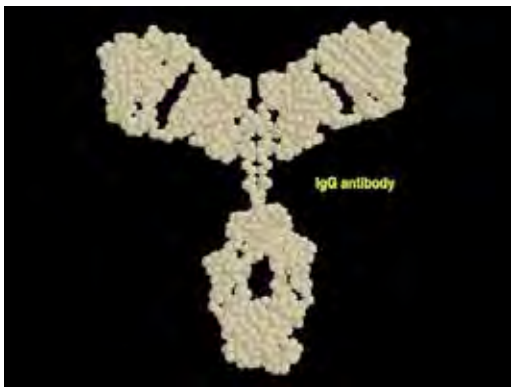
Cytotoxic T cells contain membrane vesicles called cytotoxic granules, which package the proteins that kill target cells. The most important of these are a protein called perforin, and a set of proteases called granzymes. These proteins are complexed with a scaffolding protein called serglycin. Activation of the T cell causes the release of the content of these vesicles, delivering the proteins directly onto the surface of the target cell.

Although the exact mechanism is not known, the perforin facilitates the delivery of the granzymes into the cytosol.

At this point the target cell is destined for death, and the T cell can migrate onwards to find a new target cell.

16.5 Immunoglobulin

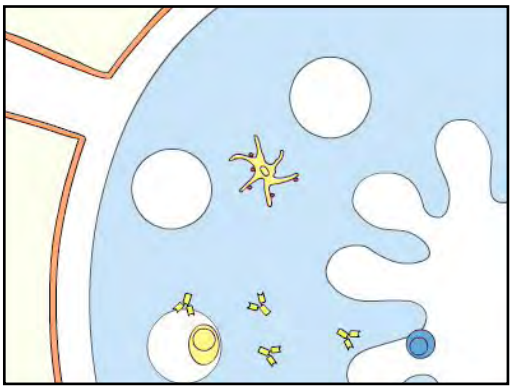
Antibodies of the immunoglobulin G class are Y-shaped glycoproteins that circulate in the blood stream. They bind to and inactivate foreign molecules—the antigens—and mark them for destruction. Each IgG molecule consists of two light chains and two heavy chains. The heavy chains have carbohydrates attached. The regions of the antibody that bind to antigens are located at the very tips of the two arms.



Antigens bind to the tip of each antibody arm, generally two molecules per antibody. In the example shown here, the antigen binds to the antibody via a large contact surface, providing a tight and highly specific association.

16.6 The Immune Response

An immune response involves events that unfold both locally, at the site of an infection, and at more distant sites, such as nearby lymph nodes. We can see the integration of the different parts of the immune response if we follow the course of a typical infection.



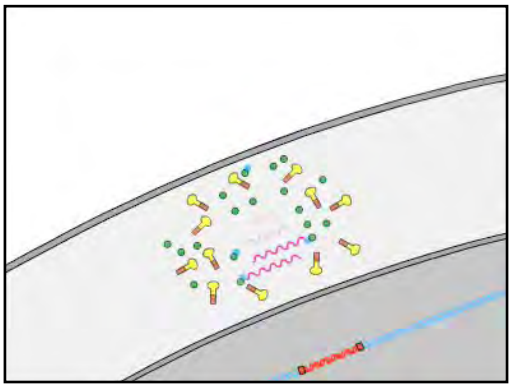
Most pathogens are kept outside of the body by epithelial barriers, such as the epidermis, and are crossed only when there is an injury or tissue damage. After an injury, bacteria cross the epidermis and establish an infection in the underlying tissue. Phagocytic cells in the tissues, such as macrophages and neutrophils, engulf the pathogen.

Dendritic cells are also phagocytic, and are activated by binding pathogens to leave the site of infection and migrate to a lymph node. The migrating dendritic cells enter the lymphatic vessels and are collected in a draining lymph node. In the lymph node, T cells are activated by antigen presented by the dendritic cells, and in turn activate B cells to secrete antibody.

Effector T cells and antibody molecules return to the circulation. They leave the circulation again at the site of infection, where inflammatory mediators have induced changes in the blood vessel endothelium.

T helper cells activate macrophages to become more cytotoxic, while antibody enhances the uptake of pathogens by phagocytes. In the case of a viral infection, activated cytotoxic T cells would kill any infected cells present.

17.1 HIV Infection



HIV infects cells via two cell surface molecules. CD4 is the primary receptor for the virus, while the chemokine receptors CCR5 and CXCR4 act as coreceptors for the viral infection of macrophages and T cells respectively.

HIV binds initially to CD4 via the envelope glycoprotein gp120.

Interactions of the virus with CD4 and the coreceptor allow virus uncoating and the entry of the nucleocapsid, containing the viral genome, into the cell.

The viral reverse transcriptase, which is an integral part of the viral particle, copies the RNA genome of HIV into double stranded DNA. The viral integrase then mediates the integration of the viral DNA into the chromosomal DNA of the host cell.

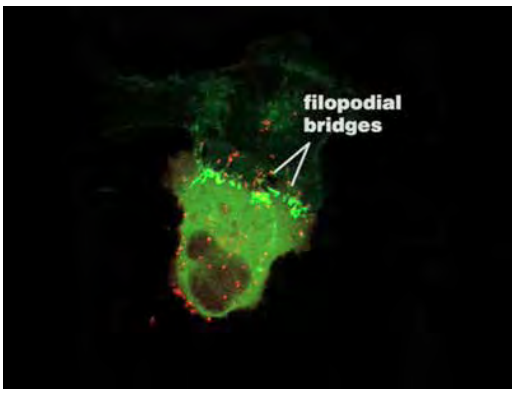
In this state the virus is latent, that is, it can persist in the cell in an inactive state.

Reactivation of the virus occurs when the host cell becomes activated and viral transcription is initiated. This results in the accumulation of viral proteins as well as genome length RNA transcripts of the virus.

Viral proteins assemble at the cell membrane with copies of the RNA genome, and bud off to create a new viral particle.

Maturation of this new virus particle continues after it has budded off from the host cell, to create a new infectious virion with its characteristic nucleocapsid morphology.

17.2 HIV Infected Cells



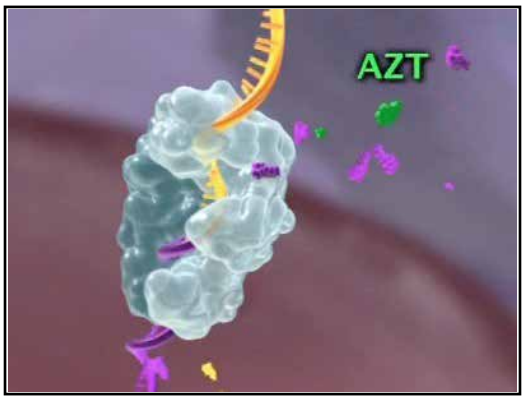
Walther Mothes
Yale University School of Medicine

In this movie retroviruses, which are colored red, are moving from an infected host cell to a target cell. The infected host cell is at the bottom and the target cell is outlined at the top.

The viruses cross from the infected host cell to the target cell by means of viral cytonemes, which are also called filopodial bridges.

Notice over time how the red viral particles move along these cytoplasmic projections. This movement is dependent upon host cell actin molecules.

19.1 AZT Action



Let's review how HIV's reverse transcriptase works. The yellow strand is HIV RNA. It's attached to the reverse transcriptase enzyme. Reverse transcriptase uses the host's cell nucleotides. It makes DNA using HIV RNA as the template and the viral RNA is destroyed in the process.

Now lets see how AZT works. Here's the RNA and the reverse transcriptase again. The purple pieces are host cell nucleotides being assembled into a DNA strand. The green molecule is AZT. Here it's shown next to a thymidine molecule, one of the four nucleotide bases A, T, C, and G. AZT is very similar to thymidine. Only one part of the molecule is different and because they are so similar, reverse transcriptase mistakes AZT for thymidine and incorporates it into the DNA chain. But, the difference is crucial because when AZT is incorporated into the DNA chain, the chain cannot be extended and the process shuts down.

When that happens, the HIV life cycle is disrupted. Here is a mutant form of reverse transcriptase that is resistant to AZT. It's resistant because its mutant molecular structure does not allow AZT to be used as a substitute for thymidine. AZT is therefore rejected, DNA synthesis can proceed, and HIV continues to infect the cell.