Anatomy and Physiology of Immune System

Supplemental Reading

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Table of Contents

The immune system is tasked with three distinct and interrelated duties. 1) Defense of the body from external invaders (pathogens and toxins). 2) Surveillance in identifying the body's cells that have mutated and may become or have already become neoplasms (tumors). 3) Maintain homeostasis by removing cellular detritus from the system to ensure uniformity of cells and function (Price & Wilson, 2003). Traditionally, immunologists were only concerned with the first duty. It is only recently that the additional tasks of the immune system came to light. In many ways, the immune system can be thought of as the body's policy enforcers. It is responsible for making sure that the body's cells look sharp and do their jobs. Cells that slack or misbehave are destroyed so as not to affect the functioning of other cells. In its role of enforcer, the immune system also makes sure that the functioning of the body's cells are not impaired by foreign invaders. When the body is damaged, the immune system leads the way preparing the injured area for the healing and reparation process. With so much power over the functioning and viability of the body's cells, it is no coincidence that some of our worst diseases come about as a result of immune dysfunction.

Describing the immune system is a difficult task. Although there are relatively clear divisions in immune function, the components that make up these divisions have overlapping roles. Any general statement is sure to have two to three exceptions, and it is practically impossible to describe or define one part of the immune system without using terms that belong in another part and have not yet been defined. After a brief historical introduction, the approach of this text is to describe the overall interaction of the immune system, and then to discuss each of the components in greater detail, and then put the physiology together.

Sidebar

We have known that the inflammatory part of the immune system plays a part in the formation of atherosclerosis (atherogenesis) in arteries for several years. In particular, we knew that macrophages ingested subendothelial cholesterol and became foam cells. In the past four years we have also learned that C-reactive protein levels confirm that inflammation plays a part in atherogenesis and myocardial infarction. It is thought that at least part of the anti-atherosclerotic benefit of statin cholesterol-lowering medications is due to their anti-inflammatory properties. But even more recently, it has been discovered that the specific (acquired) immune system plays a part in atherogenesis. Without lymphocytes, progression of an atherosclerotic plaque cannot occur (Song, Leung, Schindler, 2001; Nicoletti, Caligiuri, Hansson, 2000; Nicoletti, Caliguri, Paulsson, Hansson, 1999). Natural killer cells are also implicated in atherogenesis (Linton, Major, Fazio, 2004; Tupin, et al., 2004; Whitman, Rateri, Szilvassy, Yokoyama, Daugherty, 2004). Essential enzymes in atherogenesis that interact with immune and inflammatory cells are being identified (Boehm, et al, 2004). Most of the experimental research is currently being conducted in animals due to the ethics involved, but human research is also taking place on a limited scale. In the future, assessing immune function will become part of assessing cardiovascular health.

Introduction

A Brief History

Immunology officially began in 1796 with Edward Jenner who discovered that infection with cowpox (vaccinia) induced protection against smallpox in humans. He named the procedure vaccination. Jenner's vaccination worked, but he did not know why. It was not until the late 1800's that Robert Koch proved his germ theory of pathogenesis—a theory rejected by mother of modern nursing, Florence Nightingale (Woodham-Smith, 1996). There are four broad categories of pathogens recognized today: viruses, bacteria, fungi, and parasites.

A fifth category of pathogen may be added in the future: prions, the cause of mad cow disease and Jakob-Creutzfeldt Disease. There is still much debate in the scientific community as to whether prions should be considered as pathogens, mainly because they are not metabolically active nor are they nucleic acid based. Prions are aberrations in normal nervous proteins and are usually transmitted from parent to offspring. However, prions do reproduce, and exposure to prions can and does lead to prion disease, thus qualifying them as an infectious agent. There is currently no known treatment or cure for any prion disease (Janeway, et al., 2004).

The last two decades of the nineteenth century marked an explosion of breakthroughs in immunology beginning with the development of a rabies vaccine by Louis Pasteur and the subsequent discovery of antibodies against tetanus and diphtheria in animal blood serum by Emil von Behring and Shibasaburo Kitasato. At the same time, Elie Metchnikoff was discovering that many microorganisms could be ingested and destroyed by phagocytic cells. He called these specialized cells macrophages. In contrast to the specific responses of antibodies which required previous exposure, macrophages were able to mount an attack against a wide variety of pathogens.

It was apparent that antibodies could be induced against a wide variety of substances. These antibody generating substances were called antigens. It was not known however, where antibodies came from. As late as the 1950's, medical physiology texts labeled lymphocytes as "function unknown." Another component of the immune system, dendritic cells, was only discovered in 1973. Even today, several popular nursing pathophysiology and medical-surgical texts include no mention of dendritic cells. The horizon of immunology is rapidly expanding, but it is still like peering through a haze.

Overview of Immunity

The immune system is generally divided into two large categories, innate and acquired. **Innate immunity**, also called **natural immunity**, is present at birth and functions similarly regardless of the pathogen earning it the designation, "**nonspecific**." **Acquired immunity** refers to immunity that is not present at birth and develops either as a result of exposure or through an external source such as colostrum or injection of immunoglobulin. Acquired immunity is also called **adaptive** or **specific**, because the immune response develops and changes in response to the specific pathogen. Adaptive immune responses are considered either humoral mediated or cell-mediated. **Humoral mediated immunity** refers to immunity that is mediated by B lymphocytes, plasma cells, and antibodies. **Cell-mediated immunity** refers to immunity that is mediated by T lymphocytes.

This simple division between types of immunity is muddied by the interactions between the innate (nonspecific) and adaptive immune systems. The adaptive immune system requires the innate immune system for initial activation. Once activated, however, much of its effector mechanisms involve potentiating innate immune responses. Thus the innate system forms part of the adaptive system's response and vice versa. The innate immune system can eliminate some threats by itself, but many invaders either overwhelm it or evade detection by it. In these cases, the adaptive immune system is required. It takes four to ten days for the adaptive immune system to mount its first response. Once developed however, the adaptive immune system will retain some of its effector cells as memory cells. Upon subsequent exposures, the adaptive immune system can mount a response almost immediately.

The key characteristics of both systems are recognition and effector mechanisms. **Recognition** mechanisms are the methods by which various immune system cells recognize invading cells and toxins or aberrant host cells. **Effector** mechanisms are the methods by which the immune system destroys and eliminates these threats.

The nonspecific immune system relies on receptors that detect common pathogenic features such as bacterial cell wall polysaccharides. Most of these receptors are found on the surface of various white blood cells, but some of them are found on plasma proteins such as complement C1q and c-reactive protein. When one of the receptors is bound to its substrate, it activates a series of reactions that activates the nonspecific immune system and calls white blood cells to the site of injury. This process is called **inflammation** and will be discussed later in the chapter. The specific immune system also relies on receptors, but instead of relying on common pathogenic features, the receptors are designed to respond to only one feature, called an **antigen**. The two main receptors of the adaptive immune system are the T cell receptor (TCR), and antibodies. In a population of lymphocytes, there may be up to 1,000,000 different receptors represented. When an invader is identified, only those lymphocytes whose receptors match the invader are activated (thus the specificity). Antibodies are released by B lymphocytes and contain the same receptors that the manufacturing cell featured. (The B cell receptor is in fact a membrane bound antibody.) The antibodies are plasma borne proteins that serve much the same purpose as the plasma proteins of the innate immune system, except that instead of recognizing common pathogenic features, antibody receptors recognize only their specific antigen.

Antigen

The term antigen originally referred to a molecule that caused antibodies to be generated, but has now been refined to mean any molecule that can bind with a specific antibody. The term **immunogen** refers to any molecule that elicits an immune response. These may include viruses, bacteria, pollen, toxins, foods, transplanted organs, or transfused blood. There is a fine difference between immunogens and antigens, but practically speaking, for most purposes, they can be used interchangeably. See sidebar for more detail.

The bulk of an antigen's surface causes no immune response. Only certain portions of the surface are reactive. These reactive portions are called **epitope** (sometimes also called antigenic determinant). Most antigens have more than one kind of epitope and are called **multivalent**. Other antigens have repeated arrays of the same epitope. Antibodies are produced in response to epitope, not the antigen as a whole. Thus a multivalent antigen may react with more than one

kind of antibody. A given epitope may be present on more than one antigen, so that one antibody may potentially react with more than one antigen.

Sidebar

The difference between immunogens and antigens is that all immunogens are antigens, but some antigens are not immunogens. That is, that some antigens do not elicit an immune response (and thus antibodies) by themselves. These are called **haptens**. In order for a hapten to elicit an immune response, it needs to be bound to carrier molecule (at which point it becomes an immunogen). Two factors influence the ability of an antigen to elicit an immune response. One is the size or weight of the molecule. Smaller molecules tend to cause less reaction. The other factor is the concentration. Very small quantities of an antigen may not cause an immune response. Haptens are clinically significant because once bound to their carrier molecule, the immune system will produce three different kinds of antibodies against them. The first kind of antibody will react to the hapten regardless of whether it is bound or not. The second kind of antibody will react to the carrier molecule regardless of whether it is bound to the hapten or not. The third kind of antibody only reacts to the hapten-carrier complex. Hapten antibodies are important in immunology research, but they are also clinically significant as they form the physiological basis for penicillin allergy cross-reactivity with cephalosporins and other antibiotics. In clinical practice, immunogen and antigen are often used interchangeably, but you should know the distinction.

Self vs. Nonself

In all three of its roles, the immune system's essential requirement is the ability is to distinguish between what is self and what is foreign (nonself). A group of genes responsible for the recognition of self is called **major histocompatibility complex** (**MHC**). The MHC manufactures two major types of MHC proteins that are essential in identifying the body's cells as self. Class I MHC proteins are present on the surface of the cell membrane of almost all host cells with a developed nucleus and platelets. They are also called **Human Leukocyte Antigen (HLA)** because they were first identified on leukocytes. Substances lacking HLA are identified as nonself. Each person has an HLA that uniquely identifies them. As far as science can tell there are no two persons that have identical HLA, although twins may have very similar HLA. MHC class II molecules are found mainly on immune system cells, but can be induced in other cells by interferons. Class I and II MHC proteins also serve to present antigen to T cells. See Lymphocytes below.

Anatomy of the Immune System

Physical Barriers

The human body is constantly surrounded by pathogens in the air, on solid surfaces, and in water. Pathogens are ingested with every meal and inspired with every breath. Before ever encountering an immune system cell, a pathogen must penetrate the body's outer defenses. These consist of barriers—mechanical, chemical, and microbial—that are considered to be part of the innate immune system. In addition to the barriers themselves, each of these areas is populated with members of the innate immune system and often with lymphoid tissue.

The most obvious physical barrier is the skin. The outermost layer consists of several layers of keratinized, water-resistant squamous cells. But skin is made even more formidable by secretions of lactose that lower the pH of skin, making it a less hospitable environment. Perspiration contains salt which can be toxic to pathogens by hypertonic mechanism. Sebaceous glands secrete sebum that helps to trap invaders and actually inhibits some type of bacteria.

With regard to the digestive system, saliva in the mouth forms the first chemical barrier. The pH of saliva, combined with several enzymes, make it an unattractive place to live. The hydrochloric acid and pepsin of the stomach form the next inhospitable atmosphere that invaders will encounter. Bile salts, fatty acids, lysolipids, and other digestive enzymes are found in the small intestine. After making it through this gauntlet of digestive enzymes, the large intestines are thoroughly colonized with a wide array of flora, both bacterial and fungal. There about 400 different kinds of bacteria in the intestines, weighing over a kilogram and outnumbering the body cells ten to one. These native flora make the intestines less hospitable to invaders through competition for space and nutrients.

The respiratory system begins with nasal hair and turbinates in combination with mucous secreting membranes which all serve to trap invading pathogens and may contain IgA antibodies. Once in the bronchus, mucous secretions in combination with a cilia "elevator" serve to bring foreign particles to the karina where the cough reflex helps to expel the invaders from the body. The epthithelium of the lungs secretes two proteins called surfactants A and D which coat pathogens making them more easily phagocytosed.

The eyes are protected by lashes and the blink reflex. Tears, which contain antimicrobial factors including IgA antibody, help to wash out pathogens that make it past the lids and lashes. The vagina is colonized with lactobacillus which secrete lactic acid, lowering the pH of the vagina making it less hospitable. Yeast and lactobacillus compete with potential pathogens for nutrients, further preventing infection.

Leukocytes

The main cells of the immune system are white blood cells collectively referred to as **leukocytes.** Like all blood cells, leukocytes originate from the bone marrow. Stem cells (undifferentiated cells) in the marrow develop into the various white blood cells. In addition to serving as the birthplace for leukocytes, the bone marrow also acts as a reservoir for mature cells that may be needed in event of infection or blood loss. Although most leukocytes originate in the bone marrow along with red blood cells, most spend very little time in the blood. Leukocytes spend most of their time in storage, in lymphoid tissues (see below), or dispersed throughout the host tissues. Leukocytes use blood mainly as a transport system to travel to areas of the body where they are needed. There are six families of leukocytes that have distinct roles in the body's defense. These are the monocyte-macrophages, dendritic cells, mast cells, granulocytes, lymphocytes, and natural killer cells. All the leukocytes except the lymphocytes are considered part of the innate immune system. Lymphocytes are the only leukocytes associated with the adaptive immune system. All the leukocyte families originally come from **pluripotent hematopoeitic stem cells** in the bone marrow. The pluripotent stem cell differentiates into common lymphoid and common myeloid progenitors. All lymphocytes as well as natural killer cells are descended from the common lymphoid progenitor. The common myeloid progenitor differentiates into monocyte, dendritic cells, granulocyte, erythrocyte, and platelet precursors.

(The common lymphoid progenitor can also give rise to a dendritic cell that is indistinguishable from the myeloid derived dendritic cell.)

It is imperative to understand **progressive differentiation** in order to understand leukocytes. The leukocytes found in the blood and lymph tissues are typically not fully differentiated. As a case study, monocytes descend from the common myeloid progenitor as discussed above. Monocytes circulate in the blood until summoned to the tissues. At this time, they exit the blood vessels through specialized openings in the vessel wall and enter the tissue. Once in the tissue, monocytes differentiate yet again, maturing into macrophages which usually live in the tissues until their death. Thus the macrophage is the monocyte's final differentiation and the monocyte is simply a relatively inert circulation form of the cell. Another way to think of progressive differentiation is to think of the monocyte as an observation form and the macrophage as the functional form. Most other leukocytes also undergo progressive differentiation. The exception is the granulocytes which circulate in fully differentiated form.

Proliferation is the other concept necessary to understand some white blood cells. Although lymphocytes originate in the bone marrow from stem cells, they are also able to reproduce within lymph tissue. When activated, lymphocytes will proliferate (reproduce) first, then differentiate into their final functioning form. This allows the few cells that are able to respond to a given invader to reproduce quickly without a corresponding increase in lymphocytes that are not needed for the present threat.

Monocyte-Macrophages

Monocytes are leukocytes found in relatively small quantities in the blood, because most of them are either in the tissues or stored in the bone marrow. Arising from the common myeloid progenitor, the majority of monocytes remain in the marrow serving as a reservoir against infection. The immature stage is referred to as monocyte, while the fully differentiated stage is called a **macrophage**. Monocytes are continuously migrating to tissue and differentiating into tissue macrophages. Tissue macrophages are called different names, depending on the tissue in which they have differentiated. Tissue macrophages in the nervous system are called microglial cells, while macrophages in the liver are call Kupffer cells. No matter where they differentiate, tissue macrophages serve the same function—to monitor the surrounding tissue for invaders and foreign antigen. Collectively, they are sometimes referred to as mononuclear phagocytes.

Macrophages are one of three **phagocytic** cells in the immune system. Having differentiated in tissues, macrophages are relatively immobile, monitoring the nearby tissue for invaders. Macrophages have receptors for a wide variety of common pathogen features such as the glucan receptor and mannose receptor, scavenger receptor which binds to negatively charged ligands which are components of many Gram-positive bacterial cell walls, and the CD14 (LPS) receptor which detects bacterial lipopolysaccharide. Upon detecting an invader, macrophages attempt to engulf the invader in an amoeboid-like process called **phagocytosis**. The cell membrane distorts and wraps around the particle until the two sides of the cell membrane touch. The cell membrane edges fuse themselves together and the particle is encased in a vesicle made of membrane that was formerly part of the cell's outer membrane. This vesicle is called a **phagosome** or endocytic vacuole. Lysosomes containing destructive enzymes are then fused with the phagosome and the enzymes are released into the phagosome. The phagosome-lysosome complex is called a phagolysosome.

Macrophages are **antigen presenting cells (APCs)** and act as one of the first responders in the immune response process. Once activated, a macrophage releases cytokines and chemokines. **Cytokines** affect the way other cells act. (Cyto- "cell" and –kinein "move") **Chemokines** attract other leukocytes the area to battle the invaders in a process called **chemotaxis**. See table 42-1 for a list of selected cytokines released by macrophages and their effects. Because macrophage recognition of pathogens is so important, one of the key distinguishing features of pathogenic microbes (as opposed to non-pathogenic microbes) is the ability to overwhelm or evade macrophages and other segments of the innate immune system. For example, some bacteria coat themselves in a thick polysaccharide that is not recognized by macrophage or neutrophil receptors. Other pathogens, such as mycobacteria, can actually live and multiply inside of phagosomes by keeping the lysosomes from fusing with the phagosome.

One unique characteristic of macrophages is the ability to form giant multi-nucleated cells. When confronted with an overwhelming opponent, several macrophages can join together to form one large cell, the afore mentioned giant multinucleated cells. This allows macrophages to engulf invaders that they other wise could not engulf.

Insert Table 42-1; Important cytokines released by macrophages and their effects.

Cytokine	Local effect	Systemic effect
IL-1□	Activates endothelium Activates lymphocytes Local tissue destruction Increases access of effector cells	Fever Production of IL-6
TNF-□	Activates endothelium and increases vascular permeability, leading to increased entry of IgG, complement, and increased fluid drainage to lymph nodes.	Fever Mobilization of metabolites Shock
IL-6	Lymphocyte activation Increased antibody production	Fever Induces acute-phase protein production
CXCL8	Chemotactic factor recruits neutrophils, basophila, and T cells to site of infection	
IL-12	Activates natural killer cells Induces the differentiation of CD4 T cells into T_H1 cells.	

Dendritic cells

Dendritic cells are star-shaped cells that are so called because they resemble a neuron's dendrites. While we have been studying macrophages for more than a hundred years, we have only known about dendritic cells for less than thirty-five years, and there is still much that we do not know about them. Their lifecycle is more complicated than that of macrophages. The immature dendritic cells migrate to tissues, particularly the skin, airway, spleen, and lymph nodes. Like tissue macrophages, tissue dendritic cells are called different names depending on the tissue in which they live. Tissue dendritic cells that live in the skin are called Langerhans

cells. (Skin tissue macrophages are also called Langerhans cells.) Immature tissue dendritic cells are both phagocytic and **macropinocytic**; that is, they can ingest large amounts of surrounding intersitial fluid. Tissue dendritic cells break down proteins and display the ingested antigens on their cell membranes. At the end of their life cycle, they will migrate to lymph nodes and induce tolerance in lymphoctyes, because they do not have co-stimulatory molecules in their immature stage. The signals for maturation are either direct contact with a pathogen or inflammatory cytokines. Pathogens are ingested when they are recognized by their common features as described above. Macropinocytosis allows the dendritic cell to ingest pathogens that have some mechanism to escape detection by phagocytic receptors. As the products are degraded inside the dendritic cell, it is able to recognize bacterial DNA, bacterial heat shock proteins, and viral double stranded RNA. Once activated, they differentiate into mature dendritic cells, develop co-stimulatory molecules, and migrate to the lymph nodes to activate the lymphocytes that migrate through the nodes.

Mature dendritic cells carry high levels of Major Histocompatibility Complex (MHC) on their cell membranes in order to present antigen to T lymphocytes. When the T cell with the right receptor recognizes the presented antigen, it proliferates and differentiates. The truly amazing thing about dendritic cells is that they are able to activate only the specific T lymphocytes that are needed to respond to a given invader, whether it is a virus, bacteria, or fungus. In some cases, this may mean activating just one in 10,000 or one in 1,000,000 T lymphocytes.

The dendritic cell's strength is also a key weakness exploited by several viruses, such as HIV and measles. Instead of activating lymphocytes in lymph nodes against these viruses, the infected dendritic cell acts as a transportation system, allowing the virus to then infect the T lymphocytes.

Much of the extracellular debris that is ingested by dendritic cells is harmless, often byproducts of dead body cells. Dendritic cells are essential in inducing and maintaining tolerance to these antigens, keeping the immune system from reacting to the body's antigens (Steinman & Nussenzweig, 2002). As T lymphocytes exit the thymus gland, dendritic cells are responsible for destroying cells that are reactive to self-antigens. This process is referred to as central tolerance and removes the majority of self-reactive T lymphocytes. Dendritic cells also induce peripheral tolerance, suppressing self-reactive lymphocytes that escaped central tolerance or cells that are reactive to antigens not expressed in the thymus.

Sidebar

Dendritic cells are seen as the missing key in many immunological disorders. Dendritic cell infection is crucial in defeating the body's defenses against several viruses, including Ebola and HIV (Geisbert, et al., 2003; Janeway, et al., 2004). Both these viruses neutralize dendritic cells keeping the body from mounting a defense against them. By the time the immune system is mobilized, it is often too late. It is hoped that understanding dendritic cells will aid in the treatment and possible vaccination against these diseases. Dendritic cells are also implicated in tumor formation and research is being conducted to see if dendritic cells can be manipulated in a way as to be a kind of "vaccine" against cancer cells. In a cancer vaccine, dendritic cells would be harvested from the patient's body and cultured. Once a thriving culture has been established, tumor cells from the patient are introduced to the culture. The primed dendritic cells are then injected back into the patient where they initiate the immune response against the cancer. Trials are currently underway testing this technique in melanoma, lymphoma, prostate cancer, and

colon cancer. Because of the large expense involved in developing a dendritic culture for each patient, additional research is being done to try and up-regulate dendritic cells in the body. In the opposite direction, research is being done in the areas of immune down-regulation. It is hoped that dendritic cell research will be able to provide effective cures for some autoimmune diseases and transplant rejection (Fecci, et al., 2003).

Mast Cells

Mast cells are also descended from the common myeloid progenitor and differentiate in the tissues. Their blood borne precursor is currently unknown. Mast cells tend to live near the skin and connective of small blood vessels and contain **granules** with stored chemicals. When activated, they release substances within the granules (**degranulate**) that affect vascular permeability, particularly histamine. See table 42-2 for a list of mast cell products. Mast cells are thought to play an important part in protecting mucosal surfaces from pathogens and help the inflammatory process to begin the process of healing damaged tissue, although they are primarily known for their role in IgE-mediated allergic reactions. In fact, mice that do not have fully differentiated mast cells cannot produce IgE mediated inflammatory responses.

Insert Table 42-2. Molecules released by mast cells on activation.

Class of product	Examples	Biological effects
Enzyme	Tryptase, chymase, cathepsin G, carboxypeptidase	Remodel connective tissue matrix
Toxic mediator	Histamine, heparin	Toxic to parasites Increase vascular permeability Cause smooth muscle contraction
Cytokine	IL-4, IL-13	Stimulate and amplify T_H2 cell response
Cytokine	IL-3, IL-5, GM-CSF	Promote esosinophil production and activation
Cytokine	TNF-α	Promotes inflammation, stimulates cytokine production by many cell types, activated endothelium
Chemokine	CCL3 (MIP-1α)	Attracts monocytes, macrophages, and neutrophils
Lipid Mediator	Leuktrienes C4, D4, E4	Cause smooth muscle contraction Increase vascular permeability Stimulate mucous secretion
	Platelet-activating factor	Attracts leukocytes Amplifies production of lipid mediators

		Activates neutrophils, eosinophils, and platelets

Adapted from Janeway et al., 2004.

Granulocytes

The **granulocytes**, are so called because when stained, they have granule shaped objects visible within their cytoplasm, much like mast cells. They also have lobed irregular nuclei, earning the designation **polymorphonuclear leukocytes (PMNs)**. The granules are lysosomes—vesicles filled with destructive enzymes. These enzymes are used to destroy invaders. Neutrophils are the most numerous granulocyte and thought to be the most important. Neutrophils are the third and final phagocytic cell in immune system. Upon engulfing an invader, the granules are fused to the vesicle and the enzymes are released into vesicle—hopefully destroying the particle. Neutrophils are especially reactive to bacteria, and the number of circulating neutrophils greatly increases during bacterial infections. **Neutrophils** are the first responders to chemotaxis, and are rarely found in healthy tissue. Neutrophils are relatively fragile compared to macrophages. They can only ingest a few bacteria before dying, while macrophages can ingest a hundred bacteria. Pus is mostly made up of bacteria and dead neutrophils. Because of their expendable nature, they appear in the blood in large numbers, with several times that number in reserve in the bone marrow. They are the most numerous granulocyte and often the most numerous leukocyte. Deficiency in neutrophils, called **neutropenia**, can cause overwhelming bacterial infection.

The other two classes of granulocyte cells are **exocytic**, meaning they produce their effects on outside cells as opposed to phagocytosed cells. **Eosinophils** are found in small quantities in the blood as most of them are distributed in the tissues. Their primary effector function is to release their highly toxic granules that can kill parasites and other microorganisms. They also produce cytokines, leukotrienes, and prostaglandins. Eosinophils are involved in defense against parasites and increase in numbers when the body has a parasitic infection. They are most well known for their role in IgE mediated allergic reactions and are often present in mucous secretions during allergic reactions.

Basophils, are final and most inscrutable granulocyte. Not much is known about them, but they appear to have an effect against fungus and also play a role in inflammation. They behave very similarly to eosinophils and are distributed throughout the tissues.

Natural Killer Cells

Natural killer (NK) cells arise from the common lymphoid progenitor. They appear as large lymphocytes with cytoplasmic granules and circulate in the blood. Although lacking antigen specific receptors, they are able to detect and attack a limited number of abnormal cells such as tumor cells and cells infected with the herpes simplex virus. They are also able to kill cells that are coated in antibody, a process known as antibody-dependent cell-mediated cytotoxicity (ADCC) and is mediated by the Fc receptor (see Antibody below). Natural killer cells are also activated by interferons and macrophage-derived cytokines.

Lymphocytes

There are two major types of lymphocytes, T lymphocytes and B lymphocytes, or simply T cells and B cells. All lymphocytes originally descend from the common lymphoid progenitor, but

differentiate differently depending on where they mature. Some lymphocytes mature in the bone marrow, while others migrate to the thymus for maturation. **B lymphocytes** (also called B cells) are so called because they mature to their intermediate stage in the bone marrow. When activated, B lymphocytes complete their differentiation process and become plasma cells, releasing antibodies. **T lymphocytes** (T cells) are so called because they mature in the thymus. T cell development is more complex than that of B cells. The first division of T cells is based on receptor chains. Most T cells have receptors consisting α and β chains, but a second division T cells have receptors made of γ and δ chains. These are called α:β (alpha:beta) and γ:δ (gamma:delta) T cells respectively. The α:β T cells eventually become CD4 and CD8 T cells. The function of γ:δ T cells is poorly understood, but they appear to function as innate immune cells, rather than adaptive immune cells. (Similarly, there is a line of B cells, called B-1 cells, that also function like innate immune cells.)

Most inactivated lymphocytes are small and rather featureless with inactive nuclear chromatin. As late as the 1960's, many textbooks described these cells as having no known function. Indeed, lymphocytes do show very little activity until activated by the presence of antigen and co-stimulatory molecules, usually presented by an antigen presenting cell such as a macrophage or dendritic cell. Upon activation, lymphocytes differentiate into lymphoblasts which undergo mitosis and then differentiate into the final activated phase taking on their specialized functions. Once the infection has been eradicated, most of the lymphocytes that were produced as a result of lymphoblast proliferation undergo **apoptosis** (programmed cell death), however, a few remain as memory cells enabling the body to respond rapidly to subsequent infections by the same pathogen.

The main functional characteristic of lymphocytes is the ability to mount specific immune responses against virtually any foreign antigen. All lymphocytes have a prototype receptor that changes during the intermediate maturation process so that taken as a whole, they are able to react with almost any possible antigen. The B cell and T cell receptors are closely related in structure, but very different in function and will be discussed in more depth below. The **B cell receptor (BCR)** actually consists of the antibody that the B cell will release when activated and can recognize only one specific antigen. B cell receptors (and antibodies) are only able to detect antigen that is in the extracellular fluid. The **T cell antigen receptor (TCR)** is structurally similar to the BCR, but does not recognize whole antigens. Rather it detects fragments of antigen that are displayed by MHC molecules on the surface of host cells. Thus the T cell, detects antigen within the host cells such as viruses that have commandeered a cell's processes or a parasitic bacteria. Variability in the BCR and TCR is attained by mutation of the genes responsible for their production.

B Lymphocytes and Antibodies

B cells are lymphocytes that develop in the bone marrow. Their primary job upon activation is to produce antibodies. B cells develop in the bone marrow until they express the IgM molecule on their cell surface. Once the IgM molecule is expressed the immature B cell undergoes self tolerance testing and viability testing. B cells that bind to self antigen suffer one of four fates. The first is immediate destruction by induced apoptosis (cellular suicide); the second is receptor reconfiguration and retesting; the third is an induction of a permanently unresponsive state (anergic cells); and the last is ignorance. Apoptosis is most common when the interactive self

antigen is multivalent; this process is called clonal deletion. Anergic cells are released into the circulation but cannot be activated by any means and soon die. Ignorant cells are cells that bind to self antigen, but the antigen is either produced in small quantities or typically not available (perhaps bound in the center of a protein complex). Although the chances are small, ignorant cells can and do become activated, causing host damage. Upon passing these tests, the immature B cell migrates to the secondary lymph tissues, where it develops into a mature B cell, expressing both IgM and IgD molecules (see below). Mature B cells, also called naïve B cells because they have not encountered their specific antigen yet, recirculate through the lymph tissues waiting to encounter their antigen and become activated. Upon activation, B cells proliferate and then become plasma cells, secreting antibodies.

Antibodies are the B cell's raison d'etre. The only immune function of the B cell is to release antibodies when activated. Unlike T cells which venture out of the lymph nodes when activated, plasma cells stay in the lymph node, secreting antibody to be delivered to the systemic circulation. Antibody itself is a molecule composed of two segments, a recognition/binding segment, and an effector segment. The recognition segment binds to the antigen, and the effector segment activates other components of the immune system. Thus antibodies may neutralize threats directly by physically binding to them and keeping them from damage. At the same time, antibodies recruit other components of the immune system to attack and destroy the threat.

Antibodies are a category of protein called **immunoglobulins (Ig)**. All immunoglobulins share a similar structure. They are generally "Y" shaped molecules, with two recognition segments and one effector segment. The recognition segment is called the variable region or V region, because it changes from antibody to antibody to ensure that a wide range of antigens can be recognized. The variability of the V region can be further enhanced in the inactive B cell through actively mutating the gene responsible for V region production—a process called **somatic hypermutation**. This process takes place in the peripheral lymphoid tissues only in the presence of activated T cells. The effector segment of antibodies is called the constant region or C region, because it does not change from one antibody to the next. The arms of the Y are formed by the V regions, while the tail is formed by the C region. The three arms are bound by flexible disulfide bonds. The bonds can be cleaved, and the separated segments will function separately. The end of the V region that contains the antigen receptors is called the V terminus. The end of the C region is called the C terminus. The backbone of the immunoglobulin is formed by heavy chains. The outer portion is formed by light chains. The light chain only contains variable regions, while the heavy chain contains both constant and variable regions. See figure 42-1. The C region heavy chain (C terminus) varies with the class of immunoglobulin. There are five main classes of immunoglobulins—IgM, IgD, IgG, IgA, IgE—which are distinguished by their C terminus. The BCR is nothing more than IgM with the C terminus remaining in the cytoplasm of the B cell.

Antibodies bind to the epitope of their antigen with a lock-and-key mechanism. This means that grooves in the epitope fit ridges in the antibody and vice versa. The bond between antigen and antibody is not covalent and be can be disrupted by changes in pH, hypertonicity, detergents, and even high concentrations of epitope (i.e., the antibody breaks away from the epitope to bind to another nearby epitope). The strength of antibody-antigen complex is determined by the antibody's affinity for the antigen. Antibodies with high affinity will form stronger bonds.

The different immunoglobulins perform different effector functions in the body. B cells will be selected secrete more of a given immunoglobulin depending on the type of immune response and the location in the body of the activated B cell. IgG is the most abundant immunoglobulin in the body and is further divided into four subtypes (IgG1, IgG2, IgG3, IgG4), where IgG1 is the most abundant in plasma and IgG4 is the least abundant. IgA is divided into two subtypes (IgA1, and IgA2). IgM and IgA can form polymers in the blood. IgM forms a pentameter, a molecule composed of five IgM antibodies joined by their C terminuses (see figure 42-2). IgA appears in the blood as both a monomer (single antibody) and a dimer (two antibodies joined at the C region) (see figure 42-2).

Naïve B cells express both IgM and IgD on their surface. When activated, the B cell will produce IgM and IgD antibodies. However, later in the immune response, the B cell will change to producing IgG, IgA, or IgE by irreversibly recombining its DNA. At this point the cell can no longer produce IgM or IgD antibodies. The signal to switch antibody production is mediated by cytokines and T cells. Remember that an activated B cell proliferates before differentiating and producing antibodies, so it is likely that all antibody classes will be represented although not in equal quantities.

Antibodies work by four basic functions, neutralization, opsonization, activation of inflammation, and activation of complement. **Neutralization** accomplished solely by the variable region of the antibody, and its effectiveness is determined by the antibody's affinity for the antigen. Its mechanism is the complete binding of an antigen by the antibody, so that there are no available binding sites, effectively rendering the invader inert. This process is especially important for bacterial toxins and viruses. A special case of neutralization is agglutination. This occurs when the arms of the antibody bind to the same epitope on different antigens. For example, the IgM pentameter has ten binding sites, and could theoretically bind ten different bacteria that share the same epitope. This causes clumping, called **agglutination**. The second mechanism is called **opsonization**. When antibodies coat a bacteria or other pathogen, it can induce nearby macrophages to engulf the pathogen. This is especially important against bacteria that have natural defenses to keep macrophages from engulfing them. The third mechanism is the activation of inflammatory processes, including the activation of natural killer cells. The fourth mechanism is the activation of the complement cascade. Complement is a cascade of lytic proteins that are activated by antibodies. The activation of complement by itself can cause the death of some invaders, but it is always a signal to nearby phagocytic cells to attack the pathogen.

The C regions of the immunoglobulin classes endow them with their unique effector abilities. See table 42-3 for a summary of effector functions of various immunoglobulins. The effector regions of immunoglobulins are also called Fc portions. The Fc portions of IgG1 and IgG3 are recognized by Fc receptors on macrophages and neutrophils, inducing them to engulf pathogens covered with the IgG. The Fc portion of IgE antibodies can bind to mast cells which release inflammatory mediators. The Fc portion of antibody is what binds to complement to activate it. The Fc portion of antibodies also has a very specialized role in active transport. This allows antibodies to appear in places they otherwise could not. When IgA comes into contact with certain mucous membrane receptors, they are actively transported across the membrane. This allows IgA to be present in tears to protect the eyes, and in milk to endow breast-fed infants with an initial acquired immune system, while their own is developing. IgG antibodies also use this

mechanism to cross the placental barrier to enter the fetal circulation to protect the child in utero. Because of Fc effector threat, some pathogens have developed proteins that bind to Fc portions or destroy them in an attempt to keep them from carrying out their effector function. Staphylococcus aureus produces Protein A for this purpose.

Table 42-3 Properties of immunoglobulin isotypes. IgD is not shown because it has no known function.

Functional Activity	**IgM**	**IgG1**	**IgG2**	**IgG3**	**IgG4**	**IgA**	**IgE**
Neutralization	+	++	++	++	++	++	-
Opsonization	+	+++	+	++	+	+	-
Sensitization for killing by NK cells	-	++	-	++	-	-	-
Sensitization of mast cells	-	+	-	+	-	-	+++
Activation complement	+++	++	+	+++	-	+	-
Distribution							
Transport across epithelium	+	-	-	-	-	+++	-
Placental Transfer	-	+++	+	++	+-	-	-
Diffusion into extravascular sites	+-		+++	+++	+++	++	+
Mean serum level (mg/ml)	1.5	9	3	1	0.5	2.1	.00003

Adapted from Janeway, et al., 2004

Legend: pluses indicate a positive effect, minuses indicate an inhibiting effect. The number of pluses or minuses indicates a stronger effect.

In an immune response IgM is the first antibody to be released. IgM has a fairly low binding affinity for epitope, and it is believed that the ten binding sites of the pentameter provide a higher effective affinity for repetitive epitope such as bacterial capsule molecules. Essentially it allows the IgM molecule to stick to antigen longer until higher affinity antibodies can be manufactured. Although IgM appears in the blood as a flat pinwheel, upon binding to an antigen, the other binding sites bend toward the antigen surface like spider legs. The pentatmeric nature of IgM also makes it a particularly potent activator of the complement cascade. Thus IgM is an excellent first responder.

IgG, IgA, and IgE are produced later in the immune response. They are smaller than IgM and can diffuse relatively easily out of the blood and into the tissues. IgG is the principal antibody found in the blood and tissues, while IgA is principally found in secretions such as tears and saliva. IgG is an excellent opsonin, but IgA is not. This makes sense, as IgA works on epithelial surfaces that do not normally contain complement or phagocytes. These antibodies are produced and secreted close to where they will function. IgE is found only in very low levels, but has an extremely high affinity for mast cells and binds to mast cells even before it binds to antigen. Antigen binding to this mast cell-associated IgE triggers mast cells to degranulate and release their inflammatory mediators.

T Lymphocytes

T lymphocytes progenitors leave the bone marrow and migrate to the thymus gland where they develop into T lymphocytes instead of B lymphocytes. The T cells later develop into CD4 and CD8 T cells. CD4 and CD8 are surface proteins on the membranes of T cells. For years, CD8 has marked **"cytotoxic" (cell-killer) T cells**, and CD4 has marked "whelper" T cells, which further differentiate into two subclasses, **T_H1** and **T_H2** cells, upon activation. T cells do not produce an immune response by binding to an antigen. The antigen must be displayed by MHC. CD4 binds to MHC II, while CD8 binds to MHC I. Thus CD4 and CD8 are called co-receptors. MHC I is used to present pathogen antigen, typically viruses and cellular parasites, to CD8 cells. Because viruses can infect any nucleated cells MHC I present on almost all nucleated cells. (See sidebar.) Upon binding to antigen and MHC I, the CD8 cytotoxic cell is programmed to destroy the cell, preventing further infection. In contrast, CD4 cells are activators of other effector cells. MHC II is not usually expressed on host cells, but only other leukocytes, such as B cells, macrophages, and dendritic cells. When CD4 T cells bind to MHC II on B cells, they stimulate the B cell to produce antibodies. When they bind with MHC II on macrophages, they activate the macrophages to destroy the cells in their phagosomes. (Recall that some bacteria multiply in macrophage phagosomes.) CD4 cells are stimulated to become T_H1 cells by the accumulation of pathogens inside macrophage and dendritic cell vesicles, while large amounts of extracellular antigen tend to stimulate differentiation into T_H2 cells. T_H1 cells activate the microbicidal properties of macrophages and induce B cells to produce IgG antibodies which act as opsonins for macrophages. Both forms of helper T cells initiate the humoral immune response by inducing naïve B cells to activate and produce IgM antibodies. T_H2 cells can then direct the activated B cells to switch from IgM to IgA, IgE or IgG antibodies.

During development, T lymphocytes go through two selection process. **Positive selection** encourages lymphocytes that bind weakly to self antigens. While **negative selection** eliminates lymphocytes that bind strongly to self proteins. The reason for this twofold process is that lymphocytes must be able to bind with MHC I and II (self antigens) in order generate an immune response, but lymphocytes that bind too strongly could possibly cause autoimmune disease. The MHC antigens that the T lymphocytes recognize as self are determined the by the MHC antigens present in the thymus gland.

Red blood cells do not express MHC I. Because they have no nucleus, viral infection poses no threat to them. However, this does make them an appealing target for other cellular parasites such as Plasmodium, the organism responsible for malaria. The infected red blood cell has no way to present its infection to the immune system, and the parasite is able to flourish until such time as it leaves the cell.

Each naïve T lymphocyte can detect only one specific antigen, and it wanders the body's lymph nodes in search of its antigen. If it finds it, then proliferates and differentiates into its active phase. It takes more than simply meeting its specific antigen to activate the naïve T cell. The T cell must meet its antigen while being displayed by an antigen presenting cell that also displays co-stimulatory molecules. Naïve CD8 T cells always differentiate into cytotoxic T cells, but require more co-stimulation than CD4 cells to do so. Dendritic cells are always able to provide the needed co-stimulation, but other antigen presenting cells are not. If an activated CD4 helper cell binds to the antigen presenting cell, it can induce the APC to produce additional co-

stimulatory molecules which enables it to activate the CD8 T cell. The activated T cells, whether CD8 or CD4 must be able to bind with first an antigen presenting cell, and later with their target cell. The main two molecules involved are surface proteins LFA-1 and CD2. Primed T cells express much more of these molecules on their cell membranes that naïve T cells. CD8 T cells can bind to their target cell, degranulate and destroy it, detach, and move to the next target relatively quickly. CD4 T cells must bind to macrophages or B cells for relatively long periods of time, perhaps days, in order to activate them. This binding allows the T cell to focus its attention on the target cell, actually reconfiguring the structure of its organelles so that its effectors are close to the target cell. The binding also acts as a seal, so that the effector molecules released by the T cell affect only the target cell. Cytotoxic T cells release **cytotoxins** as their primary effector molecule, but also release cytokines that have an effect on nearby tissues. T_H1 and T_H2 cells do not produce cytotoxins; their primary effector molecules are cytokines and membrane associated proteins. Membrane associated proteins are essentially cytokines that are bound to the T cell's membrane and not released. Thus, they affect only the cell that the T cell is bound to. Table 42-4 summarizes the cytotoxins and cytokines released by T cells.

Table 42-4 Primary effector molecules produced by primed T cells

Cytotoxic CD8 cells	T_H1 **Cells**	T_H2 **Cells**
Cytotoxic Molecules: Perforin Granzymes Granulysin Fas ligand	Macrophage activating: IFN-γ GM-CSF TNF-α CD40 ligand Fas ligand	B cell activating: IL-4 IL-5 IL-15 CD40 ligand
Other cytokines released: IFN-γ TNF-β TNF-α	IL-3 TNF-β IL-2 CXCL2	IL-3 GM-CSF IL-10 TGF-β CCL11 CCL17

Primary Lymphoid Organs

Anatomically speaking, the immune system is largely identified with the lymphoid portion of the immune system. The primary lymphoid organs are the bone marrow and thymus gland because lymphocytes develop and mature within them. The thymus gland is located superior to the heart. The thymus gland also serves as a reservoir for T lymphocytes. It is believed that the thymus gland's major function is in the development of the immune system. It is larger in children than in adults. Removal of the thymus in children causes a reduction in the number of T lymphocytes and a higher number of granulocytes (Eysteinsdottir et al., 2004). The effects of the removal of the thymus gland in adults does is not well understood and only recently has begun to be studied (Haynes, Sempowski, Wells, & Hale, 2000). New evidence shows that the thymus is active in adults and that efforts should be made to preserve it during cardiothoracic surgery (Halnon et al., in press).

Secondary Lymphoid Tissue

Although lymphocytes are distributed throughout the body, they are concentrated in several tissues. The tissues where they aggregate and function are called secondary lymphoid tissues, and include the spleen, lymph nodes, and epithelial lymphoid tissues. Secondary lymphoid tissues are strategically placed in the body so that invading pathogens will encounter them as early as possible, allowing the immune system to be activated before extensive damage can be done.

Spleen

The spleen is a fist-sized organ located on the left side of the body, behind the stomach. It acts as a filter, collecting antigen from the blood and destroying senescent red blood cells. Most of the spleen is made up of tissue called red pulp which primarily serves as the site of red blood cell destruction and also houses macrophages. Interspersed throughout the red pulp, lymphocytes surround artieroles forming pockets called white pulp. The organization of white pulp consists of two layers, the periarteriolar sheath, consisting mainly of T lymphocytes, and the B-cell corona, consisting of mainly B lymphocytes. The white pulp is responsible for generating immune responses to blood borne immunogens and plays an important role in preventing septicemia. Removal of the spleen often results in life-threatening infections known as overwhelming post-splenectomy infections (OPSI) (Jirillo et al., 2003; El-Alfy & El-Sayed, 2001).

Lymph Nodes

The lymph nodes are encapsulated lymphoid structures located throughout the lymphatic vascular system and provide the tissues and lymph with the same function that white pulp of the spleen provides for blood. Ranging in size from 1 mm to 20mm, lymph nodes are responsible for generating immune responses to the immunogens in the lymph drainage and interstitial fluid that drains from local tissues into the lymph vessels. Lymph nodes are typically bean shaped with two layers, an outer cortex and an inner medulla. Several afferent lymphatic vessels enter into the cortex which is separated into several compartments called follicles. Each follicle leads to the medulla where the lymph fluid is consolidated and one larger efferent lymphatic vessel exits from the medulla. The medulla is also associated with an artery and vein that is used for incoming naïve lymphocytes. The lymph nodes also act as a pump for lymph fluid, activated by random skeletal muscle contraction.

Lymph node follicles are divided into several distinct regions. The outer portion of the follicle is made up mostly of B cells. During an immune response, areas of intense B cell proliferation are called **germinal centers**. Follicles that do not contain germinal centers are called **primary lymphoid follicles**. Once a germinal center has been established, it is called a **secondary lymphoid follicle**. Primary follicles contain inactive B cells surrounding a specialized cell of uncertain origin, called a follicular dendritic cell (FDC). The FDC secretes chemokines that attract both inactive and active B cells. The next section of the cortex is called the paracortical area and is mostly made up of T lymphocytes. The third part of the cortex which is closest to the medulla is made up of macrophages and antibody-secreting plasma cells and is called the medullary cords.

Lymph nodes are designed so that antigen presenting cells from the tissues will come into the lymph node through the afferent lymphatic vessel and encounter B lymphocytes first, then T lymphocytes, and will then take up residence in the medullary cords. This ensures that it will encounter both kinds of lymphocytes, and if the lymphocyte with the specific antigen it is presenting is not present, as that lymphocyte recirculates through the body, it will encounter it in the medullary cords. The recirculating naïve T lymphocytes enter the node through the arteriole using special **adhesion molecules** called L-selectin which allows them to stick to the artery's surface. Activated B cells remain in the lymph node and form germinal centers, but activated T lymphocytes need to travel to the site of infection. When T cells mature, they lose their L-selectin, so that they can no longer enter lymph nodes through the artery. (Systemic artery endothelium expresses other adhesion molecules so that mature T cells can adhere to the artery wall of infected tissue.) T_H1 cells either remain in the lymph node or eventually journey back to the lymph node to activate the B cells and macrophages found there, but will do so through the lymphatic vessels.

Epithelial Lymphoid Tissues

In addition to lymph nodes, there are also patches of unencapsulated lymphoid tissue located throughout the body in connective tissue. The gut-associated lymphoid tissues (GALT) include the tonsils, adenoids, Peyer's patches in the small intestine, and the appendix. GALT collects antigen from the surface of epithelial cells in the digestive tract. Peyer's patches are the most organized of the GALT and consists of a B cell center surrounded by smaller numbers of T cells. Specialized epithelial cells called multi-fenestrated cells collect the antigen from the lumen of the small intestine. Similarly, bronchial-associated lymphoid tissue (BALT) and mucosa-associated lymphoid tissue (MALT) provide the same functions in the bronchial tree and other mucosa.

Chemical Components

In addition to the leukocytes and antibodies, there are also a number of chemicals that make up the immune system. Many of these are secreted by leukocytes, but some are not. Chemical components serve several different functions. Two of these functions have already been discussed briefly in the leukocyte section—attracting cells and changing cell behavior. Chemicals that attract other leukocytes to the area are called chemokines. Chemicals that change the behavior of other cells are called cytokines. Some cytokines may induce vasodilation or increase vascular permeability. Other cytokines may activate leukocytes. The third function of chemical components is called opsonization. Opsonization is the process whereby a pathogen is coated so that phagocytosis is easier. In some cases, phagocytosis may not occur in the absence of opsonization. The lung surfactants discussed under Physical Barriers serve as opsonins as do antibodies. A fourth function of chemical components is pathogen or toxin neutralization and/or direct destruction (effector mechanisms).

Cytokines

Cytokines are small proteins that affect the behavior of cells. The cytokines may act in an autocrine manner (affecting the cell that secreted it), paracrine manner (affecting adjacent cells), or even endocrine manner (affecting distant cells). The ability of a cytokine to act on distant cells depends on its ability to enter the blood and how long it stays in the blood (half-life). An important concept in understanding cytokines is that of kinases and kinase inhibitors. These

enzymes destroy cytokine and preserve cytokine respectively. Each cytokine has its own set of kinases and kinase inhibitors which are important in the regulation of immune responses. Some diseases may not have anything to do with under or overproduction of cytokines, but rather problems with these regulatory proteins. Too much kinase or too little kinase inhibitor will result in abbreviated immune response, while too little kinase or too much kinase inhibitor will result in prolonged immune response.

When cytokines were first being discovered, they were named **interleukins** (IL), to signify that they were secreted by a leukocyte. Over time, it became apparent that the cytokines are a diverse group of molecules structurally and behaviorally. Newer nomenclatures are being developed that group the cytokines according to their structure or function. Different cytokines are released by leukocytes in response to different pathogens. For example, TNF-α (tumor necrosing factor-α) is released by macrophages in response to LPS bearing bacteria. (The effects of TNF-α are discussed in Macrophages, and Innate Immune Response and Inflammation.) See table 42-5 for a summary of common cytokines and their functions.

The interferons (IFN) are a class of cytokine that was so named because it interfered with viral replication in cells that were previously uninfected. The first two interferons discovered, IFN-α and IFN-β, are similar in function but differ substantially from the third interferon, IFN-γ. IFN-γ is produced later in the immune response and is not induced by viral infection, but does have a role in the adaptive immune response to intracellular pathogens. IFN-α and IFN-β are both induced by viral infections. They are produced by many cell types after becoming infected with a virus. It is thought that the presence of double stranded RNA is the stimulus for their production. Interferons bind to nearby cells through an interferon receptor which induces the cell to produce a variety of proteins that inhibit viral replication. In mice, the ability to manufacture the protein Mx in response to interferon confers immunity to influenza. In addition to this protein production, interferons also stimulate the immune response to viruses by inducing the synthesis of MHC class I molecules on the surfaces of infected cells. Recall that the specific immune response to viruses depends on presenting antigen bound to MHC to T cells. Finally, interferons activate natural killer cells to destroy viruses and virus-infected cells.

Insert Table 42-5 Selected Cytokines and their functions

Cytokine	**Producer**	**Action**
IL-1β	Macrophage, epithelial cells	Fever, T cell actvation, macrophage activation
IL-2 (T cell growth factor)	T cells	T cell proliferation
IL-4 (BCGF-1, BSF-1)IL-3 (multicolony CSF)	T cells, mast cells	B-cell activation, IgE switch, induces differentiation into T_H2
IL-6 (IFN-β2, BSF-2, BCDF)	T cell, macrophages, endothelial cell	T and B cell growth and differentiation, acute phase protein production, fever
IL-9	T Cells	Mast cell enhancing activity, stimulates T_H2
IL-13 (P600)	T cells	B cell grotwth and differentiation, inhibits macrophage inflammatory cytokine production and T_H1 cells,

		induces allergy/asthma
IL-15 (T cell growth factor)	Many cells	Stimulates growth of intestinal epithelium, T cells, and NK cells, enhances CD8 memory T cell survival
IL-21	T_H2	Induces proliferation of B, T, and NK cells
G-CSF	Fibroblasts and monocytes	Stimulates neutrophil development and differentiation
GM-CSF	Macrophages, T cells	Stimulates growth and differentiation of myeloid cells, especially dendritic cells
IFN-α	Leukocytes, dendritic cells	Antiviral, increased MHC class I expression
IFN-β	Fibroblasts	Antiviral, increased MHC class I expression
IFN-γ	T cells, NK cells	Macrophage activation, increased expression of MHC molecules and antigen processing components, Ig class switching, supresses T_H2
TNF-α (cachectin)	Macrophages, NK cells, T cells	Local inflammation, endothelial activation
TNF-β (lymphotoxin, LT, LT-α)	T cells, B cells	Killing endothelial activation
Fas ligand (Fasl)	T cells	Apoptosis, Ca2+-independent cytotoxicity
IL-20	Monocytes	Stimulates keratinocyte proliferation and TNF-α production

Chemokines

Chemokines are a subgroup of cytokines that attract other cells, a process called chemotaxis. They function mainly as chemoattractants, recruiting monocytes, neutrophils, and other leukocytes to the area, however, some chemokines also have roles in lymphocyte development and angiogenesis. Chemokines can be secreted by a wide variety of cells including endothelial cells and keratinocytes (skin cells). They have been discovered fairly recently and originally shared the interleukin designation with cytokines. More recently, there has been as change in nomenclature to reflect their structure. The two main families of chemokines are called CC and CXC chemokines. The chemokine itself is designated by the letter "L" and a number, while the receptor is designated by the letter "R" and the same number. Thus, IL-8 became CXCL8 and binds to the CXCR8 receptor. (Ahh, the sweet kiss of logic!) Table 42-6 shows the major chemokines and their functions

Insert Table 42-6 Properties of selected chemokines.

Chemokine	Produced by	Cells attracted	Major effects
CXCL8 (IL-8)	Monocytes	Neutrophils	Mobilizes, activates

	Macrophages Fibroblasts Keratinocytes Endothelial cells	Naïve T cells	and degranulates neutrophils Angiogenesis
CXCL7 (PBP, β-TG, NAP-2)	Platelets	Neutrophils	Activates neutrophils Fibroplasia Angiogenesis
CXCL1 (GROα) CXCL2 (GROβ) CXCL3 (GROγ)	Monocytes Fibroblasts Endothelium	Neutrophils Naïve T cells Fibroblasts	Activates neutrophils Fibroplasia Angiogenesis
CXCL10 (IP-10)	Keratinocytes Monocytes T cells Fibroblasts Endothelium	Rest T cells NK cells Monocytes	Immunostimulant Antiangiogenic Promotes T_H1 immunity
CCL3 (MIP-1α)	Monocytes T cells Mast Cells Firbroblasts	Monocytes NK and T cells Basophils Dendritic Cells	Competes with HIV-1 Antiviral defense Promotes T_H1 immunity
CCL4 (MIP-1β)	Monocytes Macrophages Neutrophils Endothelium	Monocytes NK and T cells Dendritic Cells	Competes with HIV-1
CCL2 (MCP-1)	Monocytes Macrophages Firbroblasts Keratinocytes	Monocytes NK and T cells Basophils Dendritic Cells	
CCL5 (RANTES)	T cells Endothelium Platelets	Monocytes NK and T cells Basophils Eosinophils Dendritic Cells	Degranulates basophils Activates T cells Chronic inflammation
XCL1 (Lymphotactin)	CD8>CD4 T cells	Thymocytes Dendritic cells NK cells	Lymphocyte trafficking and development
CX3CL1	Monocytes Endothelium Microglial cells	Monocytes T cells	Leukocyte-enddothelial adhesion Brain inflammation

Complement

The **complement** system is a cascade of several lytic proteins that aid in pathogen destruction. They were first observed being activated by antibodies and enhancing the action of antibodies.

Hence their discoverer called them antibody-complement proteins—later simplified to complement. However, it is now understood that complement can function even in the absence of antibodies. The complement cascade consists of enzymes that aid in the destruction of pathogen membranes. In order to keep these enzymes from destroying host cells, they circulate in the blood as **zymogens** (enZYMe proGENitors). In order to be activated, the zymogen is cleaved in into two parts, freeing the active enzyme. An example of zymogens elsewhere in the body is pepsinogen, stored in stomach epithelial cells. Once secreted into the stomach, the hydrochloric acid cleaves pepsinogen into pepsin which breaks down peptide bonds. This mechanism keeps pepsin from digesting the cell that stores it.

Complement proteins are designated by the letter "C" and then a number. The number does not represent the step in the cascade, but the order in which they were discovered. The cascade steps are C1, C4, C2, C3, C5, C6, C7, C8, C9. As each protein is cleaved to activate it, the larger section is denoted by the letter "b" (for "big") and the smaller fragment by the letter "a." (There was one exception to this convention, but many current textbooks have corrected this inconsistency.) The complement cascade can be activated by one of three different pathways, but all three converge with the production of the protease C3 convertase which cleaves complement protein C3. C3 convertase is bound to the pathogen's surface, so that when C3 is cleaved, C3b is left bound C3 convertase and thus to the pathogen, while C3a diffuses into the nearby extracellular fluid and has mild inflammatory properties. C3b combines with C3 convertase on the pathogen surface to form C5 convertase, which lyses C5. Because the converting enzyme in each step is fused to the pathogen, the next cascade reaction can only take place at the pathogen's surface. This ensures that the complement cascade does not simply occur in the blood or extracellular fluid where it could damage host cells.

It is interesting to note, that a small amount of C3 convertase can cleave a large amount of C3, and a small of amount of C5 convertase can cleave a large amount of C5. This allows for an effect called amplification, so that relatively large amounts of the later cascade proteins can be activated from a very small amount of the early cascade. Many of the non-bound fragments also have chemokine or cytokine properties. C5a, in particular, recruits of phagocytes and induces inflammation; C3a also has a similar, but weaker effect. In addition to becoming C5 convertase, C3b binds to phagocyte receptors inducing phagocytosis and removal of immune complexes.

C5b initiates the late phase that generates the **membrane attack complex**. C6 and C7 bind to the C5b on the pathogen surface to form the C5b67 complex. C8 binds to the complex and inserts itself into the membrane doing a small amount of damage. C9 then binds to C8 in a polymerization reaction. This means that up to sixteen C9 molecules bind to each other forming a ring in the plasma membrane. The C9 ring forms a pore in the cell membrane. If enough pores are present, complement alone may kill the cell. Thus the complement cascade performs all four chemical component functions (cytokine, chemokine, osponin, and effector). See figure 42-3 for a graphical representation of the complement cascade.

The three pathways for complement cascade activation are 1) the classical pathway, 2) mannose-binding lectin (MB-lectin) pathway, and 3) the alternative pathway. The classical pathway always begins with the binding of C1q to the pathogen surface. (C1q is the receptor portion of the C1 molecule.) C1q can bind to pathogens in one of three ways. The first is to bind directly to certain surface components of the cell membranes of bacteria, such as lipoteichoic acid. The

second is to bind to C-reactive protein, an acute inflammatory protein that binds to residues in bacterial polysaccharides. (First discovered in pneumococcal C polysaccharide, hence C-reactive protein.) Third, C1q can bind directly to antibody-antigen complexes. The MB-lectin pathway is initiated by the binding of mannose-binding lectin to mannose containing carbohydrates in bacteria and viruses. MB-lectin is a serum protein that increases during inflammation. The alternative pathway can be initiated by the binding of spontaneously activated C3 in plasma to the surface of a pathogen.

Because of the dangerous nature of complement (amplification and effector mechanisms), it is tightly controlled by several proteins called complement control proteins. These proteins control all three activation pathways in order to keep complement from damaging the host cells. Theoretically, because of amplification, a single activated C1q molecule could activate all of the remaining complement proteins in the entire body. The C1 inhibitor (C1INH) protein deactivates bound C1q so that it is only active for a short time. There are similar deactivation mechanisms to keep C3 convertase and C5 convertase from remaining active for extended periods. Despite the safeguards to ensure that C3 and C5 convertases are only produced on pathogens, occasionally the convertases bind to host cells. Host cells have a membrane protein called CD59, or protectin, which prevents the formation of the membrane attack complex on the host cell surface.

Physiology of the Immune System

Innate Immune Response: Inflammation

Innate immunity is dependent largely on the recognition of common pathogenic features such as mannose and glucan found in bacterial cell walls. Because macrophages live in the tissues, they are usually the first immune system cell to encounter pathogens and typically begin the innate immune system response. When a macrophage recognizes an invader in addition to attempting to phagocytose the invader, it also releases cytokines and chemokines, thus inducing the inflammatory response. Inflammation plays three roles in the innate immune response. First, it brings more effector cells to the site and augments their killing ability. Second, it provides a physical barrier, through capillary coagulation, to keep the pathogens from spreading into the blood. Third, it prepares the tissues for healing. Inflammation is characterized by localized pain, erythema (redness), heat, and edema (swelling).

The first reaction to inflammatory chemokines and cytokines released by activated macrophages is local vasodilation which causes the erythema and some of the heat. Vasodilation also serves to slow blood flow. The second reaction is the expression of adhesion molecules by the endothelium (inner layer of the arterial wall) in order bind to circulating leukocytes. The combination of slowed blood and adhesion molecules allows leukocytes the time to migrate through the arterial wall into the tissues in a process called **extravasation**. The first leukocytes to migrate to the area are neutrophils, followed by monocytes which differentiate into additional tissue macrophages. Later, eosinophils and basophils will migrate to the site.

The third reaction in the inflammatory process is increased vascular permeability. This allows fluid and plasma proteins to leak into the inflamed tissues, causing edema and pain. The plasma proteins, including complement and clotting factors, aid in the inflammatory process and immune response. For example, once the complement cascade is activated, C5a increases

vascular permeability, induces adhesion molecules, activates phagocytic cells and mast cells. Activated by C5a, the mast cells degranulate releasing the inflammatory molecules histamine and TNF-□.

This triggers the fourth reaction in the inflammatory process, causing blood clots to form, walling off the infected area from the blood supply. This allows the infectious antigens in the edematous fluid, usually inside a dendritic cell, time to travel through the lymph vessels to a lymph node where an adaptive immune response can begin. TNF-α is critical in the isolation of the infection from the rest of the body.

In addition to these local effects of inflammation, systemic effects are also evident. The release of TNF-α, IL-1β, and IL-6 (called **endogenous pyrogens**) raise the body's temperature. Elevated temperature helps the body in a number of ways. It inhibits the growth of most pathogens which tend to prefer lower temperatures; adaptive responses tend to be more effective; and increased temperature helps to protect the body from the harmful effects of TNF-α.

TNF-α is critical in the innate immune response because it is so potent in vasodilation, increasing vascular permeability, and inducing clotting. These properties make it ideal for sending leukocytes to the site of infection and then walling it off. Unfortunately, if a pathogen does make it to the systemic circulation, these same qualities make TNF-α release backfire. When **sepsis** occurs, widespread systemic release of TNF-α occurs by macrophages in the spleen and liver. This systemic release causes systemic vasodilation leading to loss of blood pressure. At the same time, TNF-α also causes increased systemic vascular permeability leading to a loss of oncotic pressure and plasma, aggravating the drop in blood pressure caused by vasodilation. TNF-α's clotting properties cause disseminated vascular coagulation throughout the systemic circulation, further impeding blood flow, while depleting the body's supply of clotting proteins putting the patient at risk for hemorrhage. This condition is known as **septic shock**. It is the spleen's job to minimize systemic TNF-α release by filtering the blood and sequestering any pathogens.

TNF-α, IL-1β, and IL-6 also induce a response known as the **acute-phase response**. The acute phase is characterized by a change in the proteins that the liver produces and secretes into the plasma. The proteins that are produced as a result of TNF-α, IL1β, and IL-6 are called acute-phase proteins. Some of the proteins act similar to antibodies, but rather than binding to specific antigens, they have broad-spectrum binding. Anything that triggers inflammation will trigger all of these proteins, so it is not a targeted response, as antibodies are. The first acute phase protein is C-reactive protein (CRP). It has already been mentioned that CRP can activate complement. CRP binds to phosphocholine in bacterial and fungal cell walls and acts as an opsonin in its own right in addition to being able to activate complement. Another acute phase protein is mannose-binding lectin which in addition to activating complement acts as an opsonin to monocytes which unlike fully differentiated tissue macrophages do not express a mannose receptor. The other two important acute-phase proteins are the lung surfactants SP-A and SP-D. These proteins bind to pathogens in the lung and act as opsonins for phagocytes.

The last systemic effect of inflammatory cytokines is **leukocytosis**, an increase in the numbers of circulating leukocytes, especially neutrophils. Additionally, TNF-α has a role in stimulating dendritic cells to migrate from the tissues in which they reside to lymph nodes. The systemic actions of TNF-α, IL-1β, and IL-6 are summarized in table 42-7.

Table 42-7 Systemic effects of the endogenous pyrogens TNF-α, IL-1β, and IL-6

Tissue affected	Action on tissue	Net result
Liver	Acute-phase protein production	Activation of complement Opsonization
Bone marrow, endothelium	Leukocytosis	Phagocytosis
Hypothalamus	Increased body temperature	See below
Fat, Muscle	Protein and energy mobilization to increase body temperature	Decreased viral and bacterial replication; increased antigen processing; increased
Dendritic cells	Migration to lymph nodes	Initiation of adaptive immune response

Adaptive Immunity

Adaptive immunity refers to the process whereby lymphocytes are activated against the specific invader that is threatening the body. It is also called specific, because only lymphocytes that are capable of countering the current pathogen are activated. The process of activation, proliferation, and differentiation takes about 4-7 days to occur. The end result of the process (if all goes well) is destruction of the pathogen and the development of lymphocytes that are able to immediately respond to the same invader during subsequent infections. There are two basic pathways by which the adaptive immune system functions. These are termed humoral mediated and cell-mediated immune responses. Both forms of immunity involve T cells and antibodies (produced by B cells), but the mechanism of activation is different.

Cell Mediated Immune Response

Cell mediated immunity refers to the activation of naïve T lymphocytes to proliferate and mature into armed effector T cells (Cytotoxic, T_H1, T_H2 cells). A naïve T cell needs to have antigen presented to it by its appropriate MHC molecule. But this alone does not activate the naïve T cell. The T cell must also simultaneously receive a co-stimulatory signal. The only cells that are able to produce both classes of MHC and co-stimulatory molecules are dendritic cells, macrophages, and B cells. These are termed professional antigen presenting cells and are the only cells that can **activate** or **prime** naïve T cells. Priming occurs in lymphoid tissues where naïve T cells are constantly recirculating.

When infection occurs, the innate immune system signals tissue dendritic cells to differentiate into mature dendritic cells that express co-stimulatory molecules. Cytokines also stimulate the dendritic cells to migrate into the lymph. The vascular changes during inflammation serve to increase lymph drainage which in turn speeds the dendritic cell's journey to the lymph nodes. There are resident macrophages in all the lymph nodes, and B cells are constantly recirculating through lymph nodes. In response to inflammatory cytokines, both can develop co-stimulatory molecules and thus be potentially able to prime T cells. Dendritic cells, however, are vastly more potent in priming T cells, and it is believed that in vivo, they are responsible for most if not all T cell activation.

As T cells migrate through lymph nodes, they transiently bind with every antigen presenting cell they meet. If the T cell recognizes its specific antigen in the presence of co-stimulatory molecules, the bond is strengthened and can last for days while the cell proliferates and differentiates into its active state. Its progeny, so far as space allows will also bind to the antigen presenting cell. The activated T cell will produce IL-2 which stimulates it and its progeny to proliferate and differentiate. Without the IL-2, the activated T cell will not proliferate. If a T cell recognizes its antigen, but co-stimulatory molecules are not present, the cell will go into an inactive state called anergy. Anergic cells cannot produce IL-2. Many transplant drugs that suppress the immune response to keep the body from rejecting the transplanted organ work by disrupting IL-2 from functioning normally.

After several days of proliferation, the activated T cells differentiate into mature effector cells that are able to produce all the effector molecules required in their roles as helper or cytotoxic T cells. These effector T cells no longer require co-stimulatory molecules in order to react to their specific antigen. They lose the adhesion receptors that allow them to recirculate in the lymph tissues and develop receptors that allow them to bind to the endothelium of infected tissue. This change ensures that they will be able to distribute to the infected tissues.

The case with naïve CD4 cells is a bit more complicated. Although, CD4 cells do not need large amounts of co-stimulation to activate, they must choose whether to become T_H1 or T_H2 cells. If T_H1 cells are preferred, cell-mediated immunity will continue. If T_H2 cells are preferred, humoral mediated immunity will be stimulated. The difference can have profound consequences on the outcome. Bacteria such as M. tuberculosis and M. leprae live inside of macrophages and other phagocytic cells. If T_H1 cells are predominantly produced, there will be relatively small amounts of bacteria found, few antibodies, and the patient will most likely live a long time. If T_H2 cells are predominantly produced, their will be large amounts of antibody produced, but because the bacteria are sequestered in macrophages, the antibody will not be able to reach them. The bacteria will multiply freely; the disease will be much more severe, and the patient will likely die very soon.

Viral and other intracellular parasites cause activation of cytoxic T cells which are selective serial killers of other cells expressing the specific antigen. Cytotoxic cells kill host cells that are infected with pathogen. This accomplishes two things. It prevents more pathogen from multiplying inside the infected cell, and it allows the pathogen to be released into the extracellular fluid where it is susceptible to antibodies, macrophages, and other components of the immune system. Destruction of the infected cell is accomplished through perforation of the cell membrane by the T cell's cytotoxins and by induced apoptosis by the membrane bound proteins. One special function of cytotoxic cells is the regulation of immune responses by killing other T cells after the pathogen has been eliminated. If this does not occur, lymphoproliferation occurs resulting in severe autoimmunity. In addition to their cytotoxic affects, CD8 T cells also release cytokines that affect the behavior of other cells. The primary cytokine released is IFN-γ which prevents infection in nearby uninfected cells. It also tells infected cells to express more MHC on their cell surfaces, allowing them to be detected and destroyed more easily. IFN-γ also recruits macrophages to the area to act as effector cells.

T_H1 cells' main effector function is to activate macrophages. Most of the time, macrophages need no help from T_H1 cells to destroy pathogens, but there are certain pathogens that live inside

phagosomes and are able to prevent formation of the phagolysome. In addition to these macrophage parasites, other pathogens are not destroyed by macrophages unless the macrophage is activated. T_H1 cells activate such macrophages to induce destruction of the already phagocytosed pathogen and to phagocytose extracellular pathogens. T_H1 cells also activate B cells to produce certain classes of antibody.

The study of T_H1 cells and macrophages has lead to the conclusion that macrophages are naturally in an inactivated state and require two signals for activation. One of these signals is IFN-γ; the other signal can take a variety of forms. In T_H1 cells, that the second signal is provided by CD40 ligand (a membrane-associated protein). The IFN-γ can be produced by CD8 T cells, the T_H1 cell itself, or natural killer cells. (T_H2 cells can produce IFN-γ, but they also produce IL-10 that deactivates macrophages). Unlike CD8 T cells which release preformed granules, T_H1 cells manufacture their effector molecules (IF-γ and CD40 ligand) upon recognizing a target cell, which can take several days. Activated macrophages become more effective in lysing ingested pathogens, express more MHC becoming more efficient at presenting antigens, produce more TNF-α that can activate nearby macrophages already in contact with IFN-γ, and synthesize oxygen radicals such as superoxide and nitric oxide that can damage extracellular (unphagocytosed) pathogens directly. Activated macrophages also use a positive feedback mechanism, secreting IL-12 which induces the selective production of more T_H1 cells. These mechanisms make activated macrophages extremely effective effector cells, but in addition to consuming large amounts of energy, their activation is associated with local tissue destruction because the proteases and oxides they release are equally destructive to host tissue. Their activation must be tightly controlled to prevent undue collateral damage. The primary mechanism seems to be the control of IFN-γ by T cells. T_H1 cells direct their IFN-γ primarily at the cell to which they are bound. As soon as they are activated to synthesize IFN-γ, another gene is activated that induces an enzyme that breaks down the IFN-γ mRNA. In this way, IFN-γ is discriminately released by T_H1 cells.

In addition to the activation of macrophages, T_H1 cells also express CD40 ligand and can kill infected macrophages. This may need to occur if the pathogens escape from the phagosome and enter the macrophage's cytoplasm. Both CD8 and T_H1 cells can kill macrophages in this case. When the pathogens are released from the dead macrophages they can be killed directly by the T_H1 cell or by CD8 T cells and are then susceptible to antibody.

T_H1 cells are also critical in recruitment of phagocytic cells to the site of infection. They produce IL-3 and GM-CSF which stimulate neutrophil and macrophage production. They also produce the TNF-α and TNF-β which continue the inflammatory process. They produce the chemokine CCL2 which attracts other T cells and macrophages to the site. Thus, although inflammation is considered part of the innate immune response, in its later stages, it is promulgated by the adaptive immune system. When pathogens are able to resist the efforts of the activated macrophages, chronic infection with inflammation occurs. This is often accompanied by a characteristic pattern where macrophages envelop the area, and T cells are present around the perimeter, and is called a **granuloma**. Giant cells, previously described under Macrophages, form in the center of the granuloma and attempt to sequester the pathogens. The purpose of a granuloma is to wall off the infection from the rest of the body, and it is sometimes surrounded with collagen tissue to aid in this purpose. The tissue in the center of the granuloma will die secondary to hypoxia and the effects of activated macrophages and is called caseation

necrosis. If nothing else happens, eventually, the infection will take over the entire body, but will take a fairly long time to do so. AIDS patients are unable to form granulomas to sequester local infections and are susceptible to rapid fulminant forms of infections that would usually take years to kill most patients.

T_H2 cell mediated immunity will be discussed below, because their primary function is to activate B cells, thus making them part of humoral immunity.

Humoral Mediated Immune Response

Humoral mediated immunity is the adaptive immunity pathway that was first discovered in the form of "antitoxins" in the blood against tetanus and diphtheria. Body fluids were once called humors, thus the term humoral immunity. All antibodies are produced by plasma cells that arise from the proliferation and differentiation of activated B lymphocytes. B lymphocytes typically require the help of a CD4 T lymphocyte, hence the designation "helper" T cells. Both T_H1 and T_H2 cells can activate B cells, but T_H2 cells are more associated with humoral immunity.

The B cell receptor has two functions in the naïve B cells; it serves to activate internal signals when bound to its specific antigen, but also serves to bring the antigen inside the cell where it is degraded and displayed on MHC class II molecules. The B cell does not typically proliferate until activated by a CD4 cell. Some pathogens, however, can directly induce B cell activation without T cell help, but the antibodies secreted will be limited in nature. Thus, just like the naïve T cell, the naïve B cell also requires co-stimulation. Protein antigens always require a T cell's co-stimulation, but many microbial constituents, such as bacterial polysaccharides and certain cell wall components do not require T cell co-stimulation. This may be an added defense mechanism against autoimmunity, because bacteria do not produce protein, but host cells do. This relaxing of the co-stimulation requirement ensures quicker responses against bacterial antigens, while still protecting host cells from accidental activation against self antigens. The T cell that activates the B cell must be able to recognize epitope associated with the B cell's specific antigen, but not necessarily, the same antigen. For example, the B cell may bind to a specific viral epitope, but ingest the entire virus. When the viral components are degraded, it may display several different viral components on its MHC class II molecules. Thus the same B cell may be activated by T cells that do not recognize the B cell's antigen, but recognize other epitopes that are associated with the B cell's antigen. This is called **linked recognition**. The activated CD4 cell expresses CD40 ligand on its surface which activates the bound B cell. T_H2 cells also secrete IL-4 directly to the B cell which helps to drive B cell proliferation. In return, the activated B cell expresses co-stimulatory molecules that increase T cell growth and differentiation. The combination of IL-4 and CD40 binding stimulates the B cell to proliferate and differentiate. Both T_H1 and T_H2 cells secrete IL-5 and IL-6 which contribute to the differentiation of B cells into plasma cells.

Antibodies serve a variety of effector functions as described in B Lymphocytes, but the production of specific antibody classes is directed by T cells. IgM is the antibody class that will naturally be secreted by plasma cells, but makes up less than 10% of circulating antibody. CD4 T cells direct the change in antibody class production, a process called **isotype switching**. Cytokines are the driving factor in isotype switching, which involves recombining the B cells' DNA, a usually irreversible process. Thus, a B cell that has switched from IgM production to

IgA, IgG, or IgE cannot go back to making IgM. Table 42-7 shows the influence of specific cytokines on antibody production.

Table 42-7 Influence of cytokines on isoptype switching.

Cytokine	IgM	IgG3	IgG1	IgG2b	IgG2a	IgE	IgA
IL-4	+	-	+		-	+	
IL-5							++
IFN-γ	-	+	-		+	-	
TGF-β	-	-		+			-

Adapted from Janeway, et al., 2004.

Legend: Minuses indicates that the cytokine suppresses switching, while pluses promotes switching. No symbol indicates no effect.

Naïve B cells are continuously recirculating through the lymph nodes, much like naïve T cells. When they encounter their specific antigen, they are arrested in the lymph node at the B cell-T cell border by the development of adhesion molecules. Because they are trapped at the border or the T cell zone, it is likely that the antigen presenting cells will also activate nearby specific T cells that can activate the B cell. Without this mechanism, the chances of a T cell that can activate a specific B cell is on the order of 108 (one in a hundred million) even with linked recognition. Once activated, B cells travel to the medullary cords to proliferate and differentiate, establishing a **primary focus**. As proliferation continues, some of the B cells will become **plasmablasts**. Plasmablasts are cells that secrete antibody even though they are still dividing and still maintain most of the surface molecules that allow their interactions with T cells. This allows antibody to be secreted early in the immune response. In a few days, the plasmablasts stop dividing and either die or differentiate into plasma cells. Plasma cells are committed to antibody secretion and lose their MHC class II molecules. They no longer proliferate and cannot undergo isotype switching. They do, however still express surface immunoglobulin. Recent evidence shows that plasma cells may die if they no longer bind to their antigen (i.e., the infection has cleared). Plasma cells have a range of life spans, some living only a few days, while others live for years contributing to the persistence of antibody response. Some plasmablasts leave the lymph nodes and spleen and travel to the bone marrow and lamina propria of the gut where they become plasma cells and continue to secrete antibodies for months or even years.

The second phase of B cell activation is the formation of the germinal center as the proliferating T and B cells move to a primary follicle. T cells make up about 10% of the cells in the germinal center, with B cells accounting for the rest. Here, B cells undergo somatic hypermutation with the goal of producing antibodies that have even more affinity for their specific antigen. **Affinity maturation** is the process whereby the B cells with the highest affinity are selected for survival. The rest undergo apoptosis. This is important, because at the rate of B cell division, if left unchecked for ten days, more than a billion B cells would be produced in one germinal center. This process of affinity maturation also allows for progressively higher affinity antibodies to be produced over time. After selection for survival, the B cells undergo isoptype switching mediated by CD4 T cells. The B cells selected for survival will differentiate further into plasma cells or memory B cells. The plasma cells are long lived and provide high affinity antibodies. The memory cells will share the properties of B cells that produced them, including their

hypermutations and isotope switch. Recall that some antigens do not require T cell co-stimulation in order to activate B cells. B cells activated without the help of T cells will not have undergone hypermutation or isotype switching, and will produce relatively low affinity, primarily IgM antibodies with some IgG antibodies. It will not be long, however, before T cells are activated and migrate to germinal centers to direct the humoral response. Non T cell activation is very important for immunity against encapsulated bacteria, such as Haemophilus influenza B, which can escape detection by phagocytic cells, and hence activation of T cells.

Summary: The Total Immune Response

In order for a pathogen to invade the body, it must first pass the epthelial surfaces of the body—skin, mucous membranes, lungs, etc.—that have their own antimicrobial properties. Once past the epithelial surface, the pathogen will soon encounter tissue phagocytes—tissue macrophages and dendritic cells—which initiate the innate immune response and inflammation. It is unknown how many infections are cleared by the innate immune system alone, because such infections are likely to cause few if any symptoms. Moreover, deficiencies in innate immunity are rare, and when they are present, individuals succumb very quickly to infection, unable to mount either an innate or an adaptive immune response.

Inflammation causes tissue dendritic cells to migrate to the lymph nodes where it will activate the specific T lymphocytes that recognize its presented antigen. CD8 and CD4 T cells proliferate and differentiate into cytotoxic T cells and helper T cells. The helper T cells differentiate into T_H1 and T_H2 subclasses. The exact stimulus for preference of T_H1 or T_H2 is currently unknown but involves the nature of the presented antigen and cytokines. Cytotoxic cells destroy parasites and host cells infected with parasites. They also release cytokines that prevent uninfected cells from becoming infected and potentiate inflammation. T_H1 cells activate macrophages and B cells. T_H2 cells activate B cells to produce antibodies. Both T_H1 and T_H2 cells produce cytokines that affect inflammation. Effector cells of both the innate and adaptive immune system are guided to the site of infection by chemokines and adhesion molecules on the vascular endothelium. Antibody production takes place in the lymph nodes and are secreted into the blood. Memory T and B cells are produced and are ready to mount an accelerated immune response upon subsequent infection with the same pathogen.

Immunological Memory

One of the key characteristics of the adaptive immune system is **memory**, the ability to remember past pathogens and mount an accelerated and heightened immune response against them. Memory responses are called secondary, tertiary, etc. Memory is the property of the immune response that is exploited by immunization. Most memory cells are in a resting state, but a few are dividing at any given time. It is not known what the signal for memory cell division is. It is known that IL-7 maintains all memory T cells, and IL-15 maintains CD8 memory T cells. When an animal protein is injected, primed T cells are available almost immediately, and are at maximum strength within five days. It takes a month before B cell and antibody production is at maximum capacity.

The responses of memory cells are different than primary immune responses. Memory T cells do not require co-stimulation, but upon recognizing their antigen, immediately begin proliferating. Memory B cells, already having been selected for their antibodies, produce primarily very high

affinity IgG, IgA, IgE as opposed to IgM. Memory B cells do not express IgM on their cell membranes, but whichever of the high affinity isotopes they will produce, IgG, IgA, or IgE. They also express higher levels of MHC and require less costimulation to form germinal centers. The increased affinity of their receptor combined with increased ability to bind to T cells allows them to respond much quicker to infection than during the primary response. Memory cells also suppress the activation of naïve B and T cells. This effect is used therapeutically in mothers who are Rh- with Rh+ babies. Rh+ antibodies can be injected into the mother, which will suppress the production of Rh+ immune response.

Mechanisms of Immunization

The effector mechanisms of the immune response will depend on the infectious agent. The primary (initial) immune response is usually sufficient to clear the infection from the body, although some pathogens can evade the immune system and live in the body as long as the host lives. In some of these cases, protective immunity may be induced against the pathogens preventing them from establishing a persistent presence in the first place. In the case of other pathogens, such as polio, even though the primary immune response can clear the infection, the tissue damage is debilitating. (Polio attacks the motor neurons which will not regenerate, leading to paralysis.) Protective immunity involves two components. The first is antibodies, and the second is effector cells such as primed T cells which can counter the infection. IgA antibodies are present in mucosal secretions and can keep some pathogens from ever entering the body, much less establishing a primary infection. This is the goal of immunization.

Immunization refers to the process first discovered by Edward Jenner two hundred years ago. It involves the stimulation of the adaptive immune system so that when a person is exposed to the pathogen, their body has already developed immunity against it. In some cases, it is the toxin that a pathogen produces that is the true threat. This is the case with tetanus and diptheria. In some cases, the toxic receptor-binding functions are located on separate portions of the toxin. In this case, it is possible to cleave the binding site from the toxin. This is called a **toxoid** and produces antibodies against the toxin but cannot harm the person. Toxoid immunizations can also take advantage of linked recognition of antigens. Once a baby has been immunized against the tetanus toxoid, the toxoid can be linked to Haemophilus influenza B (HIB) polysaccharides. B cells that bind the polysaccharide will take in the HIB polysaccharide-tetanus toxoid complex. The B cell will then be activated by T cells that recognize the tetanus toxoid.

In cases where the toxin is extremely toxic or very unusual, it may not be practical or possible to develop an immunization for human protection. Snake venom is an example of such a toxin. Snake toxin works too quickly for the adaptive immune system to be effective. Instead of immunizing a human, horses are immunized with the toxin. The horses produce antibodies against the venom which are then separated and stored. These anti-venom antibodies (**antivenin**) can then be injected into a snake bite victim. When the antibodies are against an organism such as rabies or malaria, they are not called antivenin, but generically immunoglobulins. Antibodies have a limited half-life and confer immunity only for a limited time. Use of antibodies in this manner is called **passive immunization**.

Immunization against actual pathogens can be accomplished in one of four ways. A very small amount of the pathogen, enough to cause an immune response but not enough to cause disease, can be inoculated. This depends on the virulence of the pathogen. Cholera needs several

thousand cells to be ingested to cause disease, while Shigella can cause disease by ingesting as few as a dozen. The next option is for attenuated pathogen to be used—that is, using pathogen whose potency has somehow been altered so that it produces no or less severe disease. The oral polio vaccine is a live vaccine and occasionally caused polio instead of preventing it. The next option is to use dead pathogens that will produce an immune response despite not being alive. Some pathogens will not cause an immune response when inoculated in dead form. This could possibly be due to clearance of the dead pathogen by the innate immune system before activation of the adaptive immune system is possible. The final method is to use a surrogate non-pathogen. This is the technique used by Jenner for his first vaccine. The cowpox virus does not cause disease in humans, but is close enough antigenically speaking to the smallpox virus to induce immunity to it.

Some immunizations are given as a series, usually about a month apart. This technique takes advantage of the germinal center's hypermutation. It takes about a month for germinal centers to become fully operational. Re-immunizing at this point, causes hypermutation to increase, causing a jump in the affinity of the antibodies produced. This is necessary for some dead pathogen vaccines, because it mimics what would naturally happen if there were dividing pathogen in the body. Without being able to reproduce, dead pathogens or antigens will be cleared relatively quickly, even by low affinity antibody. Thus, re-immunization serves to boost the affinity as well as the amount of antibody produced against the antigen.

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