The Immune System

Slide 2: The immune system is a defense mechanism found in vertebrate animals that consists of chemicals, cells and organs and structures. These provide two types of immunity; non specific = protection against any invader without specific identification of what it is, and specific = protection against a specific invader or molecule which is identified and targeted. The immune system provides protection from pathogens, toxins, and cancer cells. It utilizes a variety of cell types and responses, and some of these efforts can induce a memory response.

Slide 3: Nonspecific or innate immunity: This type of immunity is known as the body’s basic resistance. It consists of anatomical structures, physiological defense barriers, specialized cells, chemicals, and the inflammatory response. These do not need to recognize the enemy in order to function.

Slide 4: Examples of Innate immunity: Nonspecific immunity includes a variety of components. Intact skin, for example, is the best, first barrier to infection. The large number of layers of stratified squamous epithelial cells, with many layers of dead, keratinized cells protect the body from viruses and bacteria. In addition, the skin produces secretions such as sweat, that wash the organisms away from the surface. Saliva, and lysozyme also contain antimicrobial agents. If an organism is ingested, the pH of the stomach destroys many of the pathogens and prevent them from becoming established in the gut. In the respiratory tract, the pseudostratified ciliated columnar epithelium acts as a “mucociliary” escalator, moving organisms and particulates that might enter when you breathe up into the esophagus where they can be swallowed and eliminated. Many groups of white blood cells, which will be described in greater detail later, provide nonspecific immune response in the event of an infection. The inflammatory response utilizes a series of chemicals and cells to wall off any potential invader and prevents them from penetrating deeper into the body. Cellular chemicals, such as interferons, are produced by virally infected cells and prevent viruses from entering other cells in the area. Complement proteins circulate in an inactive form, and when stimulated, can eliminate pathogens.

Slide 5: Immune System Cells: Cells involved in nonspecific immunity include the granular white blood cells = neutrophils, basophils, and eosinophils; the Natural Killer cells, and the agranular white blood cells = monocytes and macrophage. Cells involved in specific immune responses are also agranular, and include the B and T lymphocytes.

Slide 6: Blood cell lineages

Slide 7: Monocytes/Macrophage: Monocytes are agranular white blood cells, about 10μm in diameter, which circulate in the blood. They are produced in the bone marrow and incompletely differentiated and mildly phagocytic. They can be identified in a blood smear by their characteristic “bean shaped” nucleus. When they leave the blood stream and begin to settle in the tissues, they mature and are called macrophage. Macrophage are either “free”, which means they move throughout the tissues, or “fixed” which means that they are localized in one region and often take on some special properties specific to that region. The fixed macrophage are morphologically varied, and include such cells as the microglia, alveolar macrophage in the lungs, and the osteoclasts. Macrophage phagocytose microbes and produce cellular chemicals (cytokines) that recruit other cells and ramp up the inflammatory response. They are also antigen presenting cells, which means that they act as a bridge between nonspecific and specific immunity by making the specific immune cells aware of an “invader”.

Slide 8: Neutrophils: Polymorphonuclear cells (PMN’s), or neutrophils, make up 50-60% of circulating white blood cells. They are important mediators of the earliest phases of the inflammatory response and the first line of defense (after the intact skin) against infectious agents. They have a distinctive segmented nucleus, with 3-5 lobes connected by little nuclear “bridges”, and have granules that do not stain strongly either with eosin or hematoxylin, so
The granules appear small and faintly blue and red. Healthy adults produce about $1 \times 10^{11}$ neutrophils per day, and each neutrophil circulates for only about 6 hours in the blood. If it isn’t involved in an inflammatory process within those six hours, it undergoes apoptosis and is destroyed by macrophage in the liver and spleen. Interestingly, a small “pool” of neutrophils is maintained for emergencies. They are attached loosely to the endothelium of large veins, where they remain until there is an infection or a stress reaction that quickly mobilizes them. When neutrophils are destroyed during an inflammatory engagement, they become part of what we know as pus. An enzyme in neutrophils called myeloperoxidase gives pus the “greenish” color.

**Slide 9: Basophils and Eosinophils**

**Basophils** are the smallest circulating granulocytes and make up about 0.5% of circulating white blood cells. They are produced and mature in the bone marrow, and circulate in their mature form. They are found predominately in the blood, but can be recruited to inflammatory sites. These cells are visually characterized by their large blue granules when stained. They are important in allergic reactions and contain histamine, chondroitin sulfate, and proteases, which they release into the blood and tissues when activated. Some of the effects of histamine include vasodilation, increased plasma leakage from the blood vessels, and bronchospasms. Basophils are very short lived and only survive for a few days after release from the bone marrow.

**Eosinophils** are produced in the bone marrow and make up about 2.7% of white blood cells in healthy individuals. They contain large granules rich in arginine basic proteins that stain bright orange with eosin. Although a few circulate, they are found predominately in tissues such as respiratory, gut, and urogenital subepithelium. Increased numbers of eosinophils are found in individuals with worm infections and allergies. They are also important mediators of hypersensitivity reactions. Usually they are bound with the IgE (immunoglobulin E antibodies) when stimulated, they release toxic protein granules and free radicals which kill microorganisms and parasites. These granules also damage tissues resulting in allergic reactions. Eosinophils produce prostaglandins and leukotrienes and amplify the inflammatory response by recruiting and activating other cells via production and release of specific cytokines. The major granule content includes basic protein, peroxidases, and hydrolases. They survive for days to weeks once released from the bone marrow.

**Slide 10: Mast Cell:** There is lots of misinformation about mast cells! These cells are produced in the bone marrow and mature in connective tissue. Under normal conditions, they are not found circulating in the blood. They leave the bone marrow as immature cells and differentiate in the tissues (in situ). They are found throughout the body, but tend to be located in lymphoid organs, near blood vessels, nerves, and beneath epithelial tissue. They are very diverse in appearance and contain granules, including histamine, as well as heparin, chondroitin sulfate, and various proteases. They are also involved in the inflammatory and allergic responses and last for weeks to months.

**Slide 11: NK Cells:** Natural killer cells are a subset of lymphocytes that attack virally infected cells and tumor cells. They are called natural killer cells because they don’t require the specific activation that other lymphocytes (B and T lymphocytes) need, so their ability to kill is considered innate or “natural”. They are sometimes called large granular lymphocytes because they have large cytoplasmic granules which contain granzyme and perforin. In addition, NK cells secrete interferon-gamma (IFN-γ) which activates macrophage so that they destroy microbes which they have phagocytosed.

**Slide 12: Inflammatory response:** The cardinal signs of the inflammatory response are described by four Latin terms; **rubor** = redness, **dolor** = pain, **tumor** = swelling, and **calor** = heat. The effects of inflammation are to prevent an intruder from getting into deeper tissues by confining them to the area that was initially invaded. It also sets up a mechanism for eliminating any cellular debris and/or pathogens that might have entered the body, and sets the stage for repairing any tissue that may have been damaged.
**Slide 13: Inflammatory response:** This slide contains an illustration of the events that occur when the first line of defense, the intact skin, is breeched by a wound of some kind. The wound introduces bacteria into the tissues at the site of inoculation. Stationary mast cells in the tissue release chemicals, such as histamine, that cause local vasodilation of the capillaries nearby. This causes an increase in the pore size in the capillaries, allowing immune effector cells, such as neutrophils, to move out of the blood vessels into the tissues (this is called extravasation, or diapedesis). The neutrophils are phagocytic and begin to munch up the invaders. Macrophage also appear on the scene. They release additional chemicals increasing the immune response. They also much up invaders and clean up the scene so that tissue repair can begin. The four cardinal signs are a consequence of these activities: rubor = comes from the increased blood supply to the affected area as the capillaries vasodilate; tumor = swelling as the protein free plasma leaks out of the blood vessels into the tissues; dolor = tissue swelling puts pressure on local pain receptors; calor = more blood going to an area and more metabolic activity due to the clean up in progress causes an increase in heat.

**Slide 14: Acute appendicitis:** Slide shows associated inflammation.

**Slide 15: Complement:** We have mentioned many substances that are produced by the body and kept on standby in an inactive form, so that they can be activated when necessary. These include enzymes, such as pepsinogen, and hormones, such as angiotensinogen. In the immune system, there are also circulating proteins that are found in the blood in an inactive form that can be activated when necessary. These complement proteins include over 20 circulating plasma proteins that protect the body in four ways: chemotaxis, opsonization, activation of inflammation, and cytolysis via the assembly of a membrane attack complex, or MAC. Chemotaxis refers to the establishment of a chemical gradient so that higher concentrations of a chemical are closer to the site. This sets up a “signal” that immune effector cells can follow that directs them toward enemy. Opsonization is a term that means “to make tasty” or “to butter up”. Activated complement proteins coat the outside surface of bacteria. If you recall, bacterial surfaces are negatively charged, which makes them difficult to phagocytose. When they are opsonized by complement proteins, it makes them “tastier” to the macrophage, which find them easier to engulf. Activated complement proteins also activate the inflammatory process.

**Slide 16: Complement cascade:** The complement system can be activated in two ways; the classic pathway and the alternative pathway. In the classic pathway, antibody produced by activated plasma cells binds to the antigens on the surface of bacteria producing an antibody:antigen complex. C1, the first complement protein in the series, is then activated by C reactive proteins made by the liver. This sets up a cascade of events producing a number of protein intermediaries that result in chemotaxis, opsonization, and activation of inflammation. The final series of proteins binds to the surface of the bacterium forming a membrane attack complex and destroying the invader. The complement cascade can also be initiated by the presence of the pathogen itself. This is called the alternative pathway.

**Slide 17: Image of complement cascade:** This slide shows you how lucky you are not to have to learn all of the steps this semester!!

**Slide 18: MAC:** This image shows how the activated complement proteins assemble to form a membrane attack complex. Once assembled, this forms a hole, or tube, in the plasma membrane of the organism and causes the cellular contents to leak out.

**Slide 19: Fever:** Macrophage and other white blood cells produce chemicals called pyrogens that reset the hypothalamus, increasing body temperature. This resets the set point so that your body systems work to maintain the new set point. Fever has many effects on the body, among them, it increase the metabolic rate, which helps the body to repair more quickly. It also makes the environment uncomfortable for pathogens. At the same time, the liver and spleen sequester (hide) Zn++ and Fe+, which are used by enzymes as cofactors. By doing this, they are making it difficult for the bacteria to survive. (We use these cofactors, as well, which is why some lozenges
contain Zinc!) Low grade fever is classified as a temperature between 38-39°C, moderate between 39-40°C, and high grade 40-42°C.

**Slide 20: Specific (Acquired) Immunity:** So far, we have discussed the actions the body can take against invaders without specific recognition of the invader. Now we will investigate the specific immune response, which involves recognition and selective elimination of pathogens and molecules. This branch of the immune system also has cellular and humoral (chemical) mechanisms. Unlike the nonspecific immune mechanisms, some specific responses induce memory, so that the second or subsequent time that your body is infected with the same pathogen, a response occurs much more quickly than the first time. It is important in this response that a distinction can be made between self and non-self. This means that the attack should be only against cells and chemicals that are not normally found in the body. The specific immune response is also systemic, meaning that it covers the entire body and moves through the bloodstream.

**Slide 21: What are antigens and haptens:** Antigens are substances that are found on the surface of all cells. When they are somewhere other than where they should be, they are called foreign antigens and are described as foreign substances that can induce a specific immune response. Essentially, they are cell surface markers. You have antigens on your kidneys, for example, that indicate that the kidney is yours. If your kidney is transplanted into someone else, the antigens on the surface of your kidney will be identified as foreign by the immune system of the recipient. This is why immune suppressants have to be taken by individuals who receive a transplant. Complete antigens are those that have two properties; immunogenicity = they are able to stimulate an immune response, and reactivity = they can react with immune effector cells and substances. An incomplete antigen is called a hapten. These are smaller molecules that don't elicit an immune response on their own, but can if they bind to larger molecules, such as your own native proteins. This is how lots of allergens work.

**Slide 22: Haptens:** Illustration

**Slide 23: APC's:** Antigen presenting cells are special cells that are capable of phagocytosis. These cells ingest invaders, break them up, and display parts of them on their surface on special receptors. This presentation activates the specific immune response. When specific immune cells recognize the presented antigen, they become activated, produce an army of clones, and “seek out” other invaders like the one that was presented. Cells that are classified as APC’s include dendritic cells, which are specialized macrophage that are stationary in the connective tissue and have lots and lots of processes, Langerhans cells, specialized macrophage that are found in the epidermis, free macrophage, and B lymphocytes. Some APC’s are found throughout the body, while others are localized. For example, B lymphocytes and dendritic cells are found in the germinal cells of lymph nodes.

**Slide 24: Histocompatibility antigens:** HLA antigens are surface antigens that are found on all human cells, except mature red blood cells. The genes that code for these antigens are found on chromosome #6. The specific type of antigen determines what type of cell will bind to it. These are very important to match when someone is being considered for a transplant. There are 6 major HLA antigens, and transplants can be performed when there are as few as 4 matches. The future matches, the less likely that the transplant will engraft and the more likely that it will be rejected. In all animals, the HLA is referred to as MHC, or major histocompatibility antigens.

**Slide 25: Class I vs Class II MHC:** There are two classes of MHC.

**Class I MHC** are found on all cells except for red blood cells and bind CD8 receptors (complementary determinant 8). These CD8 receptors are found on killer T lymphocytes (CTL = cytotoxic T lymphocytes). They present antigen derived from intracellular pathogens. The
pathogen is degraded and actively pumped from the cytoplasm to the endoplasmic reticulum. It is loaded into a Class I MHC binding cleft and expressed on the surface of the cell.

**Class II MHC** are found only on macrophage and B lymphocytes. They bind a class of receptor called CD4. CD4 receptors bind helper T lymphocytes. These are involved in the presentation of extracellular pathogens.

**Slide 26: Image:** This image shows how nonspecific and specific immunity are linked by presentation of antigen by macrophage or dendritic cells.

**Slide 27: Specific Immune response:** This image shows the connection between the nonspecific and specific immune response through antigen presentation by macrophage. Note that T lymphocytes can only be activated by presented antigen, while B lymphocytes can be activated by presented antigen, but are themselves antigen presenting cells.

**Slide 28: T lymphocytes:** T lymphocytes are involved in cellular immunity. These are cells that are activated only by presented antigen and are part of the specific immune response. There are three populations of T lymphocytes; helper T cells = which increase the immune response of both B and T lymphocytes, killer T cells = cells which directly attach to and destroy specific cells, and suppressor T cells = cells which wind down the immune response when it is complete. Please note that the helper T cells are the major target cell for HIV. When HIV invades these cells and becomes active, it destroys the helper T lymphocytes, severely debilitating the specific immune response. Affected individuals become very ill because their immune system is severely compromised. Infections occur with organisms that are often part of your normal body flora, such as *C. albicans*, or part of the normal environment, such as bread mold (*Rhizopus*, or *Asperigillus*).

**Slide 29: T cell stimulation:** Image shows that two signals are required (co-stimulation) between an APC and a T cell in order to activate the T lymphocyte.

**Slide 30: Clonal expansion of T cells:** This image is a graphic depiction of the clonal expansion hypothesis. This is an hypothesis which explains how specific immunity occurs. In specific immune cells (B and T lymphocytes) a special type of recombination occurs during cell division that is similar to the recombination in meiosis. The results of this genetic recombination produces a huge variety of cells with different specificities. This event occurs all the time, and occurs before a pathogen is encountered. The cells that are produced in this way go to the thymus (T cells) or to the germinal centers of the lymph nodes (B cells) where they are exposed to antigens that are commonly found in the body. Cells that respond to these antigens are eliminated or turned-off. Cells that remain either circulate (T cells) or remain stationary (B cells). If they encounter an antigen that they recognize, they expand by cell division (do you remember autocrine stimulation???) to produce an army of clones all specific for that antigen.

**Slide 31: B lymphocytes:** These are the specific immune effector cells that are involved in humoral immunity. When B cells are stimulated, they expand to produce more cells of the same specificity. These cells differentiate and become plasma cells. Plasma cells are more "egg shaped" because the contain tons of rough endoplasmic reticulum. They will produce antibodies. Antibodies, or immunoglobulins, are soluble proteins that recognize and bind to specific antigen, allowing the macrophage to phagocytose and eliminate them.

**Slide 32: Immunoglobulins:** The function of immunoglobulins is to recognize and "remove" pathogens and harmful chemicals from the body. In a primary immune response, when a foreign antigen is encountered for the first time, it takes up to 30 days to produce antibody. In a secondary or subsequent response, antibodies are produced within hours of infection. This is the premise for vaccination. Vaccines introduce foreign antigens to your body under controlled conditions, before you have encountered the antigen naturally. In that way, when you meet the foreign antigen naturally, you will be able to mount a secondary response.
There are five types of immunoglobulins. Immunoglobulin M, or IgM, is a pentamer, made up of 5 antibodies bound together in a ring. This is the type of antibody that is produced in a primary response. Because it is so large, it is only found in the bloodstream. IgG is seen in the greatest quantities in a secondary or subsequent response and it occurs as a single antibody. IgA is called secretory antibody because it is secreted into saliva, breast milk, and is also present in the digestive system. This antibody is composed of two linked together by a special "J" chain. This structure helps to minimize the likelihood of degradation by digestive enzymes. IgE is a single antibody that is elevated in allergic responses and parasitic worm infections. It is often found bound to the surface of eosinophils. IgD is a cell surface marker that is only seen during the development of B lymphocytes. It's activity in the immune response is uncertain.

**Slide 33: Immunoglobulin structure:** Please note that the image of IgA is incorrect. This should be made up of two antibodies.

**Slide 34: Active immunity:** In active immunity, you are introduced either naturally or through vaccination to the foreign antigen. So, you either get the disease, or receive a clinical vaccination against the disease. The resulting clonal expansion and formation of memory cells protects you from future infection with the same organism.

**Slide 35: Passive immunity:** Passive immunity is temporary. It provides protection against an invading organism by using someone else’s antibodies. Mothers do this with the IgA and IgG they produce and transfer to their developing fetus in utero, or through their breast milk (always a good idea to breastfeed if you can…no bottles to wash, too!) Before there were Hep B vaccines, we would receive IgG by inoculation whenever we were exposed. This provided temporary protection, but did not allow our systems to develop the immune response that would protect us in subsequent encounters. Remember that antibodies are proteins and can be degraded, so they don’t last very long and you need to be able to produce them yourself to have any long-lasting benefits. Another example is RhoGam for mothers who are Rh- carrying an Rh+ fetus.

**Slide 36: Clonal Selection Hypothesis:** read this and compare it to what I have already written for Slide 30.

**Slide 37: Illustration of Clonal selection and expansion.** Uses B lymphocytes as an example. Note that it is plasma cells, not B cells, that produce antibody.

**Slide 38: Allergic responses and hypersensitivity reactions:** Allergic reactions are classified into four categories based on the amount of time it takes to develop and the effect it has on the body. These reactions can cause significant damage and can result in death.

**Slide 39: Images of allergic reactions**

**Slide 40: Autoimmunity:** If the immune system is unable to distinguish between self and nonself, immune cells can persist in the body that will attack, damage, and destroy native cells. As we learn more about diseases and their causes, more and more diseases are being reclassified as autoimmune. Examples of diseases that are caused by your own immune system attacking yourself include myasthenia gravis, in which individuals produce antibodies that attack the acetylcholine receptors, blocking them; multiple sclerosis (MS) in which antibodies attack the myelin sheath, or white matter, surrounding the neurons; rheumatoid arthritis, in which antibodies are produced that attack connective tissue; IDDM, in which antibodies attack and destroy the beta cells of the pancreas, preventing the production of insulin; and systemic lupus erythematosus, or SLE, in which antibodies are produced that attack nuclear proteins and DNA.

**Slide 41: Rheumatoid arthritis:** This is an autoimmune disorder that disproportionately affects women. There is a genetic link to three regions of the major histocompatibility complex, but there are many variations and other factors that can influence the development of this disease. Autoantibodies called rheumatoid factors or RF’s, are produced and localize to the connective
tissues in the small joints. This activates a complement cascade and damages and destroys the joints, causing generalized pain, inflammation, and affecting mobility. The images speak for themselves.

**Slide 42: Myasthenia gravis:** This image shows an individual who is unable to raise his eyelid because the ACh receptors are blocked, preventing the binding of acetylcholine. The disease is characterized by muscular weakness and abnormal fatigability. In individuals under 40 years of age, it tends to be more common in men.

**Slide 43, 44, 45: Immune complex disease/SLE:** Antigens and antibodies can bind together and not be attached to other cells. In situations like this, they form what is called an immune complex. They are capable of activating the complement cascade and can create considerable systemic damage since they are not tethered to a particular cell. In the case of SLE, antibodies are produced against DNA and nuclear proteins, found throughout the body. More and more immune complexes form and get trapped in the kidneys, leading to glomerulonephritis. There are many serious systemic manifestations, but the disease is so varied that it is diagnosed by the presence of 4 symptoms out of a list of 11. This disease is more common in women of childbearing age. Some patients exhibit a rash on the face called a butterfly rash, but this is not the case in all affected individuals.

**Slide 46 & 47: Tolerance:** Sometimes the immune system determines that certain substances are present in either very low quantities, so that they are not important and the immune system can ignore them, or they are present in very high quantities so that the immune system things that they should be there. This is what is referred to as high level vs low level tolerance. In each case, the body will ignore the presence of the substance either by deleting cells that react or turning them off (anergy). This was the premise behind an immune experiment that was conducted on a man named Jeff Getty, who was HIV positive and had AIDS. Baboon cells cannot take up HIV, so, Jeff's entire body was irradiated, destroying all of his bone marrow, and was reconstituted with baboon bone marrow. As you can see from these slides, he lived an additional 11 years and died at the age of 49.