Immunity Rules!

Overview of the Immune System

Immunity Protects Us from Infectious Disease.

The immune system has evolved to protect us from infectious disease. The System Module Infectious Disease describes four major groups of **pathogens** (disease-causing infectious agents). **Antigen** is the name for any molecule that stimulates an immune response. Most antigens are pathogen proteins or carbohydrates that are "foreign" or "non-self" to the host.

Even simple multi-celled animals like sea squirts and starfish, as well as many plants, have defense systems that can recognize a generic "danger" signal and respond by engulfing or walling off foreign organisms. Humans have a corresponding **innate** immune response that begins immediately in response to tissue damage. **Phagocytes** are attracted to the site of infection or tissue damage and engulf and destroy pathogens, as well as our own dead or damaged cells. In some instances, innate immune responses are effective at eliminating antigen from the body.

Innate immune responses include

barriers to pathogen entry: skin and mucous membranes with tight junctions between cells.

mechanical responses to eliminate antigen: cilia, blinking, sneezing, coughing, peristalsis, vomiting.

chemical agents: fatty acids on the skin and HCl in the stomach, lysozyme and other digestive enzymes in tears and mucus, antibacterial molecules called **defensins** and **complement** in the blood that promote phagocytosis and pathogen destruction, and antiviral **interferons** that block virus replication in host cells.

phagocytes: neutrophils, eosinophils, and monocyte/macrophages in blood and in many body tissues, Langerhan's cells in the skin, M cells in the mucous membranes of the digestive, respiratory and genital systems.

inflammatory responses to attract white blood cells (**leukocytes**) to the infection site.

Natural Killer (**NK**) **cells** to kill virus-infected and cancer cells.

Adaptive immunity is antigen-specific and found only in vertebrates. The adaptive immune system responds more quickly and efficiently to a repeat infection (**immune memory**), often so efficiently that no symptoms develop. A wide range of antigens can induce adaptive immunity. Adaptive immunity can be long-lasting but is not permanent. It discriminates between "self" and 'non-self", generally attacking the latter and ignoring the former. An individual's immunity depends on both environment (experience) and inheritance. Disease can result from under-activity or over-activity of the immune system. *Innate immune mechanisms including inflammation and phagocytosis are essential for the efficient functioning of the adaptive immune system.*

Adaptive immune responses include

synthesis of antibody to bind antigen and promote its elimination.

T cell killing of virus-infected cells.

T cell activation of macrophages to destroy phagocytosed pathogens that are resistant to destruction.

Adaptive immunity is usually acquired *actively* by natural infection or by vaccination with killed or weakened (**attenuated**) pathogen or inactivated toxin (**toxoid**). **Active immunity** requires 2-3 weeks to become established and may be very long-lasting, from years to a lifetime. Adaptive immunity can also be acquired *passively* from an immune person by the transfer of antibodies or (rarely) immune cells. **Passive immunity** protects as soon as the antibodies are transferred but lasts only weeks-months as the transferred antibodies are removed from the circulation in a natural process called "turnover".

Immunity which can be transferred in serum is called **humoral immunity**. Examples include antibodies transferred across the placenta or in breast milk from mother to child and horse antibodies to rattlesnake venom used to treat snake bite. Immunity that can be transferred only with T cell transfer is called **cellular immunity**. Passive cellular immunity is limited by rejection of foreign transplanted cells, and is usually only done between inbred animals or in human bone marrow transplants where the whole blood cell-forming system is transferred.

The Immune System: Cells and Organs.

A **system** is a regularly interacting or interdependent group of items forming a unified whole (Merriam Webster Online Dictionary). The **immune system** is the collection of cells and organs that work together to provide immunity. Immune system cells, the white blood cells or **leukocytes**, wander the body to detect localized infections. Immune system organs provide locations where leukocytes mature and where they interact efficiently with antigen to become fully active effector cells and memory cells. **Effector cells** eliminate antigen, while **memory cells** make a more efficient response to a repeat antigen exposure.

The **primary (central) immune organs** are where white blood cells mature. **Hematopoiesis**, the development of white blood cells, occurs in the **bone marrow**. Pluripotent, self-renewing **stem cells** divide and differentiate into all types of functional blood cells. At each stage of differentiation (stem cell --> progenitor cell --> mature cell), cells become more restricted in their potential than their precursors. During hematopoiesis, lymphocytes acquire their specific antigen receptors (one specificity per cell), co-receptors required for response to antigen, cytokine receptors, and adhesion molecules that target the cells to particular immune organs. Hematopoiesis is regulated by growth factors, growth factor receptors, and programmed cell death (**apoptosis**). T cells complete their development in the **thymus**, an organ in the chest above the heart. The thymus is relatively large in infants and children when T cell development is highest. It begins to shrink at puberty, although some T cell maturation occurs throughout life. Antigen is NOT required for the development of mature antigen-specific T and B lymphocytes.

Phagocytes include two types of leukocytes : blood **monocytes**, called **macrophages** when they leave the circulation and enter the tissues, and **polymorphonuclear leukocytes** (**PMNs** or **granulocytes**), primarily **neutrophils**. A chart in the ToolBox lists the blood leukocytes and their normal values. Macrophages are large cells with round nuclei that can put out long pseudopodia to surround antigen. PMNs have lobed nuclei and many granules in their cytoplasm.

Macrophages and PMNs engulf and kill pathogens, especially bacteria. Eosinophils kill parasites, especially helminths (worm parasites). Macrophages and PMNs bind common surface molecules on pathogens or antibody-coated pathogens; phagocytes are not antigen-specific and are part of innate immunity. Macrophages also produce **cytokines** that attract other leukocytes and make blood vessels leaky, leading to inflammation. **Dendritic cells** (**DC**) can be phagocytic under certain circumstances and, along with macrophages and B cells, are ***A*ntigen-*P*resenting *C*ells (APC)** which help stimulate T cell activation.

Lymphocytes are antigen-specific leukocytes responsible for adaptive immunity. They are small, round cells with little cytoplasm and round nuclei. Lymphocytes have membrane receptors that bind antigen; each lymphocyte recognizes one specific antigen. Antigen receptor on B lymphocytes is called **membrane immunoglobulin** (**mIg** or **antibody**) or **BCR** (B Cell Receptor). Antigen receptor on T lymphocytes is called **T Cell Receptor** (**TCR**). Each lymphocyte has about 100,000 copies of its membrane antigen receptor. Lymphocytes specific for many diverse antigens are produced continually *in the absence of antigen exposure*. When a lymphocyte encounters its specific antigen and receives the proper costimulatory signals, it proliferates and differentiates into a clone of effector cells with the same antigen specificity. **Natural Killer** (**NK**) cells are large granular lymphocytes that lack specific antigen receptors. However, they recognize and respond to altered tissue typing (**MHC**) proteins present on virus-infected and cancer cells. NK cells are part of the innate immune system.

Secondary (peripheral) lymphoid organs are designed to bring together leukocytes and antigen. Peripheral lymphoid tissues are present throughout the body. Clusters of lymphocytes and specialized antigen-collecting epithelial cells called **M cells** line the mucous membranes of the respiratory, digestive, and urogenital systems where contact with pathogens is highest. With the **tonsils**, **appendix**, and **Peyer's patches**, they are called the *M*ucosal *A*ssociated *L*ymphoid *T*issues (**MALT**). Other peripheral lymphoid organs are the **spleen**, where blood-borne antigens (especially bacteria) encounter the immune system, and **lymph nodes**, where antigens from the tissues are collected.

Fluid leaves the blood circulation at the capillaries and bathes the tissues, supplying nutrients and washing away waste products. The fluid, called lymph, then collects in the **lymphatic vessels** and passes through the lymph nodes on its way back to the blood circulation. If the tissues are infected, antigen is carried to the nearby (**draining**) lymph nodes where it comes in contact with phagocytes and lymphocytes to initiates an adaptive immune response. Lymphatic vessels transport lymph and cells from the lymph nodes back into the blood circulation. At any given time many leukocytes **recirculate** throughout the body and are present in high numbers in the peripheral blood circulation. Expression of **adhesion molecules** on endothelial cells lining the blood vessels is increased by inflammatory cytokines to signal leukocytes to enter the tissues or the secondary lymphoid organs in response to antigen.

Immunology Rules!

Cells of the innate immune system are not antigen-specific; they have molecules on their membranes which bind antigens found on many infectious agents. Macrophages, PMNs, and NK cells also have membrane receptors for complexes of antibody with antigen (Fc receptors, FcR) or complement with antigen (complement receptors, CR), so that antigen which has bound

antibody or complement is more easily engulfed (or, in the case of NK cells, lysed). When macrophages bind certain common bacterial antigens, they are stimulated to produce small proteins called **cytokines** that signal other leukocytes. Some cytokines (**chemokines**) are **chemotactic**, attracting other leukocytes to the site of infection. Other cytokines signal the blood vessel endothelial cells to express more adhesion molecules, so that leukocytes can stick and move between the endothelial cells to enter the tissues. Other cytokines increase the amount of fluid that can leave the circulation, so that antibacterial molecules enter the infection site. Some cytokines signal the bone marrow to produce more leukocytes. This whole process, which results in redness, swelling and pain at the site of infection, is called **inflammation**. Although not antigen-specific, inflammation is often enough to eliminate small numbers of bacterial pathogens from the body. If antigen is not eliminated, lymphocytes and antibody can also participate in inflammation during adaptive immunity.

Lymphocytes bind antigen using antigen-specific membrane proteins. The antigen receptor on B cells (**BCR**) is **antibody.** Each B cell has about 10^5 BCR, all with identical antigen-binding sites. Antibodies bind (are specific for) protein and non-protein antigens. BCR and soluble antibody bind antigen in its native conformation. The T cell antigen receptor (**TCR**) is structurally related to antibody. Each T cell has about 10^5 TCR which share the same antigen specificity. T cells cannot bind antigen directly to their TCR; it must first be **processed** (cut into peptides) and **presented on** (bound to) **MHC** molecules. Three kinds of cells are **professional antigen-presenting cells** (**APC**): macrophages, dendritic cells, and B cells. Since TCR binds processed antigen, binding does not depend on natural antigen folding; it does depend on how efficiently the antigen can be processed, bind MHC, and bind TCR. We each have a limited number of MHC molecules with which to present antigen; fortunately, each MHC can present numerous peptides to TCR.

Antigen receptors are encoded in groups of gene segments arranged linearly on the chromosome, each segment encoding part of the receptor. As B cells develop in the bone marrow and T cells develop in the thymus, they must **recombine** (splice together) several of these gene segments to produce a functional antigen receptor. Cells which fail to successfully produce functional receptors die without leaving the primary lymphoid organs. Recombination occurs randomly, so the millions of lymphocytes produced each day collectively have millions of different antigen specificities. The large number of different specificities that are produced is called the **immune repertoire**. Since receptor generation is random, cells specific for self antigens are also produced. Antigen binding to self-specific immature T and B cells in the thymus and bone marrow results in their death (**clonal deletion**).

Mature naïve T and B cells (those that have not yet bound foreign antigen) leave the primary lymphoid organs and recirculate through the secondary lymphoid organs. If a lymphocyte does not bind its specific antigen in a few weeks, it dies. If it does bind antigen, it proliferates into a **clone** of lymphocytes that differentiate into antigen-eliminating **effector cells**. Memory lymphocytes specific for the same antigen are also produced. This process is called **clonal selection** and explains the specificity of adaptive immunity. The time it takes between antigen contact and our ability to detect adaptive immunity is called the **lag phase** of the immune response. In a **primary response** to antigen, the lag phase is usually almost a week long. Detectable adaptive immunity (for example, antibody in the serum) increases for a period and then declines as antigen is eliminated. On repeat exposure (**secondary response**) to the same antigen, the lag period is shorter and the measurable response is higher and longer-lasting due to

the **clonal expansion** of lymphocytes specific for that antigen during the primary response.

Antibody is a protein which serves as BCR and is also secreted to bind and remove antigen from the body. Antibody is composed of disulfide-bonded **heavy** and **light chains** in a Y shape, with two identical **antigen-binding regions** at the ends of the top of the Y. The antigen-binding regions of antibody differ in their amino acid sequence from one molecule to the next and are called **variable regions**. The stem of the Y (the **constant region**) has one of five possible structures (**isotypes**: IgA, IgD, IgE, IgG, and IgM) that determine the effector functions of the antibody.

Mature naïve (also called **resting**) lymphocytes must receive two signals to become fully functional **effector cells**. Signal 1 is always antigen: that keeps the immune response antigen-specific. B cells bind native antigen to their membrane antibody (BCR). When they receive a second cytokine or adhesion signal from helper T cells, they divide and differentiate into antibody-secreting plasma cells. T cells bind protein antigen **processed** (cut into peptides) and **presented on** (bound to) **MHC** molecules of Antigen-Presenting Cells (signal 1). APC can also deliver a cytokine or (usually) an adhesion signal 2 to stimulate T cell proliferation into effector cells.

Seeing and Eliminating Antigen

When the immune system functions optimally, antigen is eliminated before the host shows disease symptoms. **Exogenous** (extracellular) **antigens**, including most bacteria and their toxins, are most accessible to immune elimination. Phagocytes bind commonly shared antigens on the surface of bacteria, engulf the organisms and digest them inside phagocytic vesicles. Digestive enzymes, oxygen radicals, and peroxides released by macrophages and PMNs during inflammation kill pathogens. **Complement** is activated by binding to many bacterial surface molecules; once activated, it promotes **phagocytosis** by binding to phagocyte complement receptors. Other complement products attract leukocytes and increase blood vessel leakiness (promote **inflammation**) so that plasma proteins, including complement, clotting factors, and antibody, can reach the site of infection. In some cases, complement directly **lyses** the bacterium.

Once adaptive immunity is triggered, **antibody** is the primary tool for exogenous antigen elimination. **B lymphocytes** respond to antigen plus helper T cell signal by becoming **plasma cells**, which secrete antibodies. Antibodies bind to extracellular pathogens and bacterial toxins to inactivate them (**neutralization**). They also **opsonize** (coat) the pathogens to promote phagocytosis and activate complement to opsonize or lyse the pathogen and promote inflammation.

Antibodies, also called **immunoglobulins** (**Ig**), are antigen-binding proteins which are divided into five classes (**isotypes**) based on their structures. The amino acid sequence of antibodies differs from one molecule to the next in the antigen-binding site (variable region) but does not differ significantly in the rest of the molecule (constant region). Each isotype has different biological functions once antigen is bound, and some isotypes have more antigen-binding sites per molecule than others. **IgM** and **IgD** are antigen receptors (BCR) on B cells. Following antigen activation of B cells, IgM is the first isotype secreted. Secreted IgM is a very large molecule with 10 antigen-binding sites; it is very efficient at binding antigen and **activating complement** but can enter the tissues only slowly because of its size. When B cells receive

helper cytokine signals from T cells, they begin secreting **IgG** or **IgA**. IgG is the predominant antibody isotype in serum; it has two antigen-binding sites and can easily enter the tissues from the circulation. IgG binding to toxins or viruses prevents them from entering cells to kill them; this is called **neutralization**. Following antigen binding (**opsonization**), IgG activates complement and IgG-antigen complexes bind FcR. Phagocytes then use both complement receptors and FcR to facilitate phagocytosis. IgA is made predominantly in mucosal lymphoid tissues. It is present in mucus secretions of the respiratory, digestive, and urogenital tracts and in breast milk. IgG and IgA neutralize virus and toxin activities by blocking host cell binding. **IgE** is made to helminth (worm) parasites and to environmental antigens by people who have allergies. IgE binds FcRε on mast cells, which then respond to antigen by releasing histamine.

Endogenous (intracellular) **antigens**, including viruses, protozoan parasites, and bacteria which survive phagocytosis, are not directly accessible to phagocytes, complement, or antibody. They must be eliminated by destruction of infected host cells. Viruses which insert their own molecules into the host cell membrane prior to using it as an envelope are somewhat more vulnerable, since antibody can bind these viral proteins and trigger **complement-mediated lysis** of the host cell. Antibody on phagocyte and NK cell FcR binds membrane-expressed viral antigen and trigger host cell killing (**Antibody-Dependent Cell-mediated Cytotoxicity, ADCC**).

For intracellular parasites which do not express their antigens on the host cell membrane, immune recognition requires antigen peptide presentation on host cell MHC and specific recognition by T cells. **MHC** (**Major Histocompatibility Complex**) **proteins** are plasma membrane tissue typing antigens. They were identified when immunologists began inbreeding mice to try to understand graft rejection. The primary function of MHC proteins is antigen presentation. **Class I MHC** molecules are present on the membranes of all nucleated cells. When these cells become infected with viruses, they can process virus proteins synthesized in their cytoplasm into peptides, combine these peptides with Class I MHC in their endoplasmic reticulum (ER), and transport the peptide-MHC complexes to their plasma membranes. Infected cells present virus peptides on their MHC Class I to the TCR of **cytotoxic T cells** (**Tc**). Tc become activated **cytotoxic effector cells** (**CTL**) that kill the infected cells (**targets**).

Macrophages can process and present antigen from phagocytosed pathogens on Class II MHC to **helper T cells** (**Th1 or Th2 cells**). Th1 cells signal the macrophages to fuse their lysosomes with the phagocytic vesicles and kill the intracellular pathogens more efficiently. Th2 cells secrete cytokines that signal B cells to make antibody. Both B and T lymphocytes also respond to antigen plus Th cytokines by differentiating into **memory cells**, which are long-lived and respond more quickly than naïve lymphocytes when re-exposed to antigen.

Some molecules on pathogens bind lymphocyte surface molecules which are not antigen receptors. If this binding induces the lymphocytes to undergo cell division (mitosis), the molecules are called **mitogens**. At high doses, mitogens usually induce proliferation in a high frequency of lymphocytes regardless of their antigen specificity, a process called **polyclonal activation**. Some mitogens are T-independent antigens, capable of inducing B cells to secrete antibody in the absence of T cell help.

Key Concepts of Immunity

The immune systems has evolved to protect us from common pathogens.

Innate immunity is the immediate response to commonly shared microbe antigens (LPS, teichoic acid, patterns of sugars).

Innate immunity includes physical, chemical and mechanical barriers to entry, phagocytes to engulf and digest extra cellular pathogens, and interferon's and NK cells to block virus replication and kill virus-infected cells.

Inflammation attracts leukocytes to the infection site, where innate immune responses occur.

Adaptive immunity is the slower response to specific antigens which vary from pathogen to pathogen.

Adaptive immunity includes antibody, cytotoxic T cells, and inflammatory (macrophage-activating) helper T cells.

An active immune response is made to antigen exposure, either naturally acquired during infection or artificially acquired by vaccination. Passive immunity is acquired from another individual in the form of antibodies. Humoral immunity is due to antibody production, while cellular immunity is due to cytotoxic and inflammatory T cell activity.

The immune system is composed of circulating leukocytes and lymphoid organs.

Haematopoiesis, the differentiation of stem cells into mature leukocytes, occurs in the bone marrow. T cell maturation is completed in the thymus. Bone marrow and thymus are the primary (central) lymphoid organs.

The secondary (peripheral) lymphoid organs bring together antigen and leukocytes to initiate adaptive immune responses. Secondary lymphoid organs include the lymph nodes, spleen, and MALT (Peyer's patches, tonsils and adenoids, appendix, and collections of lymphoid cells in mucous membranes).

Leukocytes recirculate between lymphoid organs and the blood via the lymphatic vessels, which also collect antigen from the tissues.

Phagocytes include monocyte/macrophages, granulocytes (neutrophils, eosinophils, basophils) and dendritic cells. They have receptors for common pathogen antigens and for complement-coated and antibody-coated antigen. In addition to engulfing and destroying pathogens, macrophages and dendritic cells can make cytokines that regulate immune responses and can process and present antigen to T cells.

Lymphocytes include B cells, T cells, and Natural Killer (NK) cells. B and T cells have specific antigen receptors which are generated during lymphocyte development by a specialized gene-splicing process. BCR bind native antigen, but TCR bind only to antigen peptides presented on MHC. NK cells do not have antigen-specific receptors, but recognize altered MHC on virus-infected and cancer cells.

Cytokines are chemical messengers made by leukocytes, primarily T cells, macrophages and dendritic cells.

Antigen stimulation plus other signals stimulate specific lymphocytes to proliferate and differentiate into clones of effector cells and memory cells.

B cells are activated to become antibody-producing plasma cells. Antibodies are specific antigen-binding proteins that neutralize and opsonize antigen and activate complement to

promote inflammation and phagocytosis. Different antibody isotypes have specialized locations and functions.

Cytotoxic T cells identify and kill virus-infected cells that present virus (endogenous) peptides on Class I MHC. Inflammatory (Th1) T cells activate macrophages that present antigen peptide on Class II MHC to more efficiently kill the phagocytosed (endogenous) pathogens. Helper (Th2) T cells activate B cells that present exogenous antigen peptides on Class II MHC to divide and secrete antibody.

Immune memory is the faster, more efficient immune response made on repeat contact with an antigen. Memory B and T cells can persist for long times in the body.

Some antigens are polyclonal mitogens that promote lymphocyte proliferation.

THE IMMUNE SYSTEM

Category	Element	Function
LYMPHOID ORGANS		
Central	1.	1. B cell development
	2.	2. T cell development
Peripheral	1.	1. Antigen in tissues
	2.	2. Antigen in blood
	3.	3. Antigen in mucosal tissues
	4.	4. Antigen in mucosal tissues
	5.	5. Antigen in mucosal tissues
	6.	6. Antigen in mucosal tissues
	7.	7. Antigen in mucosal tissues
	8.	8. Antigen in mucosal tissues
	9.	9. Antigen and leukocyte transport
LEUKOCYTES		
Granulocytes	1.	1. Phagocytosis
	2.	2. Kill worm parasites.
	3.	3. Allergic reactions.
	4.	4. Unknown
Monocytes	1.	1. Phagocytosis & antigen presentation
Lymphocytes	1a.	1a .Extracellular antigen recognition
	1b.	1b. Antibody secretion.
	2a.	2a. Presented antigen recognition.
	2b	2b. Activates B cells, MF.
	2c.	2c. Kills virus-infected cells.
	3.	3. Kills virus-infected cells (innate).

Other cells	1.	1. Antigen presentation.
	2.	2. Antigen collection (gut).
	3.	3. Develops into lymphocyte.
	4.	4. Develops into any blood cell.

MEMBRANE RECEPTORS

Antigen-binding	1.	1. Binds antigen on B cells.
	2.	2. Binds antigen on T cells.
Antigen-presenting	1.	1. Presents exogenous antigen.
	2.	2. Presents endogenous antigen.

SECRETED MOLECULES

Antigen-binding	1.	1.Antigen-specific.
	2.	2.Antigen non-specific
Signalling	1.	1. Alters cell behaviour.

Vocabulary

How do immunologists use these terms?

Complement (note spelling)

Discriminate

Naïve

Memory

Neutralization

Presentation

Professional

Recognize/see

Repertoire

Tolerance

Problem

Influenza virus infects respiratory epithelial cells. It is an enveloped virus: virus hemagglutinin (H) and neuraminidase (N) proteins, which help the virus enter and leave the host cells, are expressed on host cell plasma membranes that envelope the emerging virus particles. Influenza viruses rapidly mutate their H and N antigens, so that exposure or immunization to influenza one year does not guarantee immunity the next. "Flu shots" contain virus particles which have been grown in fertilized eggs and inactivated (killed) so they do not infect vaccine recipients. The vaccine also contains **adjuvants** that attract macrophages to promote virus phagocytosis. Describe the immune effector mechanisms generated by flu shots and how they prevent influenza if an immunized person is exposed.

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