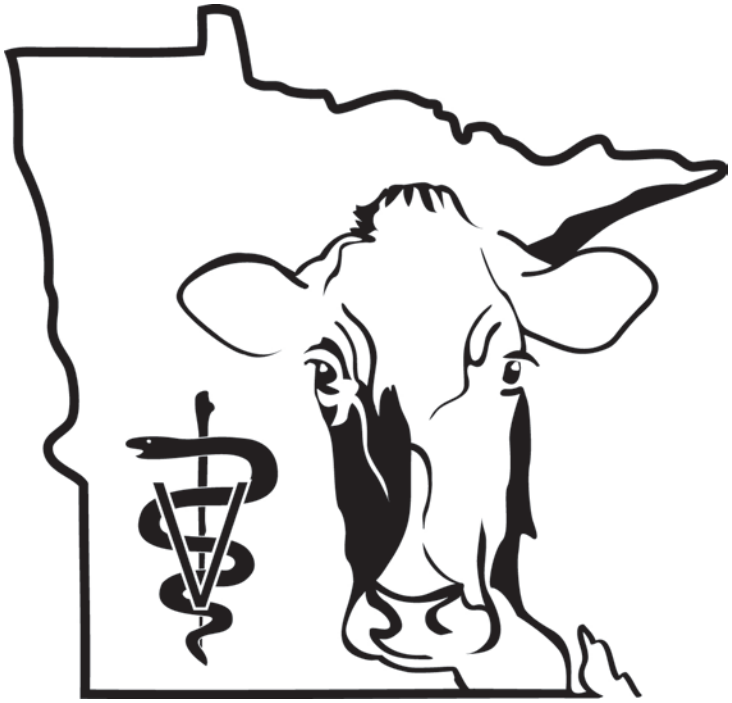
THIS ARTICLE IS SPONSORED BY THE
MINNESOTA DAIRY HEALTH CONFERENCE.

UNIVERSITY OF MINNESOTA

College of Veterinary Medicine

VETERINARY CONTINUING EDUCATION



ST. PAUL, MINNESOTA
UNITED STATES OF MINNESOTA

Basic Immunology and Principles of Vaccination

James A. Roth, DVM, PhD, Dipl. ACVM

College of Veterinary Medicine
Iowa State University
Ames, Iowa 50011

INTRODUCTION

The immune system is comprised of a variety of components that cooperate to defend the host against infectious agents. These components generally can be divided into nonspecific (or native) immune defense mechanisms and specific (or acquired) immune defense mechanisms. The nonspecific defense mechanisms are not antigen specific. They are present in a normal animal without previous exposure to antigen, and they are capable of responding almost immediately to an infectious agent. The major components of the nonspecific immune system are complement, phagocytic cells (macrophages, neutrophils, and eosinophils), natural killer (NK) cells, and some types of interferon. These components are very important in controlling an infection during the first few days of an initial exposure to an agent. This is the time when the specific immune response system is gearing up to produce antibody and a cell-mediated immune response.

B- and T-lymphocytes and their products are the components of the specific immune response system. This antigen-driven system requires two to three weeks to reach optimal functional capacity after the first exposure to antigen. Upon second exposure to antigen, the specific immune response system reaches optimal activity much more rapidly due to the anamnestic, or memory, response. A major mechanism by which B- and T-lymphocytes enhance resistance to disease is by activating the nonspecific defense mechanisms (phagocytic cells, NK cells, and complement) to be more efficient.

Providing immunity at mucosal surfaces and to the newborn are especially difficult challenges for the immune system. The nature of these special problems will be discussed as well as generalities about vaccination to improve immunity at mucosal surfaces.

If an animal is immunosuppressed due to stress, pre-existing viral infection, immunotoxicants, or nutritional factors, the nonspecific defense mechanisms may not be functioning optimally. In addition, the specific immune response may be slow to develop or inadequate. This can result in clinical disease due to an infectious agent that would otherwise be controlled by a nonimpaired immune system.

The immune system has potent mechanisms for protecting the host from infectious and neoplastic diseases. If the immune system is over-stimulated or is not appropriately regulated, it may cause hypersensitivity reactions. This can occur in response to infection, vaccination, environmental or dietary antigens, or even against normal host tissues.

PHYSIOLOGY OF THE IMMUNE SYSTEM

Native Defense Mechanisms

Physical, Chemical, and Microbial Barriers

Physical, chemical, and microbial barriers to infection at body surfaces are a very important part of resistance to disease. These factors include squamous epithelium, bactericidal fatty acids, normal flora, the mucous layer and the flow of mucous, low pH, bile and numerous enzymes. If these "non-immune" barriers are disrupted, the animal will have an increased susceptibility to infection.

Complement

The complement system received its name because it complements the action of antibody in killing bacteria. Early in this century Bordet discovered that if fresh serum containing antibacterial antibody was added to bacteria it would lyse the bacteria, however if the serum was heated to 56°C it would not lyse the bacteria. Since the antibody activity was not destroyed by heating, he reasoned that there was another factor in the serum that was heat labile that complemented the activity of the antibody. We now know that this complementing activity was due to a group of proteins which form a highly regulated enzyme cascade that can result in direct damage to infectious agents and the initiation of an inflammatory response to focus humoral and cellular defense mechanisms at the site of infection.

There are two related but different enzyme cascade pathways that can initiate or "activate" the complement system. The classical pathway is made up of four protein components (C1, C2, C3, and C4) and is predominantly activated by the presence of antibody bound to an antigen.

The alternative pathway (so named because it was described after the classical pathway, and therefore was considered to be an alternative to the classical pathway) involves six proteins (factors B, D, H, and I, properdin, and C3 which is also a component of the classical pathway). The alternative pathway can be activated on some microbial surfaces in the absence of antibody and therefore can play an important role in natural immunity. Each pathway involves the sequential activation of three different enzymes.

Each active enzyme molecule can cleave many molecules of its substrate, thereby creating multiple active molecules of the next enzyme in the cascade. This sequential activation of enzymes gives the complement system one of its important properties which is known as biological amplification. A small initiating stimulus (e.g. an antigen-antibody complex for the classical pathway or a suitable microbial surface for the alternative pathway) can lead to a vigorous response because it is amplified by the enzyme cascade.

All of the proteins that participate in both the classical and alternate pathways are pre-formed, but are circulating in an inactive state, which enables the complement system to be activated very rapidly in response to the presence of foreign antigen.

The terminal pathway (also called the membrane attack pathway) involves five proteins (C5, C6, C7, C8, and C9) and can be activated by either the classical or alternate pathway. It is initiated when C5 is split into C5a and C5b by the 3rd enzyme (C5 convertase) formed by either the classical or alternative pathway. C5a is a small peptide which diffuses away and has many important biologic activities, C5b is a larger peptide which can bind to cell surfaces immediately adjacent to where it is formed. If the C5b binds to a cell membrane, the rest of the membrane attack complex self assembles without the need for further enzyme activity. One molecule each of C6, C7 and C8 bind to the C5b molecule, then up to 15 molecules of C9 bind to this complex and form a hollow cylinder of protein inserted into the cell membrane. This effectively produces a hole in the membrane, which allows water and ions to freely diffuse into and out of the cell. If there are enough of these membrane attack complex holes in the cell, it will eventually rupture due to increased pressure from water which is osmotically attracted into the cell.

The enzymes in the complement pathway typically cleave the substrate protein molecules into a small piece that diffuses away and a large piece which binds to surfaces very near the site of cleavage. The large pieces either contribute to forming the next enzyme in the cascade (C4b, C2b, Bb and C3b), act as an opsonin to facilitate phagocytosis of the particle (C3b), or are the starting point for the terminal pathway (C5b). The small cleavage products which diffuse away are important initiators of the inflammatory response. C3a, C4a and C5a are called anaphylatoxins because they cause mast cell degranulation. This results in histamine and other mediator release leading to vasodilation and increased vascular permeability. C5a is a strong chemoattractant for neutrophils. The end result is the diffusion of serum components (including antibody and more complement) and the egress of neutrophils into the tissues to help control the infectious agents which initiated the complement cascade.

Since the complement system can be initiated rapidly, and is self-amplifying, it is very important that its activation be carefully regulated. Uncontrolled, massive activation of the complement system can cause vascular damage and initiation of the coagulation system, leading to disseminated intravascular coagulation which is rapidly fatal. Complement system activation is regulated by the fact that the enzymes formed have a short half-life, and the cleavage products which bind to membranes have only a very brief time in which to bind or they will lose their ability to do so. There is also a group of serum proteins which help to slow complement activation by inhibiting various complement components that bind to their surface or accelerate their decay. This helps to limit complement damage to normal cells. However, these protective molecules can be overwhelmed by massive activation of the complement system.

In fact, it is the presence of the complement inhibitory molecules in normal host cell membranes that protect the host from the alternative complement pathway. The alternative pathway is continuously activated by spontaneous, low level cleavage of C3 into C3a and C3b. Host cells have molecules that rapidly inactivate the C3b before it can lead to amplification of he alternative pathway. Bacterial surfaces lack the inhibitory molecules, thus allowing the alternative pathway to amplify and attack the bacteria. This is a crude method for the complement system to distinguish self from non-self.

The complement system is especially important for control of bacterial infections. People and animals that are deficient in key complement components are more susceptible to recurrent bacterial infections.

Phagocytic Cells

Phagocytic cells are responsible for engulfing, killing, and digesting invading bacteria. They also play an important role in controlling viral and fungal infections and in killing cancer cells. There are 2 main types of phagocytic cells: the granulocytes or polymorphonuclear leukocytes which include the neutrophils and the eosinophils, and the mononuclear phagocytes which include the circulating monocytes in the blood and the tissue macrophages. All these cell types are phagocytic and are capable of all the reactions which are described below for neutrophils. In addition, the macrophages play a very important role in processing antigens and presenting them to lymphocytes to initiate and facilitate the cell mediated and humoral immune responses.

Granulocytes. Neutrophils are phagocytic white blood cells that are produced in the bone marrow and released in large numbers into the blood stream. They make up 40 to 60 percent of the leukocytes in the blood stream in healthy individuals of most species and their number increases rapidly in response to many inflammatory stimuli. They have a half-life in the blood stream of approximately eight hours before migrating into the tissues. They survive for only a day or two in normal tissue and only minutes to hours at sites of inflammation. The bone marrow produces more neutrophils than any other type of white blood cell, and maintains a pool of mature neutrophils to be released rapidly in response to inflammation. The body therefore makes a major commitment in bone marrow space and protein and energy utilization to produce neutrophils in large numbers. This is necessary because neutrophils are an essential first line of defense against many bacterial infections and some viral and fungal infections. When neutrophil numbers fall below 1000 per cubic millimeter in the blood, the individual is at a high risk for severe bacterial infection. There are no examples of genetic defects in animals or humans in which there is a complete absence of functional neutrophils, whereas there are examples of absence of functional B or T lymphocytes. Therefore the absence of neutrophils is apparently incompatible with life. There are genetic defects in which specific aspects of neutrophil function are defective. Affected individuals typically have severe recurrent bacterial infections.

A mature neutrophil has a lobulated nucleus and most of the DNA is clumped. This is an indication that there is very little transcription and translation of DNA to make proteins. There are also very few ribosomes for protein synthesis in the mature neutrophil. The mature neutrophil is a short lived cell that is not able to replenish its supply of enzymes and other antibacterial substances or to repair its membranes.

The neutrophil has two or three (depending on the species) types of lysosomes (also called granules), in its cytoplasm. Lysosomes are membrane-bound vesicles containing a variety of enzymes and cationic peptides that are important for controlling bacterial infection. The primary (or azurophilic) lysosomes are formed first as the neutrophil is maturing and contain numerous enzymes and cationic peptides used for killing bacteria and for digesting the dead bacteria and/or

damaged tissues. The secondary (or specific) lysosomes are smaller and are formed later in neutrophil development. The primary lysosomes typically fuse with a phagosome, which is a membrane-bound vesicle containing phagocytized particles, such as bacteria. This fusion of the primary lysosomes with the phagosome containing bacteria concentrates the contents of the primary lysosomes where they can attack the bacteria. The secondary lysosomes mainly degranulate to the exterior of the cell when neutrophils are stimulated to migrate into and through tissues. They play a role in helping the neutrophil to adhere to vascular endothelium in order to leave the blood stream and to digest intercellular cement substances to facilitate neutrophil migration through the tissues. Ruminant neutrophils contain a third and larger type of lysosome called tertiary (or large) lysosomes which contain high concentrations of small cationic peptides that directly attack and damage the membrane of certain bacterial organisms. These cationic peptides are found in the primary lysosomes of other mammals.

The neutrophil cytoplasm also contains numerous glycogen granules. Glycogen is a storage form of glucose. The neutrophil is able to use this stored glucose through anaerobic glycolysis as a source of energy. Since it carries its own glucose with it and is able to use that glucose for energy production in the absence of oxygen, the neutrophil is able to migrate into and function at sites of severe inflammation or tissue damage where there may be very low levels of blood glucose and oxygen.

Neutrophils must be able to rapidly leave the blood stream and migrate through the tissues to sites of bacterial infection in order for them to effectively control the infection. Neutrophils normally have an adherence protein called L-selectin on their membrane that allows them to loosely adhere to normal capillary endothelial cells and slowly role along the endothelium until they traverse the capillary. This process is called margination. At any one time a large percentage of neutrophils in the blood are marginated in capillaries, so that the counting of neutrophils in a peripheral blood sample underestimates the total number of neutrophils in the blood. The presence of bacteria or other inflammatory stimuli in the tissues generates various chemotactic factors such as C5a, a byproduct of complement activation. These small chemotactic stimuli diffuse from the site of production and contact endothelial cells which causes them to express a new set of adherence proteins on their membrane. The chemotactic factors also contact neutrophils. The combination of the chemotactic factors contacting the neutrophils and the presence of new adherence proteins on the endothelial cells causes the neutrophil to rapidly transport new adherence proteins called beta-integrins to their membrane which, causes the neutrophils to stick tightly to the endothelial cells and stop rolling along through the capillary. Neutrophils then leave the capillary by diapedesis (migration between two endothelial cells) and arrive in the tissues. They then follow the gradient set up by the diffusion of chemotactic factors to the source of chemotactic factor production.

Once the neutrophils arrive at the site of bacterial infection, they must be able to phagocytize (or ingest) bacteria in order to kill them. Neutrophils are capable of phagocytosing any particle that is more hydrophobic than their own membrane. Most extracellular bacterial pathogens have a hydrophilic capsule which makes them resistant to neutrophil phagocytosis. In order for these

types of bacteria to be efficiently phagocytized, they must be coated with either antibody and/or complement components. The neutrophil membrane has receptors for the Fc portion of some classes of antibody and for the complement component C3b. The neutrophil can, therefore, bind to antibody or complement molecules that are bound to bacterial surfaces and thereby capture and phagocytose the bacteria. A particle that is coated with antibody or complement components is said to be "opsonized" for phagocytosis. The process of phagocytosis is relatively rapid and can be completed within a few minutes.

After the neutrophil ingests the bacteria through phagocytosis, it must then attempt to kill the bacteria. The neutrophil uses two basic mechanisms to attempt to kill the bacteria. Each of these mechanisms is initiated even before the bacteria is completely phagocytosed if the conditions are appropriate. One set of mechanisms is called the non-oxidative killing mechanisms because they do not require the presence of oxygen in order to function. They are due to the actions of the enzymes and cationic antibacterial substances found within the lysosomes. After the lysosomes fuse with the phagosomes, the enzymes and cationic peptides released from the lysosomes begin to break down the bacterial cell wall and damage the bacterial cell membrane. Some bacteria have very heavy capsules which make them relatively resistant to the actions of the non-oxidative killing mechanisms. The second type of bactericidal mechanisms are referred to as the oxidative killing mechanisms because they require the presence of oxygen in order to function. The neutrophil has an oxidase enzyme complex in its cell membrane which is rapidly activated during phagocytosis. As the phagosome which contains the bacteria is formed, some of the oxidase enzyme molecules are incorporated into the phagosome membrane. The oxidase enzyme converts oxygen into superoxide anion, a free radical of oxygen. The superoxide anion, under the low pH conditions which are present in the phagosome, spontaneously forms hydrogen peroxide. Some of the hydrogen peroxide reacts with additional superoxide anion to form the hydroxyl radical. Superoxide anion, hydrogen peroxide, and the hydroxyl radical are all capable of damaging bacteria trapped within the phagosome. This is referred to as the oxidative burst of metabolism because oxygen consumption by the neutrophil increases dramatically when the oxidase enzyme is activated. Oxygen radicals formed also damage the membranes of the neutrophil, which eventually destroys the neutrophil itself. A by-product of the peroxidation of the neutrophil membranes is the generation of aldehydes, which can also contribute to the bactericidal environment within the phagosome.

A very potent killing mechanism within the neutrophil involves an enzyme called myeloperoxidase, which is released into the phagosome from the primary lysosomes. This enzyme catalyzes a reaction between hydrogen peroxide and halide ions (iodine and chlorine), resulting in further damage to the bacteria within the phagosome. If chlorine is the halide ion involved in the reaction, it results in the generation of hypochlorous acid within the phagosome. Hypochlorous acid is the active component of chlorine bleach and is a potent bactericidal molecule. Neutrophils, therefore, have numerous mechanisms at their disposal for attempting to destroy bacteria.

Neutrophils may also play a role controlling viral infections through a process called antibody-dependent cell-mediated cytotoxicity (ADCC). This is a process whereby neutrophils will attach to and kill a cell that is coated with antibody. If a normal cell in the body becomes infected with a virus, antibodies may form against viral proteins on the cell membrane. A neutrophil can attach to that antibody through its Fc receptors. It may then kill the cell by damaging its membrane through the actions of lysosomal enzymes and oxygen radicals.

Neutrophils are considered to be the first line of defense against infection and are a hallmark of the acute inflammatory process. They are typically quite effective at killing extracellular bacterial pathogens if those pathogens are opsonized with antibody and/or complement. However they have more difficulty in killing pathogens that may reside within host cells, referred to as facultative intracellular bacterial pathogens. Macrophages that have been activated by cytokines from T lymphocytes are usually required to control facultative intracellular pathogens.

Neutrophils from healthy individuals are normally quite efficient at killing bacteria. However there are a number of factors that are known to be capable of inhibiting or interfering with neutrophil activity. Several viral infections have been shown to inhibit neutrophil function and to predispose to bacterial infections. Glucocorticoids released in response to stress and other hormones have also been shown to interfere with neutrophil function. In addition there are several bacterial virulence factors that are known to interfere with many aspects of neutrophil function, including inhibition of chemotaxis, phagocytosis, release of lysosomal enzymes and oxidative metabolism. Any of these factors which inhibit neutrophil function will increase the susceptibility of an individual to infection. On the other hand, several cytokines that are released by lymphocytes and macrophages are capable of enhancing neutrophil activity and increasing the resistance of an individual to bacterial infections.

The eosinophil is capable of the same phagocytic and metabolic functions as the neutrophil, but to a different extent. The eosinophil is not as active as the neutrophil in destroying bacteria but is important in the host's defense against the tissue phase of certain parasitic infections. The eosinophil is geared more toward exocytosis than phagocytosis. That is, rather than ingesting and killing small particles like bacteria, it can efficiently attach to and kill migrating parasites that are too large to be ingested. Eosinophils are also important in helping to control certain types of allergic responses.

Mononuclear Phagocytes. The mononuclear phagocytic system is made up of circulating monocytes, fixed macrophages, and wandering macrophages (histiocytes). Monocytes are produced in the bone marrow and released into the blood stream where they will circulate before migrating into the tissues to become macrophages. The fixed macrophages are found lining the endothelium of capillaries and the sinuses of organs such as the spleen, bone marrow, and lymph nodes. These fixed macrophages are important for trapping and removing foreign antigens from the blood stream and lymph. Wandering macrophages are derived from blood monocytes and are found throughout the tissues of the body. In certain locations, they differentiate into specialized

types of macrophages such as the glial cells in the nervous system, Langerhans cells in the skin, and Kupffer cells in the liver.

Macrophages are capable of all the activities described above for neutrophils. Macrophages are said to be the second line of defense. They are slower to arrive at sites of inflammation and are not as aggressive as neutrophils in the first few minutes of contact with microorganisms. However, macrophages are capable of much more sustained activity against pathogens than are neutrophils. They are able to kill certain types of bacteria that are resistant to killing by neutrophils because of this sustained activity. This is especially true if the macrophages have been activated by cytokines secreted by T-lymphocytes.

A very important function of macrophages is the processing of antigen and presentation of antigen to T-lymphocytes. This is an essential step in the initiation of a cell mediated immune response and for facilitating an efficient antibody response by B-lymphocytes. The interaction of macrophages with antigen and T- and B-lymphocytes is described below.

Natural Killer Cells

Natural killer (NK) cells are lymphoid cells which are capable of "natural cytotoxicity"; that is they can kill a variety of nucleated cells without previous antigenic stimulation. They are part of the native immune system and can kill some (but not all) tumor cells and some (but not all) virus-infected cells. NK cells in most species are also called large granular lymphocytes because of the presence of granules in their cytoplasm. NK cells in most species are part of the null cell population because they are distinct from B cells, T cells and macrophages. In most species NK cells have Fc receptors for IgG and can mediate antibody-dependent cell-mediated cytotoxicity (ADCC) against most antibody coated mammalian cells. When mediating ADCC these cells have been called killer (K) cells.

The activity of NK cells in many species is increased in the presence of gamma interferon and interleukin 2. Therefore, NK cells are an important part of the native defense mechanisms and also participate in a cell-mediated immune response by enhanced activity through cytokine activation.

Humoral and Cell-Mediated Immunity

Clonal Selection and Expansion

An important concept that is basic to understanding the immune response is the clonal selection process. Each mature T- or B-lymphocyte in the body is capable of recognizing only one specific antigen. All of the lymphocytes which recognize exactly the same antigen make up a "clone" and they have all arisen from the same ancestor cell. There are millions of clones of T- and B-lymphocytes. Each clone may contain from a few hundred to a few million cells. The lymphocytes are in a resting stage as they circulate through blood, enter the lymph nodes through the postcapillary venules, percolate through the lymph nodes and reenter the bloodstream. In the lymph nodes (or other secondary lymphatic tissues) the lymphocytes come in contact with antigens which have arrived there through the afferent lymphatics and have been trapped by

macrophages. Each lymphocyte can respond only to the one specific antigen that it can recognize through its antigen receptors. Therefore, the vast majority of lymphocytes that contact an antigen in the lymph node cannot respond to it. In an animal that never has been exposed to a particular infectious agent before, there are relatively few lymphocytes in each clone that can recognize a particular antigen. The first step, therefore, in producing an effective primary immune response is for the clone of lymphocytes that recognize the antigen to be expanded. The T- and B-lymphocytes that contact the antigen are stimulated to undergo a series of cell divisions so that within a few days there will be enough lymphocytes in the clone to mount an effective humoral and/or cell-mediated immune response. If the animal has been exposed to the antigen previously, the clone of lymphocytes has already been expanded, so not nearly as many cycles of cell division are needed to produce enough lymphocytes to mount an immune response. This can result in a degree of protection from vaccination or exposure even if there is no remaining detectable antibody. The cells present in the expanded clone are called **memory cells**. If the previous exposure has been relatively recent, there still will be circulating antibody and effector T-lymphocytes which can act immediately to begin to control the infection.

Cellular Interactions in the Induction of the Immune Response

The induction of clonal expansion and the immune response requires a complex interaction of macrophages, T-lymphocytes, and B-lymphocytes. The cells interact with each other through their surface molecules (CD molecules) and through cytokines which they secrete. Macrophages attempt to phagocytize and destroy infectious agents. After the infectious agent is partially degraded by the macrophage, peptide fragments from it appear on the macrophage surface bound to major histocompatibility class II (MHC II) molecules where they can easily be contacted by T-lymphocytes. Macrophages (and other specialized antigen-presenting cells) have a high density of Class II MHC molecules on their surface. T helper (T_H) cells (also called CD4 cells)are needed to help initiate the immune response. They can only recognize foreign antigens that are on a cell surface bound to a Class II MHC molecule. Therefore, T_H cells cannot respond to free soluble antigen or to whole bacteria or viruses. In addition to contacting the antigen and a Class II MHC molecule, the T_H cell requires a third signal to be fully activated: That signal is interleukin 1 (IL-1). IL-1 is a protein molecule (formerly referred to as **lymphocyte activating factor** and **endogenous pyrogen**) that is released by macrophages while they are processing antigens. IL-1 is a key mediator of the host response to infection through its ability to induce fever and neutrophilia, among other things. A very important function of macrophage-produced IL-1 is its action on T_H cells, which have recognized an antigen, to cause them to secrete interleukin 2 (IL-2). IL-2 is a protein molecule (formerly called **T-cell growth factor**) secreted by activated T_H cells. The T_H cells that have recognized an antigen will also synthesize IL-2 receptors in their membranes. The IL-2 binds to the IL-2 receptor and induces the T cells to undergo mitosis and produce more cells in the clone.

T_H cells also secrete other factors that are very important in initiating the B-cell response resulting in antibody production. B cells contact antigen through immunoglobulins bound to their surface which act as receptors. Antigens do not have to be presented on MHC class II molecules by macrophages for a B-cell to recognize them. An optimal B-cell response to antigen

requires the help of soluble factors (cytokines) released by T_H cells. These cytokines are needed for B-cell mitosis and clonal expansion and for switching the class of antibody produced from IgM to IgG, IgA, or IgE.

Lymphocyte Circulation

Recirculation of lymphocytes from blood to lymphoid tissues is very important for bringing antigen into contact with the rare lymphocytes that are able to recognize it. Circulation of B cells, T cells, and macrophages through lymph nodes is also important for facilitating cellular interactions needed for the induction of the immune response as described above. Lymphocytes are produced primarily in the bone marrow. Lymphocytes are released from the site of production into the bloodstream. T- and B-lymphocytes circulate in the blood for an average of approximately 30 minutes before entering the tissues. Lymphocytes enter the lymph node through two routes. Lymphocytes which leave the bloodstream and enter the subcutaneous tissues are carried to the lymph node in the afferent lymphatics. Lymphocytes may directly enter the lymph node by adhering to high endothelial cells in the venules of the lymph node. The high endothelial cells will phagocytize the lymphocytes and transport them into the lymph node. The lymphocytes exit the lymph node in the efferent lymphatics and are carried through the thoracic duct back to the circulatory system. The emigration of lymphocytes from blood into lymph nodes can be increased by antigenic stimulation.

Acquired Immune Defense Mechanisms

An important component of lymphocyte activity in host defense is mediated by soluble products released by stimulated lymphocytes. T-lymphocytes secrete a variety of cytokines, and B-lymphocytes differentiate into plasma cells that secrete antibody (B-lymphocytes are also able to secrete some cytokines). Antibodies are specific for the antigens which induced them whereas cytokines are not. These soluble products produced during the immune response play an important role in orchestrating host defense against pathogens partially through their direct activities and partially by enhancing the activity of the nonspecific defense mechanisms (i.e., complement, phagocytic cells, and NK cells).

The cytotoxic T lymphocytes (Tc cells) are an important part of the cell-mediated immune response to virus infection and tumors. Most Tc cells have the CD8 marker on their surface and only recognize antigen associated with MHC class I molecules on a cell surface. They directly attack host cells that have foreign antigen (e.g., viral antigen or tumor antigen) on their surface. These cells do not attack free bacteria or viruses.

Immunoglobulins

Antibody Structure. Antibodies (or immunoglobulins) are the protein molecules produced by B lymphocytes and by plasma cells derived from B lymphocytes. There are five major classes of antibody molecules in mammals: IgM, IgG, IgA, IgE, and IgD. The basic structure of an antibody is a molecule consisting of two "heavy" peptide chains and two "light" peptide chains. These four peptide chains combine to make a Y-shaped molecule. Antigen binds at the ends of

the arms of the Y-shaped molecule, and these arms are called the Fab (fragment antigen binding) portion of the antibody molecule. The tail of the Y-shaped molecule is called the Fc (crystalizable fragment) of the antibody molecule. The different classes of antibody molecules have different amino acid sequences in their Fc portion. The Fc part of the molecule gives different classes of antibody molecules some of their important functions. It allows the antibody molecule to bind to different types of Fc receptors on different types of cells; it determines if that class of antibody will initiate the classical complement cascade, and it determines if that class of antibody will cross the placenta or be concentrated in the colostrum (depending on the species).

The heavy and light peptide chains of antibody molecules are both made up of similar homology regions or domains. Each domain is approximately 110 amino acids long and has one internal disulfide bond, which helps to maintain its globular structure. The light chains have two domains, one variable and one constant and the heavy chains have four or five domains, one variable and three or four constant domains. The amino acid sequence in the constant domains determines the class or isotype of the antibody. These domains are called constant domains because the amino acid sequence is highly conserved for a single class of antibody molecule in all members of a species. For example, all IgG1 molecules from a particular species have essentially identical amino acid sequences in the constant domains of their heavy chain. There are only two possible amino acid sequences for the constant domain of light chains within a particular species. The light chain constant regions are designated as either kappa or lambda. There is no apparent difference in biological function between the Kappa and Lambda types of light chains.

In contrast to the constant domains there is tremendous variability in amino acid sequence in the variable domains of both the light and heavy chains. This variability is essential because it is the amino acid sequence in the variable domains that determines the antigen specificity of the antibody molecule. It is estimated that there may be as many as 100 million different antigens that antibody molecules must be able to distinguish between. This means that there would need to be approximately 100 million different amino acid sequences within the combined variable regions of the light and heavy chains.

Within the sequence of 110 amino acids which make up the variable domain of the light and heavy chains, there are three or four subregions consisting of a few amino acids each where most of the variation in amino acid sequence occurs. These are called hypervariable regions. The hypervariable regions of the heavy and light chains come together at the end of the antibody molecule when the heavy and light chains are folded into their globular three-dimensional structure. These hypervariable regions which come together at the end of the antibody molecule form the antigen binding region of the antibody molecule. If the amino acids making up the hypervariable region change this results in a change in the antigen specificity of the antibody molecule.

When an antibody molecule binds to an antigen, it only interacts with a small portion of the antigen molecule. This small portion of the antigen molecule which binds to the antibody is

called an epitope. A typical epitope on an antigen molecule would be approximately the same size as a six to eight amino acid peptide. The epitope will interact with only a few amino acids from the variable region of the light and, or heavy chain on the antibody molecule.

A single monomeric antibody molecule can bind two epitopes, one on each arm of the Y-shaped molecule. The binding of antigen by the antibody molecule will often induce a conformational change in the Fc region of the antibody molecule. This conformational change may allow the antibody molecule to bind tightly to an Fc receptor on a cell surface, or to bind the C1 portion of complement.

General Properties of Immunoglobulin Classes. Immunoglobulin G (IgG) is the class of antibody found in the highest concentration in the plasma of animals. It is a monomer with molecular weight of approximately 160,000 to 180,000 Daltons. It's relatively small size allows it to diffuse into tissue fluids, especially sites of inflammation. Since each IgG molecule is able to bind two antigen molecules, IgG is capable of precipitating antigen molecules or agglutinating large particulate antigens. Some subclasses of IgG can activate the classical complement pathway by binding the C1 molecule. Neutrophils and macrophages have Fc receptors for some subclasses of IgG. This facilitates phagocytosis of IgG coated particles. IgG is the class of antibody that is passively transferred from the mother to the offspring either by crossing the placenta, through the colostrum, or through the yolk sack (in birds). IgG has a half life of approximately two to three weeks in the blood stream.

Immunoglobulin M is a very large molecule of approximately 800,000 to 900,000 Dalton molecular weight. It is made up of five monomers, each of which consists of two heavy and two light chains. The five monomers are held together by a J-chain, which is a peptide of approximately 15,000 molecular weight. IgM is always the first class of antibody to appear in a primary immune response. Since it is such a large molecule, it does not readily leave the blood stream and enter the tissues unless there is a major disruption in vascular integrity. Since the IgM molecule has ten potential antigen binding sites, it is very efficient at precipitation and agglutination of antigens. It is also much more efficient at activating the classical complement pathway than is IgG.

Immunoglobulin A may occur as either a monomer, dimer, or trimer. IgA is especially important for protection at mucosal surfaces. IgA in the serum is primarily found as a monomer, whereas IgA on mucosal surfaces is primarily found as a dimer held together by a J-chain. Dimeric IgA on mucosal surfaces also has secretory component attached to it. Secretory component is a peptide chain of approximately 70,000 Daltons in molecular weight. It helps to transport the dimeric IgA on to mucosal surface, to protect the IgA form proteolytic digestion, and to anchor the IgA in the mucus layer on the mucosal surface.

IgA is typically found in relatively low concentration in the serum. However since IgA is found in high concentration on mucosal surfaces, and since the body has a large mucosal surface area which needs protection, there is more total IgA produced in an animal than any other class of

antibody molecule. IgA functions on the mucosal surface primarily by attaching to toxins or microorganisms and preventing them form attaching to the epithelial cells at the mucosal surface. It does not opsonize particles to promote phagocytosis and it does not efficiently activate the classical complement pathway.

Immunoglobulin E occurs as a monomer and is found in only low concentrations in the serum of normal healthy individuals. IgE, which is not bound to antigen has a high affinity for FC receptors of mast cells and basophils. For this reason it is sometimes called homocytotropic antibody. Most of the IgE in a normal, health individual is found bound to the surface of mast cells with only small amounts in the circulation. IgE plays an important role in resistance to migrating parasites and it is responsible for inducing the symptoms of some types of allergies. Individuals who are heavily parasitized, or that are highly allergic will often have high levels of IgE in their circulation.

Immunoglobulin D is found only on the surface of B lymphocytes and plays and important role in B cell maturation. IgD is not usually detectable in the serum and is not thought to play an active roll in defense against infectious agents.

Production of Immunoglobulins. B-lymphocytes from clones that have never been stimulated by antigen have monomeric IgM antibody molecules on their surface that act as antigen receptors. All of the IgM molecules on one B cell are specific for the same antigen. When a B cell is appropriately stimulated by the antigen it recognizes (along with soluble products from a T_H cell) it begins to undergo mitosis. This results in the formation of many more B cells with IgM receptors that also recognize the same antigen. Some of these newly formed B cells differentiate into plasma cells that secrete IgM antibody. As the antigen-specific IgM antibody concentration begins to increase in the blood, it signals the T_H cell to cause it to in turn signal some of the B cells to switch from IgM production to IgG, IgA, or IgE production. These B cells then rearrange their genetic material which codes for antibody production and produce antibody molecules with the same antigenic specificity (i.e., the same light-chain structure and variable portion of the heavy chain) but of a different antibody class (i.e., the constant heavy portion of the antibody molecule is changed). Changing the antibody class gives the antibody molecules different properties. The class of antibody that the T_H cells cause the B cells to switch to depends to a large extent upon the nature of the antigen and the location in the body where the antigen was trapped. T_H cells located in lymph nodes and the spleen tend to induce B cells to switch to IgG production. T_H cells located in Peyer's patches or under other mucosal surfaces tend to induce B cells to switch to IgA and/or IgE production, depending on the nature of the antigen and the genetic predisposition of the individual.

Antibody molecules have a variety of activities in host defense, although they alone cannot kill infectious agents. Antibody molecules can coat infectious agents to prevent them from attaching to or penetrating host cells, they can agglutinate infectious agents to reduce their infectivity, and they can directly bind to and neutralize toxins. A very important function of antibody is that it marks infectious agents for destruction by complement, phagocytic cells, and/or cytotoxic cells.

Polyclonal and Monoclonal Antibodies. Antibody produced by an animal in response to an infection or vaccination is polyclonal antibody. Infectious agents are complex antigens with many different antigenic specificities on their surface; therefore, they stimulate many clones of B- and T-lymphocytes to respond. This results in a heterogenous mixture of antibodies which recognizes a wide variety of surface molecules on the microorganism. This broad spectrum of antibodies that are produced and are present in the serum are most helpful to the animal in overcoming infection. It is sometimes a disadvantage, however, if one wishes to use the serum for developing diagnostic reagents. The polyclonal antibodies produced in response to one infectious agent may cross react with another infectious agent and thus interfere with the specificity of the assay. The majority of the protein present in a polyclonal antiserum produced against an infectious agent are not antibodies directed against the agent. Therefore, the amount of specific antibody in relation to the amount of protein present is low. This is a disadvantage when attempting to protect an animal from disease by administering antisera.

Monoclonal antibodies are now commonly produced in research laboratories and are used to overcome many of the disadvantages of polyclonal antisera for diagnostic and (less commonly) for therapeutic purposes. Monoclonal antibodies are produced by one clone of B-lymphocytes and therefore are all identical. All of the antibody molecules present in a monoclonal antibody preparation are specific for the same antigenic determinant; therefore, the antibody can be present in extremely high concentrations. This reduces the problem of cross reactivity between microorganisms in diagnostic tests. If monoclonal antibodies can be produced against a protective antigen on a microorganism, the monoclonals can be used in therapy or prevention of disease. Since they can be produced in very high concentration and purity, a much lower volume of monoclonal antibody than polyclonal antibody solution can be used to passively immunize animals. This reduces the risk of serious reaction to the passively administered antibody and its extraneous protein.

Antigen Antibody Binding

There are four types of chemical bonds involved in antigen antibody binding: ionic bonds, hydrogen bonds, hydrophobic bonds, and Van der Waals forces. All four of these types of chemical bonds are reversible and require close association between the antigen and antibody molecule in order to have strong binding. Therefore the antigen molecule must be highly complementary to the structure of the antibody molecule in order to get high affinity binding. The shape, charge distribution, and hydrophobic regions on the antigen and antibody molecules must all be complementary for binding to occur.

Since antigen antibody binding does not involve covalent bonds, it is always reversible. Therefore antigen antibody molecules in solution are continually associating and disassociating. If the antibody has a high affinity for the antigen molecule, it will spend a much greater portion of time bound to the antigen than separated from the antigen. Antigen and antibody molecules in solution will come to equilibrium between the bound and unbound state within a few minutes to a few hours depending on the temperature and viscosity of the solution. Since ionic bonds are an important component of antigen antibody binding, disruption of ionic bonding by altering the pH

or ionic strength of solutions away from physiologic pH and ionic strength may dissociate antigen antibody binding. The ability to dissociate antigen antibody binding by altering pH and ionic strength is often used to the scientist's advantage for purifying either antigens or antibodies using affinity chromatography.

Not all molecules make good antigens. In general, molecules that are foreign to the animal, large, rigid, and chemically complex make the best antigens for inducing an antibody response. The molecules must be foreign in order to induce an antibody response because typically animals are tolerant to molecules normally found in the body. Size of the antigen molecules is important because, in general, molecules of less than 10,000 molecular weight are not very antigenic. Small molecules can be made to be antigenic, that is they can induce an antibody response, if they are covalently bound to larger molecules. Small molecules which are covalently bound to larger molecules in order to induce an antibody response are called haptens. Complex molecules make better antigens because they have a greater variety of chemical structures on their surface for forming different types of non-covalent bonds needed for binding to antibody. An antigen molecule must be rigid in order to induce a strong antibody response since the antibodies bind to a characteristic stereochemical shape on the antigen molecule. If the antigen molecule is highly flexible the stereochemical shape will continually change.

In general, molecules containing peptide sequences make the best antigens, therefore, proteins, glycoproteins, and lipoproteins tend to be good antigens for inducing an antibody response. Polysacaharides, lipids, and nucleic acids are typically not as good for inducing an antibody response.

Cytokines

Cell-mediated immunity is mediated through the collective action of the cytokines and cytotoxic T cells (described below). T-lymphocytes secrete a variety of cytokines which are important in regulating the activity of the entire immune system. It is estimated that more than 100 molecules have been described already as cytokines. The T_H cells are the most active lymphocytes for cytokine secretion. The T_{H1} lymphocytes secrete cytokines which mainly enhance cell-mediated immune responses. The T_{H2} lymphocytes secrete cytokines which enhance antibody responses.

A brief description of some of the more well-characterized molecules involved in a cell-mediated immune response is included here.

Interleukin 1. IL-1 is a protein secreted by stimulated macrophages (it was previously known as **lymphocyte activating factor**). IL-1 facilitates the production of IL-2 by T_H cells and is, therefore, necessary for lymphocyte proliferation. In addition to its role in triggering lymphocyte proliferation, IL-1 causes fever (it was also known as **endogenous pyrogen**) and stimulates the liver to secrete acute-phase proteins (an important part of the acute inflammatory response).

Interleukin 2. IL-2 is a glycoprotein secreted by T-lymphocytes after antigen and IL-1 stimulation. IL-2 is required for the proliferation of activated T cells, NK cells, and other cytotoxic effector cells.

Interferons. There are three general types of interferon: alpha, beta, and gamma. Alpha interferons are produced by leukocytes and other cells in response to a variety of inducers, such as viruses, bacterial products, polynucleotides, and tumor cells. At least 15 sub-types of human alpha interferon have been described. Even though alpha interferons are secreted by T- and B-lymphocytes, they are not considered to be part of the acquired immune response because their production is not limited to those clones of cells that specifically recognize the antigen. Beta interferon is produced by fibroblasts and epithelial cells (as well as other cell types) in response to the same types of inducers (viruses, bacterial products, polynucleotides) as alpha interferons. Gamma interferon is produced by T-lymphocytes in response to antigenic stimulation. It therefore is considered to be the a component of cell-mediated immunity.

All three types of interferon control replication of certain viruses by inhibiting production of viral protein in infected cells. The interferons can also modify a variety of biologic activities and therefore, have important regulatory functions. Gamma interferon is an especially active biologic response modifier. It is one of the cytokines capable of activating neutrophils and macrophages to be more efficient. Gamma interferon also enhances the activity of NK cells.

Tumor Necrosis Factor. Tumor necrosis factor is a soluble protein secreted by macrophages or lymphocytes that have been appropriately stimulated. It was named for its ability to cause necrosis of subcutaneously transplanted tumors in mice. Tumor necrosis factor is preferentially cytotoxic for transformed cancer cells and is believed to be an important mediator of tumor cell killing. Tumor necrosis factor also may play a role in controlling virus infection and chronic intracellular bacterial infections.

Colony-Stimulating Factors. Colony-stimulating factor refers to a group of glycoproteins that stimulate leukocyte production by the bone marrow. Colony-stimulating factors may also enhance the antimicrobial activity of mature neutrophils and macrophages. Many cell types have been shown to produce colony-stimulating factor without an apparent stimulus, including macrophages, fibroblasts, and endothelial cells. Lymphocytes stimulated by antigen also produce various colony-stimulating factors.

Cytotoxic T cells. Cytotoxic T cells (Tc cells) have the CD8 molecule on their surface and can only recognize peptide antigens that are bound to an MHC class I molecule on the surface of cells. MHC class I molecules can only bind peptide molecules that are about 8-10 amino acids long. All nucleated cells in the body are capable of presenting foreign peptides on MHC class I molecules on their surface. Normal nucleated cells have the intracellular machinery for processing proteins in their cytoplasm into peptide segments that are 8-10 amino acids long, then transporting these segments into the endoplasmic reticulum where they become bound to MHC I

molecules and are transported to the cell surface. The T_C cell recognizes both the peptide antigen and the MHC I molecule.

Both T_H cells and T_C cells can only recognize antigen presented by MHC II or MHC I molecules that are found in the same individual that the T cells came from. MHC molecules are highly variable between individuals of the same species. Therefore, antigen presentation by MHC I and MHC II molecules is said to be genetically restricted. This means that the antigen presenting cell must come from an individual that is genetically identical to the individual that the T lymphocytes came from.

After a T_C cell recognizes a foreign peptide bound to an MHC I molecule, it will begin to respond. In a primary immune response, it will be stimulated by IL-2 and other cytokines to undergo mitosis to expand the clones of T_C cells that recognize the antigen. In a secondary immune response, the T_C cell will proceed to kill the target cell that has the foreign antigen on it's surface. It will kill the target cell using perforins and other molecules called granzymes that are stored in granules in its cytoplasm and by inducing apoptosis (programmed cell death) in the target cell.

Mucosal Immunity

Protection at mucosal surfaces provides a difficult challenge to the immune system. Mucosal surfaces are exposed to the environment where they are frequently in contact with pathogens. Mucosal surfaces are moist and often have a high level of nutrients (such as in the GI tract and mammary gland) which favor microbial growth. In addition, many of the cells and molecules of the immune system either are not present in sufficient concentration or have difficulty functioning on mucosal surfaces.

Native defense mechanisms are extremely important for protection at mucosal surfaces. One of the most important native defense mechanisms is the presence of normal microbial flora which helps to compete with and exclude pathogens from colonizing. If the normal microbial flora is disrupted due to antibiotic treatment or for other reasons, then pathogens can more easily colonize the mucosal surface. Other factors that help to protect mucosal surfaces include antibacterial peptides in mucosal secretions, the presence of a mucus layer over the epithelial cells, the flow of the mucus, low pH in the stomach, and an anaerobic environment in the intestines. Coughing, sneezing, vomiting and diarrhea are all protective mechanisms which help to physically remove pathogens from mucosal surfaces. If any of these native defense mechanisms are disrupted, it is likely to result in increased susceptibility to mucosal infections.

An important component of specific immunity at mucosal surfaces is the presence of secretory IgA in mucosal secretions. Secretory IgA is made up of two molecules of IgA held together by a J chain and bound to a molecule of secretory component. Secretory component is a protein molecule that helps to protect the IgA dimer from digestion by proteases which are frequently present on mucosal surfaces. The proteases can damage other classes of antibody which may

find their way to mucosal surfaces. The secretory IgA is concentrated in the mucus layer covering the epithelial cells and functions largely by attaching to and blocking microorganisms and toxins from binding to the epithelial cells. Blockage of attachment to the epithelial cells will prevent clinical signs from occurring for most bacterial, viral and toxin-induced diseases at mucosal surfaces.

IgA makes up only approximately 5-15% of the total antibody in the plasma. However, it is the predominant class of antibody on mucosal surfaces. Since the body has a very large amount of mucosal surface area to protect, IgA is actually the predominant class of antibody produced by the immune system. It is estimated that 60-70% of the total antibody present in an individual is IgA and the majority of it is found on mucosal surfaces.

The lymphoid tissues and cells that lead to IgA production on mucosal surfaces are somewhat separate from those that produce IgG in the plasma. Antigens to which an individual is exposed at a mucosal surface tend to induce the production of IgA on mucosal surfaces. Antigens that an individual is exposed to in deeper tissues either through systemic infection, injection of a vaccine, or natural exposure tend to induce an IgM and an IgG class antibody response. Thus, the type of antibody response induced tends to be optimal for protection at the particular site of infection.

In order for the immune system to respond to antigens present on a mucosal surface, the antigens must cross the mucosal surface so that antigen presenting cells and lymphocytes can interact with them. Mucosal surfaces, especially the intestine and respiratory tract have aggregates of lymphoid tissues under the epithelium; in the intestine these are called Peyers Patches. The epithelium over these lymphoid aggregates at mucosal surfaces is a specialized epithelium that is capable of transporting small amounts of material from the mucosal surface to the underlying lymphoid tissue where it can be processed and presented to lymphocytes. These specialized epithelial cells (M cells) are called membranous epithelial cells or dome cells. The T helper lymphocytes present in the submucosal lymphoid tissues tend to secrete cytokines that induce B cells to become IgA producing cells. When the B cells in the submucosal lymphoid tissues recognize an antigen they will proliferate to make more B cells specific for that same antigen and they will rearrange their DNA (class switching) to become IgA-producing cells. These B cells will then leave the lymphoid tissues through the lymphatic drainage and eventually reenter the blood stream by flowing with the lymph through the thoracic duct and into the anterior vena cava. These B cells will circulate through the blood for a brief period of time, then leave the blood stream in post-capillary venules at mucosal surfaces and enter the submucosal tissue. Most of the B cells that originate in intestinal Peyers Patches will return to the intestine. However many of them will end up at other mucosal surfaces, such as the upper respiratory tract, the salivary glands, the mammary glands in pregnant females, and the reproductive tract. The B cells which home to submucosal surfaces will then differentiate into plasma cells and produce secretory IgA specific for the antigens that were originally encountered at a mucosal surface.

This dimeric IgA needs to be transported across the epithelial barrier and onto the mucosal surface in order to provide protection. This transport is accomplished by the epithelial cells which line mucosal surfaces. These epithelial cells produce the secretory component protein on the submucosal side of their membrane, near the plasma cells. The J chain of the dimeric IgA binds to the secretory component in the epithelial cell membrane, then the complex of dimeric IgA with secretory component is transported through the epithelial cell and released onto the mucosal surface with the secretory component still attached to the dimeric IgA. The secretory component has a high affinity for mucus, therefore it serves to hold and concentrate the IgA in the mucus layer overlying the epithelial cells. This provides a protective blanket of IgA overlying the mucosal surface where it can effectively block microbes and toxins from penetrating the mucus layer and attaching to the epithelial cells.

Since some of the B cells that originate in the Peyers Patches in the intestines travel to the mammary gland, this results in the production of IgA in the milk that is specific for the pathogens in the mother's intestinal tract. This IgA in the milk provides rapid protection to the newborn against pathogens that the mother is carrying in her intestinal tract.

The IgA on mucosal surfaces serves to prevent pathogens from penetrating the mucosal surface. However, if a pathogen is successful in penetrating, then the T lymphocyte-directed cell mediated immune system can play a role in attempting to control these invading pathogens. Mucosal epithelial surfaces contain numerous intraepithelial lymphocytes. These lymphocytes can detect the presence of intracellular pathogens in the epithelial cells and pathogens which invade between epithelial cells and that have been captured by macrophages or other antigen presenting cells. They can respond by secretion of cytokines which activate macrophages, neutrophils, and natural killer cells or they can destroy the infected epithelial cells through cytotoxic activity. The T lymphocytes which serve to protect mucosal surfaces also tend to have separate circulatory patterns from the T lymphocytes which protect from systemic infection, much like the separate circulatory patterns described for B cells that produce IgA. This separate set of T and B lymphocytes which protect mucosal surfaces is called the common mucosal immune system. The separate circulatory patterns for lymphocytes that protect mucosal surfaces enables the immune system to provide optimal types of protective mechanisms for pathogens that enter through mucosal surfaces and different types of immune responses for pathogens that tend to produce systemic infections.

Immunity in the Fetus and Neonate

All components of the native and acquired immune systems develop in utero and are functional at birth in domestic animals. However, they are generally less efficient than in the adult. Since the normal newborn has not yet been exposed to antigen, it has not yet developed a humoral or cell mediated immune response to any infectious agents. After exposure to infectious agents it will take 7-10 days for a primary antibody or cell mediated immune response to develop. During this time resistance to infection depends upon the actions of the native defense mechanisms and antibody which is passively transferred from the mother to the neonate. In ruminants, pigs and

horses there is virtually no transfer of antibody across the placenta. The placenta in these species has several epithelial layers between the maternal and fetal circulation which prevents antibody transfer. In the large domestic species, passive transfer of antibody from mother to offspring occurs through the colostrum. The dam concentrates antibody in the colostrum during the last several days of gestation. This antibody is largely transferred intact across the gut epithelial cells into the circulation of the newborn. Intestinal absorption of antibody from the colostrum normally ceases by 24 to 36 hours after birth. The passive transfer of antibody from mother to newborn in the colostrum and milk is very important for neonatal survival. In dogs and cats, small amounts of antibody do cross the placenta. The newborn puppy or kitten will have circulating antibody concentrations that are about 10% of the mothers. They depend on colostrum for the rest of the passive antibody that they need.

Native Defense Mechanisms

The newborn has low levels of hemolytic complement activity at birth. In colostrum-deprived pigs the hemolytic complement activity gradually increases during the first 36 days of life. Piglets allowed to suckle colostrum have higher titers of hemolytic complement than colostrum-deprived piglets during the first 3 weeks of life. This suggests that some of the complement components that are present in limiting amounts are transferred through the colostrum to the piglet.

Phagocytic cells are present in newborn animals but generally have reduced phagocytic activity as compared to adult animals. In calves, neutrophil function does not reach adult levels until four to six months of age. Since phagocytes depend on complement and/or antibody to opsonize many infectious agents, the overall efficiency of phagocytosis may also be reduced due to inadequate levels of complement and antibody.

HYPERSENSITIVITIES

Hypersensitivities are conditions in which there is excessive responsiveness to antigen which the animal has previously been exposed to. The clinical signs are due to the immune response to the antigen rather than to a direct action of the antigen. The hypersensitivity conditions can be divided into 4 types based on their mechanism of action.

Mechanisms of Immune-Mediated Hypersensitivity

Type I or immediate type hypersensitivity involves the synthesis of specific IgE (reaginic or cytotropic) antibodies. The IgE preferentially binds to Fc receptors on the surface of tissue mast cells. When the same antigen is encountered subsequently it will bind to the IgE on the mast cell surface (if there is a sufficiently high concentration of IgE specific for the antigen) and cause the mast cell to release numerous pharmacologically active substances which are responsible for the clinical signs (e.g., histamine, serotonin, kinins, prostaglandins and others). Type I hypersensitivities may be localized to a particular region or organ or may be systemic (anaphylaxis).

Type II hypersensitivity (or cytotoxic type hypersensitivity) involves the presence of antibodies directed against cell membrane antigens. These may be normal tissue antigens in the case of autoimmune diseases or foreign antigens (e.g. drugs, viral or bacterial antigens) which have adhered to the cell surface.

Type III hypersensitivity (or immune-complex type hypersensitivity) involves the presence of antigen-antibody complexes in the circulation or tissue. These immune complexes can fix complement and, therefore, may initiate the inflammatory response, attract neutrophils to the site, and damage cell membranes.

Type IV hypersensitivity (or delayed-type hypersensitivity) is mediated by sensitized T cells releasing cytokines. It does not involve antibody. The tuberculin skin test is a classic Type IV hypersensitivity reaction.

It is not unusual for clinical hypersensitivity conditions to involve more than 1 of the 4 types of hypersensitivity.

IMMUNODEFICIENCY AND IMMUNOSUPPRESSION

Primary or secondary immunodeficiency increases the susceptibility of animals to infectious disease. A primary immunodeficiency is defined as a disorder of the immune system for which a genetic basis is proven or suspected. A secondary immunodeficiency is a disorder in which the animal is genetically capable of normal immune function, but some secondary factor is impairing resistance to disease.

Clinical findings that are associated with immunodeficiency include:

1. Illness from organisms of normally low pathogenicity or from an attenuated live vaccine.
2. Recurrent illnesses that are unusually difficult to control.
3. Failure to respond adequately to vaccination.
4. Unexplained neonatal illness and death affecting more than one animal in a litter.
5. A variety of disease syndromes occurring concurrently in a herd.

A large number of primary immunodeficiencies have been reported in man and a few have been reported in other domestic species;

A common cause of secondary immunodeficiency is failure of passive transfer of adequate levels of maternal antibody through the colostrum to the newborn. Other potential causes of secondary immunodeficiency (or immunosuppression) include:

1. Physical or psychological distress
2. Immunosuppressive infectious agents
3. Inadequate nutrition
4. Immunotoxic substances

Physical and Psychological Distress

There is ample evidence that both physical and psychological distress can suppress immune function in animals, leading to an increased incidence of infectious disease. Excess heat or cold, crowding, mixing, weaning, limit-feeding, shipping, noise, and restraint are stressors that are often associated with intensive animal production and have been shown to influence immune function in various species. Distress-induced alterations in immune function are mediated by interactions between the neuroendocrine and immune systems. The study of these multi-system interactions initially focused on the secretion and influence of glucocorticoids, which suppress several aspects of immune function. It is now recognized that there are many mechanisms by which the neuroendocrine system can alter immune function; in addition, the immune system is capable of altering the activity of the neuroendocrine system.

The neuroendocrine and immune systems communicate in a bidirectional manner via direct neural as well as hormonal signaling systems. Neuroendocrine signals that are capable of directly altering the function of cells of the immune system include: 1) direct sympathetic innervation to the parenchyma of the thymus, spleen, and bone marrow, 2) glucocorticoids produced by the adrenal cortex after pituitary adrenal corticotrophic hormone (ACTH) stimulation, 3) catecholamines produced by the adrenal medulla, 4) endogenous opiates (endorphin and enkephalins) produced by the pituitary, adrenal medulla, sympathetic terminals, and lymphocytes, 5) vasoactive intestinal peptide released by sympathetic neurons of the intestine and perhaps other sites, and 6) substance P released by sympathetic nerve terminals. Receptors have been detected on lymphocytes and thymocytes for a variety of hormones including corticosteroids, insulin, testosterone, estrogens, B-adrenergic agents, histamine, growth hormone, acetylcholine, and metencephalon. Some of these substances have been demonstrated to stimulate lymphocyte differentiation and affect their activity.

Conversely, the immune system can influence the function of the neuroendocrine system. Upon antigenic stimulation, lymphocytes have been shown to produce small amounts of ACTH, beta endorphin, metencephalon, thyroid stimulating hormone (TSH), and other classically "neural" peptides. Activation of the immune system, as during the response to an immunizing antigen, results in a change in neural firing rates in certain parts of the hypothalamus. Some evidence indicates that certain cytokines (interleukins) can promote hormone release by pituitary cells. Thymic hormones (thymosin alpha 1 in particular) seem to affect the central nervous system (CNS) as well as the immune system and, in turn, are regulated by the CNS. Thus, the interaction between the immune and neuroendocrine systems is reciprocal and feedback loops have been described.

Immunosuppressive Infectious Agents
Certain infectious agents are capable of suppressing immune function sufficiently to make the animal more susceptible to secondary infections. Viral infections often suppress phagocyte cell function, leading to increased susceptibility to bacterial infection.

Nutritional Influences on Immunity
Both malnutrition and overfeeding may result in impairment of immune function and increased susceptibility to disease due to a deficiency or excess of proteins or calories, or a relative imbalance in vitamin or trace mineral content. Animals under intensive production conditions typically have a completely controlled diet. Therefore, it is very important that the diet, especially the vitamin and trace mineral content, be optimally formulated. Key vitamins and minerals for optimal immune function include vitamins A, C, E, and the B complex vitamins, copper (Cu), zinc (Zn), magnesium (Mg), manganese (Mn), iron (Fe), and selenium (Se). The balance of these constituents is especially important since an excess or deficiency in one component may influence the availability or requirement for another.

It is difficult to predict the optimal diet for immune function. The dietary requirements for optimal immune function may differ from the requirements to avoid deficiencies as judged by traditional methods. Relatively slight imbalances of a particular nutrient may suppress immune function, whereas a more severe deficiency must occur before the classical clinical evidence of deficiency of that nutrient is recognized. In addition, stress or the demands of rapid growth may change dietary requirements for optimal immune function.

Immunotoxic Substances
Various compounds, including heavy metals, industrial chemicals, pesticides, and mycotoxins, have been shown to be immunosuppressive at very low levels of exposure. These compounds may be detrimental to the immune system and predispose animals to infectious diseases at levels that do not cause other symptoms of toxicity.

IMMUNOMODULATION

Immunomodulators are a new form of preventive or therapeutic treatment that show promise for enhancing immune function, thereby increasing resistance to infectious diseases in domestic animals. Immunomodulators, also referred to as biologic response modifiers, can be divided into two categories: 1) endogenous immunomodulators, which are cytokines that are normally produced by the host and are products of the host genome, and 2) exogenous immunomodulators, which are not products of the mammalian genome. The exogenous immunomodulators include bacteria, bacterial derivatives, and pharmacologic compounds, which tend to act either by inducing the release of endogenous immunomodulators or by a direct pharmacologic affect on cells of the immune system.

Advances in protein chemistry and recombinant DNA technology have made possible the production of endogenous immunomodulators in large quantities and of high purity.

Endogenous immunomodulators that have been produced in quantities sufficient for in vivo testing include: interleukin 2, alpha interferon, gamma interferon, tumor necrosis factor, granulocyte-macrophage colony stimulating factor, granulocyte colony stimulating factor, and several peptide hormones from the thymus. There are species differences in these cytokines. A cytokine from one species will sometimes work in a different species.

GENERAL PRINCIPLES OF VACCINATION

For nearly 100 years scientists have known that animals may develop immunity to diseases if exposed to either the killed infectious agent or a live strain of the agent which has been modified so it does not cause disease. This approach led to the development of many successful vaccines in the early 1900s. However, it soon became apparent that for certain diseases this simple approach was not effective. An animal, for example, might produce antibody in response to vaccination, but still develop the disease. These are diseases for which circulating antibody alone is not protective or for which the vaccines do not induce antibody against the important antigens of the pathogen. The challenge for these diseases is to understand the basis for successful immunity, then to develop vaccines which induce this type of immunity.

The basic types of immune defense mechanisms against infectious agents (as discussed earlier in this manuscript) are:

1) Native defense mechanisms, the first line of defense and already operational, even in the non-vaccinated animal.
2) Humoral immunity, due to the presence of antibodies in the bloodstream.
3) Cell-mediated immunity, caused by the action of various types of white blood cells and orchestrated by T lymphocytes.
4) The secretory IgA system, important for resistance to diseases at mucosal surfaces such as the gastrointestinal tract, the respiratory tract, the mammary gland, and the reproductive tract.

It is apparent that different diseases require different types of immunity for protection and the type of vaccine (modified live versus killed), route of administration, and type of adjuvant make a difference in the type of immune response. General principles regarding vaccine efficacy and vaccine failure will be discussed here. It must be remembered that there are exceptions to these general principles for specific vaccines and specific diseases.

Selective Induction of Different Types of Immunity

It is relatively easy to develop a vaccine which will cause the production of IgG and IgM antibody in the bloodstream. However, the vaccine may not induce antibodies against the important antigens of the infectious agent. Antibody alone is not capable of killing infectious agents. The presence of circulating IgG and IgM may help to control disease by:

1) Agglutinating infectious agents thereby reducing the number of infectious particles (for viruses) and facilitating removal by phagocytosis.
2) Binding to and neutralizing toxins.
3) Binding to the infectious agent and blocking attachment to cell surfaces.
4) Binding to the infectious agent and initiating the classical pathway of complement activation.
5) Opsonizing infectious agents and facilitating phagocytosis.
6) Mediating attachment of cytotoxic cells to the surface of infected cells so the infected cells may be destroyed by antibody-dependent cell-mediated cytotoxicity.

Some disease-causing organisms, however, are resistant to control by these activities of circulating antibody. These organisms must be controlled by the cell-mediated immune system or the secretory IgA system. It is more difficult to develop a safe and effective vaccine which induces these types of immunity.

Protecting the animal from infection at mucosal surfaces such as the intestinal tract, respiratory tract, mammary glands, and reproductive tract is especially difficult for the immune system. The antibodies responsible for humoral immunity and the white blood cells responsible for cell-mediated immunity are found in the blood stream and in the tissues to some extent. However, they are not found on some mucosal surfaces. Therefore, they can help to prevent invasion through the mucosal surface, but are not very effective at controlling infection on the mucosal surface. Even in the lung and the mammary gland, where IgG and white blood cells are found in relative abundance, they are not able to function as effectively as in the blood stream and tissues. Protection on mucosal surfaces is due in large part to secretory IgA. Secretory IgA is secreted onto mucosal surfaces where it may bind to mucus and be present in fairly high concentration. Secretory IgA is resistant to destruction by the proteolytic enzymes on mucosal surfaces that are capable of breaking down IgG and IgM.

The nature of the vaccine and the route of administration are important for influencing the type of immunity induced. Subcutaneous or intramuscular injection of a killed vaccine will stimulate the immune system to produce IgM and IgG classes of antibody. However, there is very little production of IgA to protect the mucosal surfaces. In addition, the killed vaccines are not very effective at inducing cell-mediated immunity.

The induction of cell-mediated immunity generally requires a modified live vaccine capable of replicating in the animal or a killed vaccine with a highly effective adjuvant. Adjuvants which have traditionally been used in animal vaccines are not very effective at inducing cell-mediated immunity. New adjuvants are being developed which show promise for inducing cell-mediated immunity using killed vaccines. There are killed vaccines that have been available for many years and have been effective in controlling certain systemic type diseases. These are generally diseases that can be controlled by the presence of IgG in the circulation.

The route of vaccine administration is important when attempting to induce mucosal immunity. To get secretory IgA produced at mucosal surfaces, it is best for the vaccine to enter the body through exposure to a mucosal surface. This can be accomplished by feeding the vaccine to the animal, aerosolizing the vaccine so the animal will inhale it, or intramammary exposure. If a dam is exposed to an infectious agent in her intestinal tract, she may respond by producing secretory IgA not only in her own intestinal tract, but also in her mammary gland. The dam passes the IgA against the infectious agent to the newborn when it suckles. Therefore, the secretory IgA in the dam's milk can protect the newborn from infectious agents present in the dam's intestine. This protection will only last as long as the newborn continues to suckle. Enteric infections by many organisms are not controlled by the presence of IgG and IgM in the bloodstream or by cell-mediated immunity. If a modified live vaccine is given by injection, but goes to a mucosal surface to replicate, it may also induce a secretory IgA response.

Vaccination Failure

There are many reasons why animals may develop disease even though they have been vaccinated. Disease may occur because:

1) The animal may have been incubating the disease when it was vaccinated;
2) Something may have happened to the vaccine to make it ineffective;
3) The physiologic status of the host may make it unresponsive or hyporesponsive to the vaccine; or
4) The host may be exposed to an overwhelming challenge dose of infectious agent.

By being aware of these factors, veterinarians and producers can help to minimize the occurrence of vaccine failures.

Occurrence of Disease Shortly After Vaccination. The host requires several days after vaccination before an effective immune response will develop. If the animal encounters an infectious agent near the time of vaccination, the vaccine will not have had time to induce immunity. The animal may come down with clinical disease resulting in an apparent vaccination failure. In this situation, disease symptoms will appear shortly after vaccination and may be mistakenly attributed to vaccine virus causing the disease. Modified live vaccine viruses have been attenuated to be of reduced virulence. The attenuation must be shown to be stable, therefore, reversion to virulence is thought to be a rare event. However, the attenuated vaccine strains may be capable of producing disease in immunosuppressed animals.

Alterations in the Vaccine. Improperly handled and administered vaccines may fail to induce the expected immune response in normal, healthy animals. Modified live bacterial and viral vaccines are only effective if the agent in the vaccine is viable and able to replicate in the vaccinated animal. Observing proper storage conditions and proper methods of administration are very important for maintaining vaccine viability. Failure to store the vaccine at refrigerator temperatures or exposure to light may inactivate the vaccine. Even when stored under appropriate conditions, the vaccine loses viability over time. Therefore, vaccines that are past

their expiration date should not be used. The use of chemical disinfectants on syringes and needles can inactivate modified live vaccines if there is any residual disinfectant. The use of improper diluent or the mixing of vaccines in a single syringe may also inactivate modified live vaccines. Diluent for lyophilized vaccines are formulated specifically for each vaccine. A diluent which is appropriate for one vaccine may inactivate a different vaccine. Some vaccines and diluents contain preservatives which may inactivate other modified live vaccines. For these reasons, multiple vaccines should not be mixed in a single syringe unless that particular combination has been adequately tested to insure there is no interference.

Host Factors Contributing to Vaccine Failure. Vaccine failures may occur because a vaccinated animal is not able to respond appropriately to the vaccine. Vaccine failure in young animals may be due to the presence of maternal antibody which prevents adequate response to vaccination. It can also be due to immunosuppression from a variety of causes.

Maternal antibodies derived from colostrum are a well known cause of vaccine failure. These antibodies in the young animal's circulation may neutralize or remove the antigen before it can induce an immune response. Typically, virulent infectious agents are capable of breaking through maternal immunity earlier than modified live or killed vaccines. This means that even if young animals are immunized frequently, there still may be a period when they are vulnerable to infection. Vulnerability occurs between the time that young animals lose their maternal antibody and before they develop their own active immune response. This period can be shortened by the use of less-attenuated modified live vaccines or the use of killed vaccines with high antigenic mass. A high challenge dose of infectious agents will break through maternal immunity sooner than low exposure to infectious agents. Therefore, overcrowding and poor sanitation exacerbate the problem of inducing immunity in young animals before they come down with clinical disease.

Veterinarians commonly recommend that puppies and kittens be vaccinated every 3 weeks between approximately 6- and 18-weeks of age. However, for large domestic animals, a single vaccination is commonly recommended to induce immunity during the first few weeks or months of life. There is no inherent difference between large and small domestic animals in their response to vaccination in the face of maternal immunity. The frequent vaccinations recommended in puppies and kittens minimizes the period of vulnerability to infectious diseases.

Because only one vaccination is commonly recommended for large domestic animals, the timing of the vaccination is important. If the vaccine is administered too soon, it may be ineffective because of the presence of maternal antibody. If the vaccine is administered after all maternal antibodies are gone from animals in the herd, there may be a prolonged period of vulnerability before they develop their own immune response. Most veterinarians and producers decide that because of time and expense considerations it is impractical to vaccinate young food producing animals frequently. However, frequent vaccination may be justified in cases of unusually high disease incidence.

Immunosuppression due to a variety of factors including stress, malnutrition, concurrent infection, or immaturity or senescence of the immune system may also lead to vaccination failure. If the immunosuppression occurs at the time of vaccination, the vaccine may fail to induce an adequate immune response. If the immunosuppression occurs sometime after vaccination, then disease may occur due to reduced immunity in spite of an adequate response to the original vaccine. Therapy with immunosuppressive drugs (e.g., glucocorticoids) may also cause this to occur.

Another concern is that some modified live vaccines are capable of inducing disease in the immunosuppressed animal. Modified live vaccines are typically tested for safety in normal, healthy animals. They are not recommended for use in animals with compromised immune systems. Therefore, these vaccines should not be used in animals that are immunosuppressed for any reason. This includes animals in the first few weeks of life unless the vaccine has been specifically tested in animals this young. When it is necessary to vaccinate animals under these conditions, killed vaccines should be used.

Overwhelming Challenge Dose. Most vaccines do not produce complete immunity to disease. They provide an increased ability to resist challenge by infectious agents. If a high challenge dose of organisms is present due to overcrowding or poor sanitation, the immune system may be overwhelmed resulting in clinical disease.

Vaccine Efficacy

Vaccines that are licensed by the United States Department of Agriculture have been tested to determine that they are safe and effective. However, "effective" is a relative term. It does not mean that the vaccine must be able to induce complete immunity under all conditions which may be found in the field. This would not be realistic since the immune system is not capable of such potent protection under adverse conditions.

To be federally licensed, the vaccine must have been tested under controlled experimental conditions. The vaccinated group must have had significantly less disease than the non-vaccinated control group. This testing is typically done on healthy, non-stressed animals under good environmental conditions and with a controlled exposure to a single infectious agent. Vaccines may be much less effective when used in animals that are under stress, incubating other infectious diseases, or exposed to a high dose of infectious agents due to overcrowding or poor sanitation.

It is important to remember that for most diseases the relationship between the infectious agent and the host is sufficiently complicated that vaccination cannot be expected to provide complete protection. The vaccine can increase the animals resistance to disease, but this resistance can be overwhelmed if good management practices are not followed.