

Immune System

Rithik Sogal

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Table of Contents

1	Introduction	3
1.1	Immune Cells	3
1.2	Cytokines	3
2	Innate Immunity	4
2.1	Invertebrate	4
2.2	Vertebrate	5
2.2.1	Barrier Defenses	5
2.2.2	Innate Immune Cells	6
2.2.3	Toll-like Receptors	7
2.3	Inflammation	8
2.3.1	Vasodilation	8
2.3.2	Chemotaxis	8
2.3.3	Phagocytosis	10
2.3.4	Complement System	11
2.3.5	Tissue Repair	12
3	Adaptive Immunity	12
3.1	Lymphoid Organs and Lymphopoiesis	13
3.1.1	Lymphoid Organs	13
3.1.2	Lymphopoiesis	14
3.2	Humoral and Cell-mediated Responses	15
3.2.1	Antibody-mediated (Humoral) Response	15
3.2.2	Cell-mediated Response	15
3.3	Lymphocyte Receptors	16
3.3.1	B Cell Receptors	16
3.3.2	T Cell Receptors	17
3.4	NK Cells	19
3.5	Development of Immune Tolerance	19
3.6	Antibody-mediated Immune Responses: Defenses Against Bacteria, Extracellular Viruses, and Toxins	19
3.6.1	Antigen Recognition and B Cell Activation	20
3.6.2	Antibody Secretion	20

3.6.3	The Attack	21
3.6.4	Direct Enhancement of Phagocytosis	21
3.6.5	Activation of the Complement System	22
3.6.6	Antibody-dependent Cellular Cytotoxicity (ADCC)	22
3.6.7	Direct Neutralization of Bacterial Toxins and Viruses	22
3.6.8	Active and Passive Humoral Immunity	22
3.6.9	Defenses Against Virus-infected Cells and Cancer Cells	23
3.6.10	Role of Cytotoxic T Cells	23
3.6.11	Role of NK Cells and Activated Macrophages	24
3.7	Graft Rejection	24
4	Conclusion	25

1 Introduction

The immune system is a complex and vital defense mechanism that protects our bodies against harmful pathogens, such as bacteria, viruses, and parasites. It comprises a network of specialized cells, tissues, and organs working together to identify and neutralize foreign invaders while distinguishing them from healthy cells. Through a coordinated response of innate and adaptive immunity, the immune system plays a crucial role in maintaining overall health and protecting us from infections and diseases.

1.1 Immune Cells

- Immune cells are often referred to as white blood cells or leukocytes. For the sake of simplicity, we will refer to them as WBCs.
- WBCs can exit the circulatory system to enter tissues where they function.
- There are two groups of WBCs based on the stem cells from which they differentiate, **myeloid cells** and **lymphoid cells**
- The myeloid cells consist of neutrophils, basophils, monocytes, eosinophils, and mast cells. The monocytes further differentiate into macrophages and dendritic cells.
- The Lymphoid cells consist of B lymphocytes (B cells), T lymphocytes (T cells), and natural killer cells (NK cells).

1.2 Cytokines

- **Cytokines** are messengers that regulate mitosis, and they tend to be produced by individual cells, functioning in both adaptive and innate immune responses.
 - Cytokines often act as autocrine or paracrine substances, meaning they act locally. However, they sometimes circulate in the blood (i.e., as endocrine substances) to exert hormonal effects.
- Cytokines often link different aspects of the immune system together. They mediate cross-talk, the chemical communication between immune system cells, which is essential for precise timing.
- One class of cytokines called **interleukins** are responsible for the communication between WBCs. A majority of interleukins are synthesized and released by helper T cells (discussed in the Adaptive Immunity section). Interleukins aid in lymphocyte differentiation and development.
- Another class of cytokines, called **interferons (IFNs)**, are grouped into two subgroups: type I and type II.
- **Type I interferons** include several proteins that inhibit viral replication inside host cells nonspecifically. These interferons bind to receptors both on the secreting cell and on other cells, infected or not.

- Type I interferons trigger the synthesis of antiviral proteins by the cell, which interferes with viral replication. They also act to kill tumor cells and produce fevers as part of the immune response.
- **Type II interferons:** The only type II interferon, called **interferon-gamma**, is produced by immune cells. Interferon-gamma potentiates type II interferons, enhances bacteria-killing by macrophages, and acts as a chemokine in the inflammatory response.

Table 15.7 | Some Cytokines That Regulate the Immune System

Cytokine	Biological Functions
Interleukin-1 (IL-1)	Induces proliferation and activation of T lymphocytes
Interleukin-2 (IL-2)	Induces proliferation of activated T lymphocytes
Interleukin-3 (IL-3)	Stimulates proliferation of bone marrow stem cells and mast cells
Interleukin-4 (IL-4)	Stimulates proliferation of activated B cells; promotes production of IgE antibodies; increases activity of cytotoxic T cells
Interleukin-5 (IL-5)	Induces activation of cytotoxic T cells; promotes eosinophil differentiation and serves as chemokine for eosinophils
Interleukin-6 (IL-6)	Stimulates proliferation and activation of T and B lymphocytes
Granulocyte/monocyte-macrophage colony-stimulating factor (GM-CSF)	Stimulates proliferation and differentiation of neutrophils, eosinophils, monocytes, and macrophages

Figure 1: Cytokines. (Source: Fox Human Physiology)

2 Innate Immunity

In this section, we will outline innate immunity, a set of defenses common to all animals. It is the first line of defense and provides immediate protection against foreign invaders.

2.1 Invertebrate

- Insects, the largest group of invertebrates, often live in habitats with many pathogens, evidence of their effective innate immunity.
- Insects have chitin-based exoskeletons, which provide a strong barrier to the entry of pathogens. Chitin also lines the intestine of insects, where it helps prevent infection.
- Insects also have lysozymes, enzymes that break down bacterial cell walls, essentially acting as a chemical barrier for pathogens ingested with food.

- If pathogens breach these barriers, insect immune cells produce a set of recognition proteins that bind to a molecule common to many pathogens.
- These molecules function as identity tags. Once a recognition protein binds to one of these molecules, it triggers an innate immune response.
- The majority of immune cells within insects are **hemocytes**. Some of these cells are phagocytic, meaning they ingest and break down microorganisms via phagocytosis.
- Other hemocytes can produce defense molecules that entrap larger pathogens. However, most hemocytes release **antimicrobial peptides** that circulate through the body and inactivate/kill bacteria and fungi by disrupting their plasma membranes.
- The innate immune response in insects is pathogen-specific.
 - When dealing with fungi, the binding of recognition proteins activates a transmembrane receptor called a Toll receptor. This receptor causes antimicrobial peptides to be secreted, which kills the fungi.
 - Infection by a virus is followed by the replication of single-stranded viral RNA, which temporarily becomes double-stranded during this process.
 - A host enzyme, Dicer-2, recognizes this double-stranded structure and cuts the viral RNA into fragments.
 - A protein complex, Argonaute, binds to an RNA fragment, displacing one strand. Argonaute then matches this strand with the viral mRNA and cuts it, inactivating it.

2.2 Vertebrate

2.2.1 Barrier Defenses

- In mammals, the mucous membranes and the skin serve as the most important barrier defenses.
- Few pathogens are able to infiltrate intact skin. Furthermore, the hairs that line the nose and the cough/sneeze reflex prevent pathogen accumulation in the respiratory tract.
- Mucosal membranes that line the digestive, respiratory, urinal, and reproductive tract produce a viscous fluid known as **mucus**.
- The viscosity of mucus allows it to entrap pathogens. In airways, ciliated epithelial cells sweep the mucus upward, preventing infection of the lungs.
- Tears, saliva, and mucus contain many antimicrobial proteins, such as lysozymes, which break down bacterial and fungal cell walls.
- Within the stomach, acidic secretions prevent the growth of bacteria. Similarly, oil and sweat glands give the human skin an acidic environment, preventing bacterial growth.

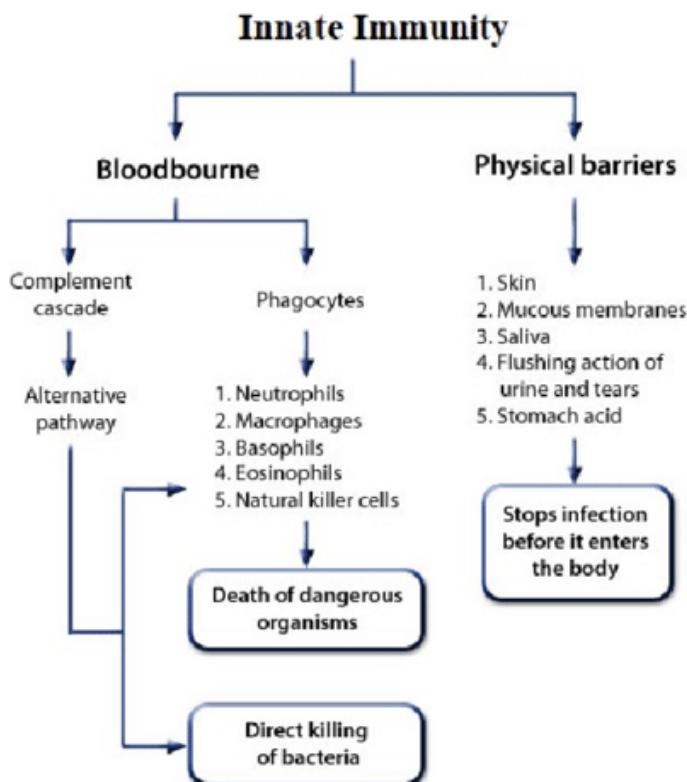


Figure 2: Innate Immunity Flowchart. (Source: Sujata Paul et al.)

2.2.2 Innate Immune Cells

- **Neutrophils** circulate in the blood, attracted to infected tissues by chemokines. Upon arrival, they engulf and destroy the foreign invaders.
- **Macrophages** tend to reside in organs. However, some macrophages circulate in the blood. Neutrophils and macrophages also help regulate inflammation.
- **Dendritic cells** tend to occupy areas in which the internal and external environment (digestive tract, skin, etc.) meet. Though different, they exert similar effects to macrophages.
- Upon activation, they process phagocytized pathogens and travel through lymph vessels to lymphoid organs, where they stimulate adaptive immune cells.
- **Eosinophils** are often found beneath the epithelium, where they deal with multicellular pathogens such as parasites. In order to neutralize these pathogens, eosinophils release destructive enzymes.
- **Mast cells** are found in connective tissue and play an important role in inflammation. They also help stimulate innate immunity.
- **Natural-Killer Cells(NK Cells)** are differentiated from similar progenitors to lymphocytes (discussed in the Adaptive Immunity section) however they play an integral role in the innate immunological response. They are often classified as group I Innate Lymphocytes(ILCs) and play a role in killing virally-infected cells and tumors. (These cells are also discussed in the Adaptive section)

Cell	Image	% in adults	Nucleus	Functions	Lifetime	Main targets
Macrophage*		Varies	Varies	<ul style="list-style-type: none"> Phagocytosis Antigen presentation to T cells 	Months – years	<ul style="list-style-type: none"> Various
Neutrophil		40-75%	Multi-lobed	<ul style="list-style-type: none"> Phagocytosis Degranulation (discharge of contents of a cell) 	6 hours – few days	<ul style="list-style-type: none"> Bacteria Fungi
Eosinophil		1-6%	Bi-lobed	<ul style="list-style-type: none"> Degranulation Release of enzymes, growth factors, cytokines 	8-12 days (circulate for 4-5 hours)	<ul style="list-style-type: none"> Parasites Various allergic tissues
Basophil		< 1%	Bi- or tri-lobed	<ul style="list-style-type: none"> Degranulation Release of histamine, enzymes, cytokines 	Lifetime uncertain; likely a few hours – few days	<ul style="list-style-type: none"> Various allergic tissues
Lymphocytes (T cells)		20-40%	Deeply staining, eccentric	<p>T helper (Th) cells (CD4+): immune response mediators</p> <p>Cytotoxic T cells (CD8+): cell destruction</p>	Weeks to years	<ul style="list-style-type: none"> Th cells: intracellular bacteria Cytotoxic T cells: virus infected and tumour cells Natural killer cells: virus-infected and tumour cells
Monocyte		2-6%	Kidney shaped	Differentiate into macrophages and dendritic cells to elicit an immune response	Hours – days	<ul style="list-style-type: none"> Various

Figure 3: Innate Immune Cells. (Source: Richard Warrington et al.)

2.2.3 Toll-like Receptors

- The receptors of mammalian phagocytes rely on numerous receptors to identify pathogens, some of which operate incredibly similarly to Toll receptors, found in insects.
- Mammalian **Toll-like receptors (TLRs)** bind to molecular motifs characteristic of a set of pathogens. These sequences are referred to as **pathogen-associated molecular patterns (PAMPs)**.
- TLRs belong to a family of proteins called pattern-recognition receptors (PRRs), all of which bind to a wide variety of ligands found in pathogens that are vital for their survival.
- Examples of these PAMPs are lipopolysaccharides, viral and bacterial nucleic acids, and flagellin.
- The binding of immune cells to PAMPs activates secondary messengers, leading to the creation and release of cytokines and antimicrobial peptides.
- There are 11 distinct TLRs. Below, I will outline 4 of the most important ones, along with their respective targets:
 - TLR4** binds to lipopolysaccharides (LPS) with the help of a helper protein. LPS overload results in the massive release of IL-1 (Interleukin-1) in an event known as cytokine storm, thus causing septic shock.
 - TLR5** binds to flagellin.
 - TLR9** binds to CpG DNA.
 - TLR3** binds to dsRNA.

2.3 Inflammation

- Inflammation is the local response to injury or infection that is mediated by phagocytes and acts to destroy or inactivate foreign invaders to set the stage for tissue repair.
- Inflammation is often exemplified by redness, swelling, heat, and pain.
- Inflammation is mediated by a set of chemicals.
- Different inflammatory responses can be mediated by multiple chemicals and one mediator can induce multiple responses.
- These mediators fall into two general categories: (1) polypeptides produced in the infection site via enzymatic reactions on proteins circulating in the plasma and (2) substances secreted into the extracellular fluid by immune cells.

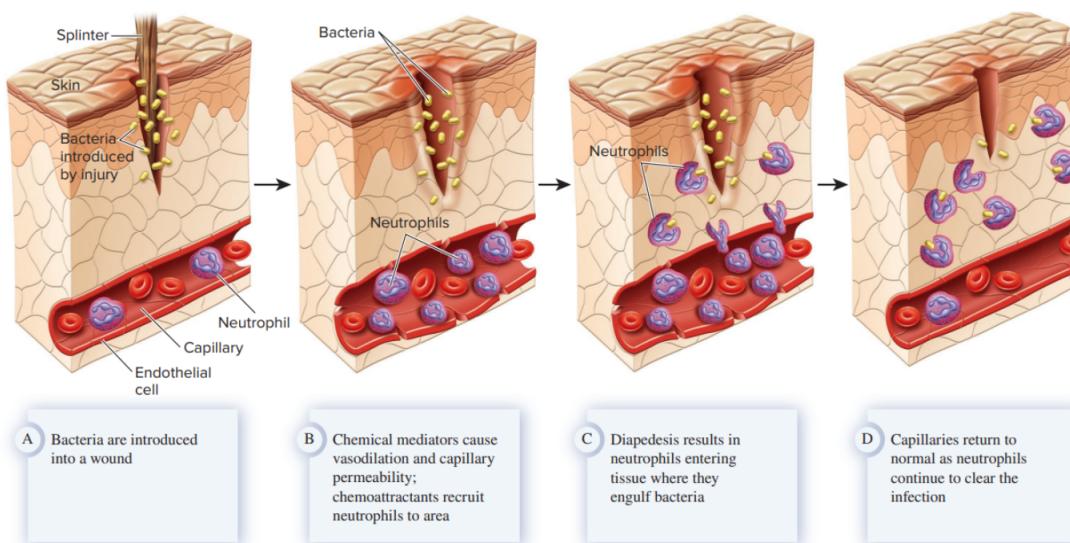


Figure 4: Inflammation Overview. (Source: Vanders Physiology)

2.3.1 Vasodilation

- Many inflammatory mediators dilate the blood vessels in an infected or damaged area. This allows for increased blood flow to the area, which hastens the transport of WBCs and proteins to the area.
- Vasodilation also causes the epithelial cells that line local venules and capillaries to contract, allowing for increased permeability to plasma proteins that play a role in inflammation.

2.3.2 Chemotaxis

- Inflammation attracts neutrophils to infected or damaged areas.
- **Chemotaxis** is the gradual migration of cells as a result of a chemical gradient.
- Chemotaxis is generally regulated by molecules called **chemoattractants**, which are released by epithelial cells in inflamed areas.

- This process also involves a variety of protein and carbohydrate adhesion molecules on vascular endothelial cells and neutrophils.
- The first stage of chemotaxis is **margination**.
 - Margination occurs as neutrophils roll across the surface of blood vessels.
 - Neutrophils are exposed to chemoattractants, which induce rapid formation of adhesion molecules in their cell membranes.
 - These molecules adhere tightly to their complements, located on endothelial cells.
 - This allows neutrophils to gather at the site of an injury or infection.
- The second stage is **diapedesis**.
 - A narrow projection of the neutrophil is inserted into a gap between two endothelial cells. From here, the entire neutrophil squeezes through the vessel wall into the interstitial fluid.
 - It is important to note that neutrophils are polymorphonuclear, meaning their nuclei are segmented into several lobes. This allows them to squeeze between endothelial cells more easily.
 - Once within the interstitial fluid, neutrophils undergo chemotaxis and migrate toward the site of tissue damage.
- Monocytes often follow the same process as neutrophils and transform into macrophages upon arrival in the interstitial fluid.
- Lymphocytes undergo chemotaxis in adaptive immune responses, as do basophils and eosinophils under certain conditions.
- Chemokines, cytokines that have chemoattractant properties, are released to trigger chemotaxis.
- Adhesion molecules generally fit the specific type of chemoattractant as well.

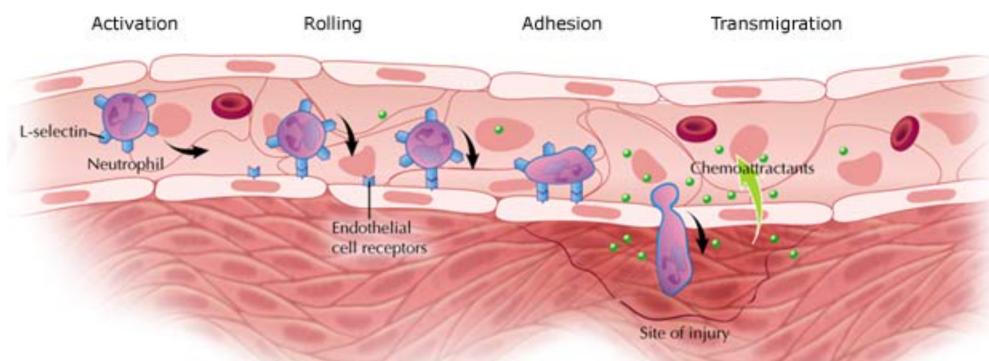


Figure 5: Chemotaxis in the Immune System. (Source: Harvard University)

2.3.3 Phagocytosis

- Upon reaching the infection site, neutrophils and other WBCs begin to phagocytize the invading pathogens.
- The first step is contact between the surface of the WBC and the pathogen.
- Contact is triggered by the interaction between WBC surface receptors and carbohydrates or lipids embedded in the pathogen cell wall.
- Substances that cause the binding of phagocytes to the pathogen are called **opsonins**.
- After being engulfed by the WBC, a small sac, called the **phagosome**, forms around the pathogen.
- The phagosome eventually fuses with the lysosome, forming a **phagolysosome**.
- Now, the lysosome's hydrolytic enzymes break down the macromolecules of the pathogen. Some enzymes produce nitric oxide, hydrogen peroxide, and other oxygen derivatives, which are destructive to macromolecules.
- Phagocytes can also release antimicrobial substances into the extracellular fluid to destroy pathogens.
- Positive feedback mechanisms draw more phagocytes to the injury site.

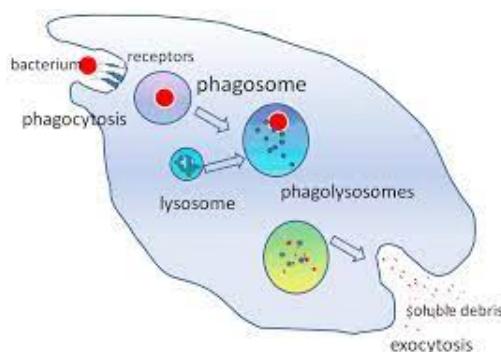


Figure 6: Phagocytosis Process (Source: TeachMePhysiology)

Example 2.1: (USABO Opens Exam 2011) John is suffering from a *Yersinia pestis* (a pathogenic bacterium) infection. These bacteria have a type III secretion system, involving an injectosome (a proteinaceous syringe) which injects Yop toxins into a certain class of John's cells. The Yop proteins prevent these human cells from performing phagocytosis, from producing toxic (bacteria-killing) forms of oxygen, and from producing cytokines. Which of John's cells are being targeted?

- A. Cytotoxic T cells.
- B. Dendritic cells.
- C. Glia Helper
- D. B cells.
- E. Macrophages.

Solution: Phagocytes tend to be involved in innate immune processes. This eliminates the answer choices A and D, which are involved in adaptive immune processes (explained later). Secondly, macrophages release hydrolytic enzymes and can form many oxygen derivatives for phagocytosis. This leaves us with option **E as the correct answer.**

2.3.4 Complement System

Complement proteins are an extremely complex family of more than 30 distinct plasma proteins that provide a means for killing pathogens without phagocytosis.

- Some of these proteins circulate in the blood in an inactive state.
- Upon activation, a cascade effect occurs (i.e., the activated protein activates a second protein, and so on).
- This cascade system ensures that multiple proteins are present at the infection site.
- Although many of these proteins exist, C3 is the most important. Activation of C3 initiates the following sequence of events:
 - First, C3b, a component of C3, is deposited on the surface of the pathogen.
 - C3b acts as an opsonin that is recognized by receptors on phagocytes, which target the pathogen for destruction.
 - C3b is also part of a proteolytic enzyme (an enzyme that breaks down proteins) that amplifies the cascade and leads to the development of a multiprotein complex referred to as the **membrane attack complex (MAC)**.
 - The MAC embeds itself into the viral protein coat or the bacterial plasma membrane and forms pore-like channels.
 - This allows water, ions, and other small molecules to flood the microbe, disrupting its intracellular environment and leading to death.
- The complement system can also initiate vasodilation.
- Interestingly, antibodies are integral to the activation of the first complement protein, **C1**, in a sequence known as the **classic complement pathway**.
- However, the complement system can be activated in another system, referred to as the **alternative complement pathway**.
 - This pathway is activated by interactions with carbohydrates on microbial surfaces and inactive complement proteins. However, not all microbes are conducive to this form of activation.
 - These interactions lead to the formation of active C3b molecules.
- Other opsonins, such as the hepatogenic **C-reactive protein (CRP)**, are crucial to the control of inflammation.

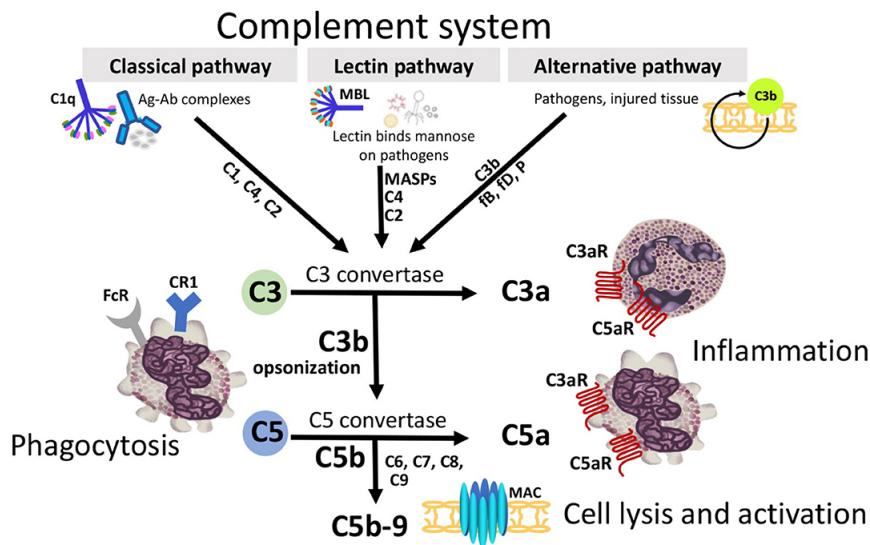


Figure 7: Complement System Pathways. (Source: Girardi et al.)

2.3.5 Tissue Repair

- Tissue repair is the final stage of inflammation.
- Fibroblasts, a type of cells that reside in the injured or infected area, rapidly divide and secrete large quantities of collagen.
- Vascular endothelial cells begin to proliferate in a process called **angiogenesis**.
- Tissue repair is mediated by locally produced growth factors. If not completed properly, tissue repair can lead to scarring.

3 Adaptive Immunity

Adaptive immune responses are generally regulated by lymphocytes, the other class of WBCs. Lymphocytes can be classified as either B cells or T cells. Their function is to identify foreign invaders by recognizing **antigens**. These are molecules that tend to be large polysaccharides or proteins and trigger adaptive immune responses. In essence, antigens are any molecule that binds to lymphocyte receptors or antibodies. The adaptive immune response is split into three stages:

1. Recognition of an Antigen by Lymphocytes

- During development, each lymphocyte synthesizes multiple copies of a receptor that can bind to a specific antigen.
- If that specific antigen is ever encountered, these receptors bind to it. Each lymphocyte is specific to one type of antigen.

2. Lymphocyte Activation

- This stage concerns the binding of the antigen to the specific receptor.

- Upon binding, the activated lymphocyte undergoes multiple rounds of cell division. This is referred to as **clonal expansion**.
- Some of the daughter cells will join the parent lymphocyte in later attacking the antigens. However, some will be set aside as **memory cells**, which function to recognize the antigen if it returns in the future.

3. Attack

- The activated daughter lymphocytes begin an attack on the antigens that are recognized by the antigen-specific receptor.
- B cells, a type of lymphocyte, differentiate into plasma cells and begin to secrete antibodies into the bloodstream.
- These antibodies opsonize the pathogen, marking them for death by innate immune cells.
- Activated cytotoxic T cells, a type of T cell, directly attack the cells that contain the antigens.
- After the attack is complete, the T cells, B cells, and plasma cells often undergo apoptosis. This prevents immune reactions from causing extensive damage. Memory cells generally persist.

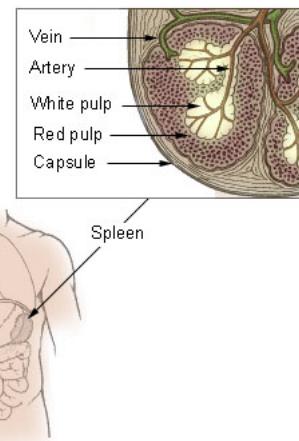
*Note: A lot of the terms outlined above may be somewhat unfamiliar. however, keep in mind that they will be further explained in later sections.

3.1 Lymphoid Organs and Lymphopoiesis

3.1.1 Lymphoid Organs

- A majority of lymphocytes reside in groups of organs collectively called **lymphoid organs**.
- **Primary lymphoid organs**, such as the bone marrow and thymus, are the initial sites of lymphocyte development. These organs supply the body with naive (unactivated) lymphocytes.
 - **The thymus**, situated in the upper chest, primarily consists of immature lymphocytes. These lymphocytes eventually grow into mature T cells upon reaching secondary lymphoid organs. T cells are so named because they are predominantly produced in the thymus.
- **Secondary lymphoid organs**, such as lymph nodes, the spleen, tonsils, and Peyer's patches, are where these naive lymphocytes are activated.
- Most of these cells are descendants of lymphocytes that matured in primary lymphoid organs. These cells that are derived from a single lymphocyte are referred to as **clones**.
- **Lymph nodes** are linked throughout the body by lymphatic vessels. They filter lymph to identify and fight pathogens.

- The tissue of a lymph node is structurally similar to a honeycomb with the walls being reticular fibers and the cells consisting of lymph and lymphocytes.
- Lymph nodes contain dendritic cells and macrophages, as well.
- The spleen** is the largest of the secondary lymphoid organs and is situated on the left-hand side of the abdominal cavity, between the stomach and diaphragm.
- Blood filters through the spleen in a vascular meshwork in which large lymphocytes, macrophages, and dendritic cells can be found.
- Macrophages phagocytize old red blood cells (RBCs) as well.
- The tonsils and adenoids** are a group of small, rounded lymphoid organs located in the pharynx.
- These organs have openings to the pharynx called **crypts**.
- The lymphocytes present in these organs respond to microbes that enter via ingested food and air.
- At any given moment, lymphocytes are on their way from primary lymphoid organs to secondary lymphoid organs. However, the vast majority of lymphocytes participate in lymphocyte trafficking, traveling between secondary lymphoid organs, lymph, body tissue, and the blood.
- Some lymphocytes are pushing through vascular endothelial cells to enter the interstitial fluid.
- They then move into lymph vessels, where they travel to lymph nodes, their "home".
- This process of recirculation is constant, regardless of whether or not infection is present.
- Similarly to phagocytes, lymphocyte migration is sped up by chemotaxis.
- Lymphocyte trafficking also increases the chance that the lymphocyte meets its target antigen.

Spleen**Figure 8: Spleen Diagram**

(Source: Wikipedia)

3.1.2 Lymphopoiesis

- B cells mature in the bone marrow, hence their name. All subsequent generations of B cells arise from these original B cells through cell division in secondary lymphoid organs.

- Other lymphocytes produced in the bone marrow leave while still immature during fetal and neonatal development. These cells travel to the thymus, where they mature into T cells or become B-cell clones.
- NK cells are also produced in the bone marrow. However, they do not operate like most lymphocytes.

3.2 Humoral and Cell-mediated Responses

3.2.1 Antibody-mediated (Humoral) Response

- Activated B cells differentiate into plasma cells, which produce and secrete antibodies. These antibodies travel throughout the body until they reach antigens identical to those that stimulated their production.
- In a process known as agglutination, antibodies combine with these antigens, guiding attacks that kill the antigens and the cells that carry them.

3.2.2 Cell-mediated Response

- All T cell responses are cell-mediated.
- There are two main functional subsets of T cells: **cytotoxic T cells** and **helper T cells**.
- Another type, **regulatory T cells (TRegs)**, which are sometimes considered a unique type of helper T cell, inhibits the function of B cells and cytotoxic T cells.
- Immune cells can be classified by the presence of specific cell surface molecules called clusters of differentiation (CDs). The most important CDs to know are CD8 and CD4, whose presence is used to classify T cells.
- Cytotoxic T cells are CD8+ cells, while regulatory T cells and helper T-cells are CD4+ cells.
- **Cytotoxic T cells** are generally known as "attack" cells. Upon activation, they travel to sites of inflammation and bind to antigens, secreting chemicals to kill their target. They tend to target cancerous cells and virus-infected somatic cells.
- **Helper T cells** assist in the activation and function of B cells, macrophages, and cytotoxic T cells.
- To help B cells, they travel to sites of B cell activation, where corresponding B-cells present a bound antigen to them. The helper T cells make contact with the B cell, communicating through surface receptors. Along with cytokine release, this allows activates the B cells.
- To help activate naive cytotoxic T cells, activated helper T cells give dendritic cell permission to secrete cytokines.
- Helper T cells are classified by the cytokines they release.

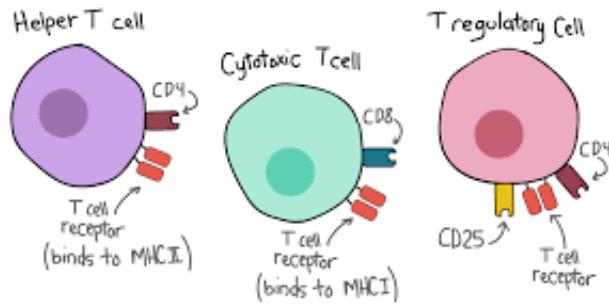


Figure 9: Types of T Cells (Source: Khan Academy)

3.3 Lymphocyte Receptors

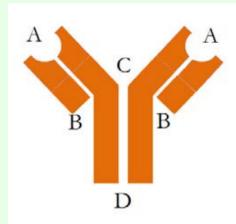
The ability of cells to distinguish between antigens is primarily a result of the specificity of lymphocyte receptors.

3.3.1 B Cell Receptors

- Plasma cells, which are derived from B cells, can secrete only one type of antibody. Each B cell displays copies of this antibody on its membrane.
- This glycoprotein (i.e., the antibody) acts as the receptor for the corresponding antigen.
- These antibodies are part of a family of receptors known as **immunoglobulins**.
- Only secreted immunoglobulins are called antibodies. Each immunoglobulin molecule is composed of four interlinked polypeptide chains. The two long chains are called heavy chains, and the two short ones are called light chains.
- There are five classes of immunoglobulins, determined by the amino acid sequences in the heavy chains and a portion of the light chains: A, D, E, G, and M.
- Immunoglobulins have a “stem” called the **Fc portion**, which comprises the lower half of the two heavy chains.
- The amino acid sequences of the Fc portion and of the light chain are identical within each immunoglobulin class.
- The Fc portion is important for the interaction of the immunoglobulin with phagocytes and the complement system.
- **The antigen-binding site** consists of the upper part of each heavy chain along with its associated light chain.
- The amino acid sequences of the antigen-binding sites vary for each immunoglobulin in a given class and are therefore known as variable ends. Each class can have millions of unique immunoglobulins

- Similar to enzymes, immunoglobulins work through lock-and-key interactions. B cell receptors can bind to antigens whether the antigen is a molecule dissolved in the extracellular fluid or simply present on the surface of a foreign cell.
- The body arms itself with millions of clones of different B cells to ensure that specific receptors exist for the vast number of different antigens the organism might encounter during its lifetime.
- The receptor that the B cell will display is determined during its maturation in the bone marrow.
- Despite the genome having only roughly 200 genes that code for immunoglobulins, the body can produce immunoglobulins having millions of different antigen-binding sites.
- The DNA in each of the genes that code for immunoglobulin binding sites is cut into small segments, randomly rearranged along the gene, then rejoined to form new DNA molecules.

Example 3.1: (USABO Opens Exam 2018). Consider the following figure that depicts the structure of an antibody.



Select the letter for the area where an antibody bound to a pathogen in the bloodstream would be recognized by a hunting macrophage.

- (A) A
- (B) B
- (C) C
- (D) D

Solution: As mentioned earlier, macrophages identify antibodies at the Fc portion, which is the "stem" of the antibody. In this diagram, D is the only area that illustrates the "stem". Thus, **the correct answer is D.**

3.3.2 T Cell Receptors

- Millions of distinct T cell clones exist due to DNA rearrangements during maturation in the thymus.
- T cell receptors for antigens are two-chained proteins that, like B cells, have variable regions that differ from one T cell clone to another. However, the receptors are not secreted like antibodies.

- The T cell receptor cannot combine with an antigen unless the antigen is first complexed with a protein from the **majority histocompatibility complex (MHC)** protein class.
- MHC proteins act as cell identity tags, as no two individuals have the same MHC proteins on the plasma membranes of their cells.
- MHC proteins are often called “restriction elements” because the ability of a T cell to recognize an antigen is *restricted* to situations in which the antigen is first complexed with an MHC protein.
- There are 2 main classes of MHC proteins: MHC class I proteins and MHC class II proteins.
- MHC class I proteins are found on the surface of all cells except for RBCs.
- MHC class II proteins are primarily found on macrophages, B cells, and dendritic cells. Under certain conditions, other cell types are induced to express MHC class II proteins.
- Cytotoxic T cells require their antigens to be associated with class I MHC proteins. Helper T cells, on the other hand, require MHC class II proteins.
- One reason for this difference stems from the presence of CD4 proteins and CD8 proteins on helper and cytotoxic T cells, respectively.
- Antigen presentation is the process by which antigens end up on the MHC complexes.
- T cells can bind antigens only when they appear on a host cell complex with MHC proteins. Cells bearing these complexes function as antigen-presenting cells (APCs).
- Presentation to Helper T Cells
 - Helper T cells require class II MHC proteins, which are only expressed in macrophages, B cells, and dendritic cells. Therefore, these cells can function as APCs for helper T cells.
 - These APCs form a link between innate and adaptive immune responses. After an antigen is phagocytized, proteolytic enzymes break it down into smaller polypeptide fragments called **epitopes**. These fragments bind to the MHC class II proteins synthesized by the cell.
 - The complex is displayed on the surface by the membrane.
 - Following the binding of an APC and a helper T cell, interactions occur between proteins on their surfaces, providing a necessary **costimulus**.
 - The antigenic binding and the stimulus cause the APC to secrete large amounts of the cytokines **interleukin I (IL-1)** and **tumor necrosis factor-alpha (TNF-alpha)**. These cytokines act as paracrine substances on the attached helper T cell to provide another important stimulus for activation.
 - The activated helper T cell now secretes various cytokines that have both autocrine effects on itself, as well as paracrine effects on adjacent B cells and nearby cytotoxic T cells, NK cells, and other cell types.

- Presentation to Cytotoxic T Cells
 - Virtually any cell can act as an APC for a cytotoxic T cell because they all synthesize MHC class I proteins.
 - This illustrates the major function of cytotoxic T cells (i.e., to destroy infected or cancerous body cells).
 - The antigens that complex with MHC class I proteins are formed within body cells.
 - When a cell is infected by a virus, the viral proteins that it produces are hydrolyzed and complexed with MHC class I proteins.
 - When a cell becomes cancerous, it encodes proteins from **oncogenes** that are not normally found in the body. These proteins act as antigens.

3.4 NK Cells

- NK cells constitute a distinct class of lymphocytes and tend to function similarly to cytotoxic T cells.
- However, unlike cytotoxic T cells, NK cells are not antigen-specific.
- The exact nature of the surface receptors that permit NK cells to identify their targets is unknown. However, MHC proteins are not involved.
- NK cell participation in an immune response is enhanced by certain antibodies and by cytokines that are secreted by helper T cells during the adaptive immune response.

3.5 Development of Immune Tolerance

- Recall that the diversity of lymphocyte receptors is due to random DNA cutting and recombination.
- **Clonal deletion**, the destruction of T cells that are able to bind to self-antigens, and **clonal inactivation**, which causes the inactivation of self-reacting T cells, are the reason why there are no active lymphocytes responding to self-antigens. This process also occurs in the bone marrow with B cells.

3.6 Antibody-mediated Immune Responses: Defenses Against Bacteria, Extracellular Viruses, and Toxins

One classic antibody-mediated response is that which results in the destruction of bacteria.

3.6.1 Antigen Recognition and B Cell Activation

- B cell activation begins with bacteria entering the interstitial fluid, then the lymphatic system and/or the bloodstream, where they are directed to the secondary lymphoid organs (eg., the spleen and lymph nodes).
- There, a B cell identifies the bacterial antigen and binds to the bacterium. As mentioned earlier, helper T cells release cytokines into the interstitial fluid to activate B cells.
- This cytokine release is initiated by the binding of helper T cells to an APC (e.g., macrophage) that displays an antigen with an MHC class II protein.
- The APC provides the costimulus, releasing IL-1, TNF-alpha, and another cytokine, **interleukin-2 (IL-2)**.
- This secretion of IL-1 and TNF-alpha causes T cells to express more IL-2 receptors.
- IL-2 acts in an autocrine nature and provides what is called a proliferative stimulus to the activated helper T cell, causing the formation of clones that release more IL-2 and other cytokines.
- Upon activation, the helper T cells migrate to the lymph nodes, where they interact with antigen-presenting B cells.
- Direct contact and the release of more cytokines cause the B cell to differentiate into a plasma cell, which secretes antibodies specific to the antigen detected.
- Some of the B cells also differentiate into memory cells, allowing for a faster and more vigorous response in the future.

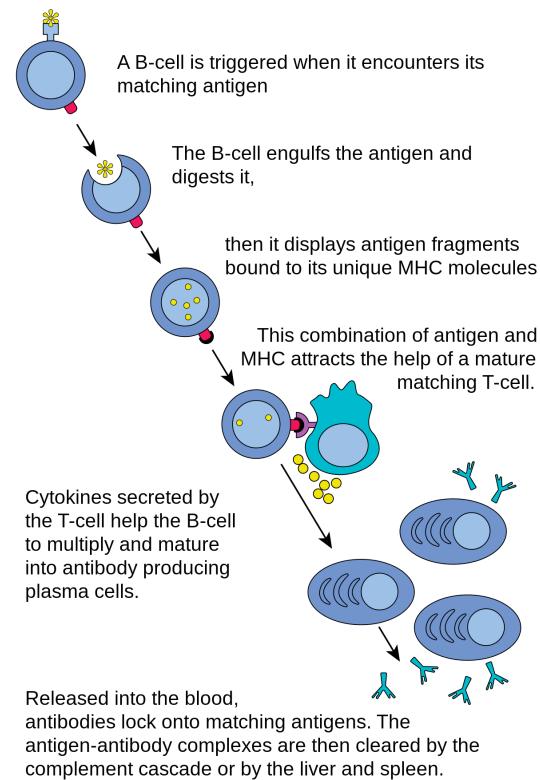


Figure 10: B Cell Activation Process.
(Source: Wikipedia)

3.6.2 Antibody Secretion

- The most abundant classes of antibody are **IgG**, commonly called gamma globulin, and **IgM**.
- Together, they provide the bulk of specific immunity against bacteria and viruses in the extracellular fluid.
 - In the bacterial infection previously mentioned, antibodies (mostly IgG and IgM) produced by plasma cells circulate through the lymph and blood to return to the infected site.

- At the infection site, the antibodies leave the blood and combine with bacterial surface antigens of the same type that initiated the immune response.
- These antibodies then direct the attack against the bacteria to which they are now bound.
- **IgE** mediates responses to multicellular parasites and allergies.
- **IgA** is secreted by plasma cells in the linings of the GI, respiratory, and genitourinary tracts. It acts locally in the linings or on their surfaces. IgA is also secreted by the mammary glands and is the major antibody in milk.
- **IgD** still has a very obscurely understood function.

Name	Properties	Structure
IgA	Found in mucous, saliva, tears, and breast milk. Protects against pathogens.	
IgD	Part of the B cell receptor. Activates basophils and mast cells.	
IgE	Protects against parasitic worms. Responsible for allergic reactions.	
IgG	Secreted by plasma cells in the blood. Able to cross the placenta into the fetus.	
IgM	May be attached to the surface of a B cell or secreted into the blood. Responsible for early stages of immunity.	

Figure 11: Antibody Types. Note that IgA is a dimer while IgM is a pentamer.
 (Source: Concepts of Biology)

3.6.3 The Attack

- Antibodies physically link the microbe to the killing mechanisms (e.g., phagocytes, complement proteins, or NK cells)
- Linkage to antibodies helps protect adjacent normal structures from the toxic effects of the chemicals employed by the killing mechanisms.

3.6.4 Direct Enhancement of Phagocytosis

- Antibodies can also act as opsonins. To elaborate, linkage with the phagocyte promotes attachment of the antigen to the same cell, triggering phagocytosis of the bacterium.

3.6.5 Activation of the Complement System

- As mentioned earlier, in adaptive immune responses, the presence of IgG or IgM bound to antigens activates the classical complement pathway.
- C1 only binds to the Fc portion of an antigen-bound antibody that is part of the IgM or IgG class.
- This binding results in the activation of the enzymatic portions of C1, initiating the classical pathway.
- At the end, the polypeptides that combine to form the MAC kill the cells that the antibody is bound to by making the membrane leaky.
- Antibodies can also enhance phagocytosis by activating C3b.

3.6.6 Antibody-dependent Cellular Cytotoxicity (ADCC)

In this process, antibodies link antigens to NK cell, which then kill the antigen by secreting toxic chemicals.

3.6.7 Direct Neutralization of Bacterial Toxins and Viruses

- Toxins secreted by bacteria into the extracellular fluid can also act as antigens to induce antibody synthesis. Synthesized antibodies then combine with free toxins, preventing interaction with somatic cells in the infected area.
- Each antibody has two binding sites, allowing clumplike chains of antibody-antigen complexes to form. These complexes are later phagocytized.
- This process is similar to that of viral neutralization.

3.6.8 Active and Passive Humoral Immunity

- Antibody production occurs slowly over several weeks following initial exposure to an antigen. However, invasion by the same pathogen causes the immediate production of additional specific antibodies, a process mediated by memory B cells.
- This aspect of memory is one of the key defining differences between innate and adaptive immunity.
- There are two types of immunity: (1) **active immunity**, resistance built up through past contact with toxins, microbes, and other antigens, and (2) **passive resistance**, which is provided by the direct transfer of antibodies from one person to another.
- An example of passive immunity is between mother and fetus. IgG can cross the placenta, and children can receive IgA through breast-feeding.

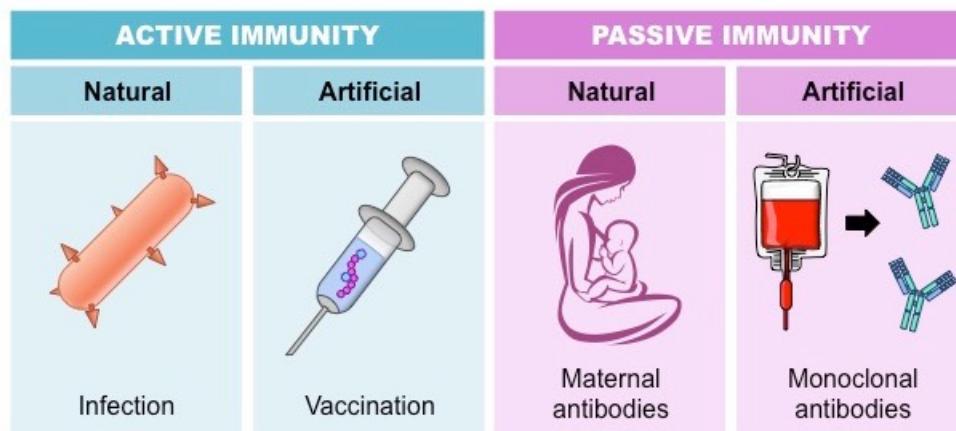


Figure 12: Active vs Passive Immunity. (Source: BioNinja)

3.6.9 Defenses Against Virus-infected Cells and Cancer Cells

- Destruction of host cells results in the release of viruses into the extracellular fluid, where they can be directly neutralized by circulating antibodies.
- If viruses are given too long to replicate and spread from cell to cell, so many virus-infected host cells may be killed by the body's own defenses that organ malfunction may occur.

3.6.10 Role of Cytotoxic T Cells

As described earlier, a virus-infected cell or cancer cell produces foreign proteins, “endogenous antigens,” which are processed and presented on the plasma membrane of the cell in complexes with MHC class I proteins.

- Binding to the antigen alone does not activate a cytotoxic T cell. Instead, additional cytokines released from adjacent activated helper T cells are also required.
- Macrophages phagocytize free extracellular viruses or antigens released from the surface of cancer cells.
- Macrophages process and present the antigen in association with MHC class II proteins to a helper T cell.
- Macrophages act as the APC and provide a costimulus while also secreting IL-1 and TNF-alpha. The helper T cell releases IL-2 and other cytokines, which stimulates its own proliferation.
- Here, IL-2 acts as a paracrine substance, causing the cytotoxic T cell bound to cancer/virus-infected cells to proliferate.
- This increases the chances of other infected cells being encountered by this type of cytotoxic T cell.
- In order to actually kill cancer cells and virus-infected cells, cytotoxic T cells release the contents of their secretory vesicles, which include **perforin**, a protein similar in structure to the MAC, into the extracellular space between themselves and the target cell via exocytosis.

- Perforin facilitates the transport of cytotoxic enzymes called **granzymes** into the infected cell. These granzymes further activate intracellular enzymes that induce apoptosis.
- Releasing these enzymes into the region between the T cell and the infected cell prevents damage to other cells.
- Some cytotoxic T cells also remain as memory cells, ensuring that an active immune response is ready in the future.

3.6.11 Role of NK Cells and Activated Macrophages

- NK cells and activated macrophages also destroy virus-infected/cancer cells by secreting toxic chemicals.
- As viral infections and cancerous growths do not trigger ADCC, NK cells must directly bind to their target without antibodies.
- IFN-gamma and IL-2, secreted by helper T cells, signal the NK cells to proliferate and secrete their toxic chemicals.
- NK cells also secrete IFN-gamma, forming a positive feedback loop (if you are unfamiliar with this term, read the endocrine system handout).
- IL-2 and IFN-gamma act on neighboring macrophages, activating them and allowing for the secretion of large amounts of chemicals that are capable of killing the cells in many ways.
- This feedback loop runs until the pathogens are gone.

3.7 Graft Rejection

The major obstacle to successful transplantation of tissues and organs is graft rejection. The immune system recognizes the transplants, called grafts, as foreign and launches an attack against them.

- Cytotoxic and helper T cells are primarily responsible for graft rejection. Except for between identical twins, the MHC class I and II proteins on the cells of a graft differ from those of the recipient.
- Consequently, the MHC proteins of both classes are recognized as foreign by the recipient's T cells, leading to the cells bearing these proteins being destroyed.
- Some of the tools aimed at reducing graft rejection are radiation and drugs that kill actively dividing lymphocytes and thereby decrease the recipient's T-cell population.
- **Hyperacute rejection** occurs a few minutes after the transplant when antigens are completely unmatched. The tissue must be removed right away so the recipient does not die. This type of rejection is seen when a recipient is given the wrong type of blood.
- **Acute rejection** may occur at any time from the first week after the transplant to 3 months afterward. All recipients have some amount of acute rejection.

- **Chronic rejection** takes place over many years. The body's constant immune response against the new organ slowly damages the transplanted tissues or organs.

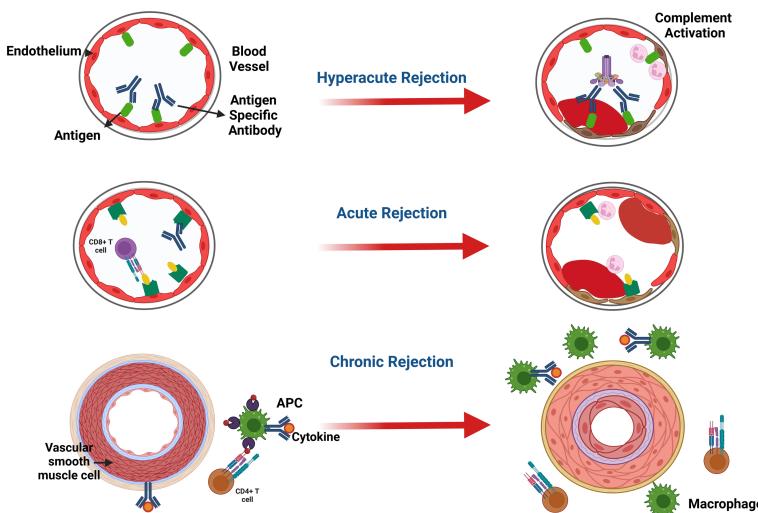


Figure 13: Rejection Types. (Source: Cureus)

Example 3.6: (USABO Semifinals Exam 2017) Studies show that prior exposure to donor MHC molecule leads to accelerated graft rejection. What can you conclude based on this outcome?

- T lymphocytes can mediate graft rejection
- B lymphocytes can mediate graft rejection.
- Graft rejection shows memory and specificity.
- Both B and T lymphocytes mediate graft rejection.
- Graft rejection is mediated by the innate immune system.

Solution: As shown in the Graft Rejection section, E cannot be correct, as cytotoxic T cells and helper T cells are significant in graft rejection, and both of them are part of the adaptive immune system. In addition, early MHC exposure accelerates graft rejection. This means that this process has some form of memory (i.e., exposure to an antigen the second time will cause a faster response thanks to memory cells). In addition, MHC molecules indicate specificity, meaning that the best answer is C.

4 Conclusion

Welcome to the end of the immune system handout! Understanding how the immune system works is crucial to the advancement of our treatment of certain diseases, such as Alzheimer's, cancer, and HIV/AIDS. I know that this handout is somewhat long, but take your time and try to understand all the nuanced connections within the immune system. Understanding the function of the immune cells helps you understand the general process of immunological response. I wish you the best of luck on the USABO exam, and, as David Goggins says, STAY HARD.

-Rithik Sogal