

3 Immune System

J. A. Roth

THE IMMUNE SYSTEM comprises 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, 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 2-3 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. The immune response in mammals has been shown to be influenced by genes in the major histocompatibility complex. Immunogenetics and the major histocompatibility complex of the pig are discussed in Chapter 56 of this book.

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

If an animal is immunosuppressed due to stress, preexisting viral infection, immunotoxins, 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, which 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 pig from infectious and neoplastic diseases. If the immune system is overstimulated or is not appropriately regulated, it may cause hypersensitivity reactions 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 mucus, low pH, bile, and numerous enzymes. More detailed information on these barriers to infection can be found in chapters dealing with specific organ systems.

COMPLEMENT. The complement system is an enzyme cascade system similar to the coagulation system and is composed of at least 20 serum proteins. In a cascade system something activates the first component, which in turn activates the next component which in turn activates the next component, etc., until the reaction is completed. The components of the mammalian complement system can be divided into different groups: classical pathway, alternative pathway, membrane attack pathway, and regulatory proteins. All nine components of the classical and membrane attack pathways have been individually titrated in swine sera (Barta and Hubbert 1978). The complement system is very important in mediating the inflammatory response and controlling bacterial infection. It also plays a prominent role in many types of allergies and hypersensitivity diseases. The classical pathway is triggered primarily by antigen antibody complexes (IgG and IgM); the alternative pathway may also be activated by antigen antibody complexes (IgA and IgE) and by certain bacterial products, such as endotoxin, and by proteases released by damaged tissue. Both the classical and alternative pathways end in the

splitting of the third component of complement (C3) and start the formation of the membrane attack complex.

The complement system has many important biologic activities. Activation of either complement pathway causes vasodilation and increased vascular permeability, resulting in serum components (including antibody and complement) entering the tissues to help control infection. Complement components produced during activation are chemotactic and attract phagocytic cells to the site of infection; they also coat infectious agents so they can be more easily phagocytized. A very important function of the membrane attack pathway of complement is the destruction of cell membranes including some bacterial cell membranes.

The complement system is important for mediating inflammation and controlling bacterial infection. However, since it is so potent it is also capable of causing serious and even life-threatening damage if it is activated in an unregulated fashion. Therefore, numerous regulators of complement, which help to control and stop the complement reaction once it has started, are present in the serum.

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 two main types of phagocytic cells: 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 described below for neutrophils. In addition, 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 produced in the bone marrow and are released into the blood. The half-life of neutrophils in the bloodstream is approximately 8 hours; they then enter the tissues. In healthy individuals the neutrophils are lost primarily into the intestinal tract and lung. Neutrophils migrate into the intestinal tract very rapidly in response to *E. coli* infection in the pig (Sellwood et al. 1986). Neutrophils in the circulation tend to marginate in the capillaries by loosely associating with the endothelial cells. In swine, neutrophils seem to have a high affinity for margination in the capillaries of the lung (Ohgami et al. 1989).

The principal function of neutrophils is to phagocytose and destroy invading microorganisms. Neutrophils are well-equipped with several mechanisms to perform this function. To be effective,

neutrophils must first come into the vicinity of the invading microorganism by the chemotactic attraction to the site. Chemotactic factors may be produced directly by certain microorganisms, generated by the cleavage of certain complement components, or released by sensitized lymphocytes at the site of infection or inflammation. Chemotactic factors will diffuse away from the site to form a gradient, and when they reach a capillary, they cause the endothelial cell membrane and the neutrophil membrane to increase the expression of adhesion proteins. Neutrophils then adhere to the endothelial cells and leave the capillary by diapedesis. Once in the tissues, the neutrophils migrate along the chemotactic factor gradient toward the source of the chemotactic factor and thus arrive at the site of infection; they may begin to ingest the microorganisms if those agents are susceptible to phagocytic activity. Most pathogenic microorganisms must be opsonized before they can be ingested; bacteria are opsonized by the attachment of specific antibody and/or complement to their surface. The opsonization process facilitates ingestion. When a neutrophil comes into contact with an opsonized particle, it attempts to surround the particle with pseudopodia and ingests it by phagocytosis. The ingested particle will be within a membrane-bound vesicle called a phagosome.

The neutrophil cytoplasm contains two main types of membrane-bound lysosomes or granules: primary (or azurophilic) granules and secondary (or specific) granules. These lysosomes contain numerous hydrolytic enzymes and other substances that are important to the bactericidal activity of the neutrophil. After a particle is ingested and is inside a phagosome, the neutrophil "degranulates"; some of the lysosomes will fuse with the phagosome and release their contents into the phagosome with the ingested particle. Hydrolytic enzymes function under both aerobic or anaerobic conditions in an attempt to destroy the ingested microorganisms. Neutrophils die after a short time at sites of inflammation. Hydrolytic enzymes are released and contribute to the inflammatory response and tissue destruction.

In addition to having hydrolytic enzymes in its granules, the neutrophil has potent bactericidal mechanisms that can function under aerobic conditions only. These mechanisms are related to the oxidative metabolism of the neutrophil. When a neutrophil is stimulated by an opsonized particle, oxygen usage increases rapidly. This burst of oxidative metabolism results in the production of some highly reactive short-lived oxygen species, specifically hydrogen peroxide (H_2O_2), superoxide anion (O_2^-), the hydroxyl radical (OH^\cdot), and perhaps singlet oxygen (1O_2). All of these components can damage microbial organisms. The H_2O_2 formed after phagocytosis may also react with halide ions in a reaction catalyzed by a myeloperoxidase enzyme that is released from the primary

granules. This reaction is one of the most potent bactericidal mechanisms of the neutrophil and is also potentially fungicidal and virucidal.

In addition to its important role in the phagocytosis and destruction of pathogenic bacteria, the neutrophil may also be important in controlling certain viral infections via a mechanism referred to as antibody-dependent cell-mediated cytotoxicity. As the name implies, this mechanism requires antibody, which presumably forms a bridge between the neutrophil and the virus-infected target cell. The neutrophil will then attempt to destroy the target cell. The mechanism of this cell destruction is not known but is thought to involve a direct membrane-to-membrane interaction. Porcine neutrophils have a high level of antibody-dependent cell-mediated cytotoxicity, even in the fetus and newborn (Zarkower et al. 1982; Yang and Schultz 1986). They are the only cell type capable of antibody-dependent cell-mediated cytotoxicity against African swine fever virus (Norley and Wardley 1983b).

Neutrophils therefore undergo several steps in attempting to control invading microorganisms: (1) adherence to vascular epithelium and exit from blood vessels via diapedesis, (2) random and directed migration along a chemotactic gradient, (3) the engulfment of opsonized microorganisms, (4) degranulation, (5) generation of oxygen-free radicals and H_2O_2 , (6) myeloperoxidase-catalyzed reaction, and (7) antibody-dependent cell-mediated cytotoxicity. Assays can be conducted for each of these processes. If any of these processes are impaired in the neutrophil, one would expect that the neutrophil would not be able to perform its function of controlling microbial infection as efficiently, which would make the animal more susceptible to microbial infection. Depression of neutrophil function has been associated with increased susceptibility to experimentally induced *E. coli* mastitis in sows (Lofstedt et al. 1983).

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. Mononuclear phagocytes comprise circulating monocytes, fixed macrophages, and wandering macrophages (histiocytes). Monocytes are produced in the bone marrow and released into the blood stream where they circulate before migrating into tissues to become macrophages. Fixed macrophages are

found lining the endothelium of capillaries and sinuses of organs such as the spleen, bone marrow, and lymph nodes. 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 but 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, and thus are able to kill certain types of bacteria that are resistant to killing by neutrophils. This is especially true if the macrophages have been activated by lymphokines 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 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.

Natural killer cells in the pig differ markedly from NK cells found in other species. NK activity in swine is mediated by small granular lymphocytes that have the cluster of differentiation (CD)2 T-cell marker (Ferguson et al. 1986; Duncan et al. 1989) and are, therefore, not null cells (Duncan et al. 1989). Swine NK cells initiate the lytic process against typical target cells (YAC-1 lymphoma or K-562 myeloid leukemia cells) more slowly than cells responsible for NK activity in other species (Ferguson et al. 1986). In swine there is evidence that the NK-cell activity and the K-cell activity

are from two distinct populations of lymphocytes (Kim and Ichimura 1986; Yang and Schultz 1986).

The activity of NK cells in many species is increased in the presence of gamma interferon and interleukin-2. Swine NK cells have been shown to respond to an interferon inducer (poly I:C) with enhanced NK activity (Lesnick and Derbyshire 1988). 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 lymphokine 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 that recognize exactly the same antigen make up a "clone," all of which arise 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), lymphocytes come in contact with antigens that arrive through the afferent lymphatics and are 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 to expand the clone of lymphocytes that recognize the antigen. 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 that 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 to phagocytize and destroy infectious agents. After the infectious agent is partially degraded by the macrophage, antigenic fragments appear on the macrophage surface where they can easily be contacted by B and T lymphocytes. Macrophages (and other specialized antigen-presenting cells) have a high density of class II major histocompatibility complex (MHC) molecules on their surface. T helper (T_H) cells are needed to help initiate the immune response. They can only recognize efficiently 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: 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 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 IL-2 is needed for 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, which act as receptors, bound to their surface. 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 released by T_H cells. These factors 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 SUBPOPULATIONS. Lymphocyte subpopulations in the peripheral blood of pigs are markedly different from other species. Young pigs have high blood lymphocyte counts compared to most other mammals (approximately 10^7 /ml). Up to 50% of these lymphocytes are null cells, which lack all surface markers specific for B or T lymphocytes (Duncan et al. 1989). These null cells do not recirculate between the blood and lymphatic tissues, and they differ from null cells in other species in that they do not have NK cell activity. The functional role and fate of this large population of null lymphocytes is unknown (Duncan et al. 1989).

Swine T lymphocytes have at least three unusual properties compared with other species (Lunney and Pescovitz 1987): (1) Approximately 25% of swine peripheral blood T cells express both the CD4 and CD8 antigens on their surface. (2) The ratio of CD4+/CD8+ T cells is normally approximately 0.6 in pigs, which is a reversal of the expected ratio in other species. A normal ratio of CD4+/CD8+ in humans is 1.5–2.0. Both of these properties are very unusual in other species and only occur in pathological conditions in humans. (3) Resting CD8+ cells in swine preferentially express class II MHC antigens. Since swine have relatively normal antibody production and cell-mediated immune responses, these unique properties of swine lymphocytes do not seem to have a negative impact on resistance to disease.

LYMPHOCYTE CIRCULATION. Lymph node structure and lymphocyte circulation are markedly different in the pig compared with humans or other domestic species. 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 in the bone marrow as well as in the thymus and in all secondary lymphoid tissues in the pig. 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. Null cells in the pig apparently remain in the bloodstream and do not recirculate between blood and lymphoid tissues. Porcine lymph nodes are structurally inverted compared with other domestic species. Lymphatics enter the node through the hilus and the lymph passes through the node with the lymph leaving through the periphery. The lymph node has a dense medulla, which lacks sinuses and cords. The germinal centers are located in the interior of the node. Other lymphoid organs such as the Peyer's patches, tonsils, and spleen are similar to those found in other species (Binns et al. 1986; Pabst and Binns 1986). Lymphocytes in swine and other species enter the lymph node through two routes. Lymphocytes leaving the bloodstream and entering the subcutaneous tissues are carried to the lymph node in the afferent lymphatics. Lymphocytes may enter the lymph node directly by adhering to high endothelial cells in the venules of the lymph node and then traversing the endothelial barrier. In other species, the lymphocytes exit the lymph node in the efferent lymphatics and are carried through the thoracic duct back to the circulatory system. In swine, the efferent lymph contains very few lymphocytes; the lymphocytes in the lymph node directly reenter the circulation

(Binns et al. 1986). The emigration of lymphocytes from blood into lymph nodes can be increased by antigenic stimulation. In addition to migrating from blood to lymphoid tissues, lymphocytes in swine migrate into most other tissues as well (Binns et al. 1986). Lymphocyte subpopulations in swine show a distinct preference for circulation to either gut-associated lymphoid tissues or surface nodes (Binns et al. 1986). For instance, mesenteric lymph node cells (both T and B lymphocytes) preferentially home to the gut (Salmon 1986). In rodents the majority of the lymphocytes found in the mammary gland also come from gut-associated lymphoid tissue, whereas in swine approximately equal numbers of lymphocytes in the mammary gland come from gut-associated lymphoid tissue and from peripheral lymph nodes. The dual origin of mammary lymphocytes in swine suggests that the local mammary immune response may not depend solely on oral immunization (Salmon 1986, 1987).

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 lymphokines, and B lymphocytes differentiate into plasma cells that secrete antibody (B lymphocytes may also be able to secrete some lymphokines). Antibodies are specific for the antigens that induced them, whereas lymphokines 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 (T_c cells) are an important part of the cell-mediated immune response to virus infection and tumors. Most T_c 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

Production of Immunoglobulins. B lymphocytes from clones that have never been stimulated by antigen have surface monomeric IgM antibody molecules 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 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 that 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 where in the body 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 antibody alone cannot kill infectious agents, it has a very important function to mark such agents for destruction by complement, phagocytic cells, and/or cytotoxic cells. 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.

Classes of Immunoglobulins. Characteristics of the various classes of porcine immunoglobulin were thoroughly reviewed in the previous edition of this book (Porter 1986).

The predominant Ig class in the pig and other species is IgG. It accounts for more than 80% of the Ig in serum and colostrum (Table 3.1). The two main subclasses of IgG are IgG₁ and IgG₂ (Metzger and Fougereau 1968), with IgG₁ predominating in serum and colostrum. IgG₃ and IgG₄ subclasses are found in lesser concentrations. An 18S Ig has been described that is antigenically similar to IgG₂ and is found in low levels in normal serum and colostrum (Kim et al. 1966). Newborn piglets also possess a 5S IgG, which may not have light chains and may not be functional (Stertzl et al. 1960; Franek and Riha 1964).

IgM accounts for approximately 5–10% of the total Ig in serum and colostrum (Table 3.1). The IgM is a pentamer held together by disulfide bonds and has a sedimentation coefficient of 17.8S (Porter 1969).

IgA is present in swine serum as 6.4S monomers and as 9.3S dimers, which are two monomers bound together with a J chain (Halpern and Koshland 1970; Mestecky et al. 1971; Porter and Allen 1972). IgA at mucosal surfaces is mostly dimeric IgA with a J chain and associated secretory component.

As discussed by Porter (1986), an analog of IgE has apparently not yet been fully defined in the pig. Antibodies against human IgE and bovine IgE have been shown to react with a homocyto-

Table 3.1. Concentration of porcine immunoglobulins (mg/ml) in body fluids

	IgG	IgG ₂	IgM	IgA
Adult sow	24.33 ±0.94	14.08 ±0.49	2.92 ±0.20	2.07 ±0.20
Colostrum	61.8 ±2.5	40.3 ±1.6	3.2 ±0.2	9.6 ±0.6
Milk				
(24 hr)	11.8 ±4.8	8.0 ±3.2	1.8 ±0.3	3.8 ±1.0
(48 hr)	8.2 ±3.2	5.0 ±1.8	1.8 ±0.4	2.7 ±0.6
(3–7 days)	1.9 ±0.6	1.3 ±0.3	1.2 ±0.2	3.4 ±1.0
(8–35 days)	1.4 ±0.6	1.00 ±0.45	0.90 ±0.25	3.05 ±0.74
Intestinal fluid				
Piglet	0.002		0.065	0.033
Sow	>0.001		0.001	0.091
Urinary tract	4.7			0.77
Follicle				
Diestrus	18.1			0.7
Estrus	25.1			0.7
Uterine secretions				
Diestrus	0.32			0.20
Estrus	0.34			0.12
Cervicovaginal mucus				
Diestrus	6.7		0.60	1.1
Estrus	2.0		0.06	0.6

Source: Veterinary Clinical Immunology, R. E. W. Halliwell and N. T. Gorman, editors. W. B. Saunders, 1989, with permission.

tropic immunoglobulin in swine serum (Barratt 1972; Nielsen 1977).

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 heterogeneous mixture of antibodies that 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 is 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) 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; thus, the antibody can be present in extremely high concentrations, which 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.

LYMPHOKINES. Cell-mediated immunity is mediated through the collective action of the lymphokines and T_C cells (described above). T lymphocytes secrete a variety of lymphokines that are important in regulating the activity of the entire immune system. It is estimated that more than 100 molecules have already been described as lymphokines.

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 subtypes of human alpha interferon have been described. Even though alpha interferons are secreted by T and B lymphocytes, they are not considered to be lymphokines 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 a lymphokine.

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, an especially active biologic response modifier, is one of the lymphokines 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.

Mucosal Immunity. Mucosal surfaces are frequently exposed to infectious agents and providing immunity at mucosal surfaces is a difficult problem. The components of the immune system described previously may not function well in the microenvironment on the mucosal surface. The degree to which the various components of the immune system contribute to protective immunity varies with the mucosal surface. For instance IgG class antibody, complement, and phagocytic cells may function efficiently in the lower respiratory tract and in the uterus but not in the lumen of the gut.

An important component of immunity at mucosal surfaces is the secretory IgA system. Antigen that enters the body through a mucosal surface tends to induce an IgA class antibody response at the mucosal surface. It may also induce secretory IgA at other mucosal surfaces. Specialized epithelial cells called dome cells or M cells are found overlying aggregations of gut- and bronchus-associated lymphoid tissues. These dome cells pinocytose antigen and transport it across the epithelial layer. The antigen may then be processed by antigen-presenting cells and presented to T and B lymphocytes.

Lymphocytes in the bloodstream tend to segregate into two populations: those that circulate between the bloodstream and the systemic lymphoid tissues of the lymph nodes, spleen, and bone marrow; and those that circulate between the bloodstream and lymphoid tissues associated with mucosal surfaces. Because of the nature of the T-helper and T-suppressor cells that home to mucosal surfaces, antigens entering through mucosal surfaces tend to induce an IgA or IgE class antibody. In some cases antigens entering through the intestinal tract may induce oral tolerance, resulting in suppression of IgG class antibody responses.

In the mucosal lymphoid tissues, B cells that have been stimulated by antigen and induced by T-helper cells to switch to IgA class antibody production will leave the submucosal lymphoid tissue and reenter the bloodstream. These lymphocytes exit the bloodstream at submucosal surfaces and locate in the lamina propria where they differentiate into plasma cells that secrete dimeric IgA. Many of these cells return to the same mucosal surface from which they originated, but others can be found at other mucosal surfaces. There is a special affinity for lymphocytes that have been sensitized in the gut of the sow to migrate to the mammary gland to become plasma cells and se-

crete IgA into the milk. The IgA in the milk helps to protect the piglet from intestinal pathogens while it is nursing. This is an important mechanism for transferring immunity from the sow to the piglet for enteric pathogens that the sow has been exposed to.

The dimeric IgA secreted by the plasma cells in the lamina propria binds to secretory component on the basal membrane of mucosal epithelial cells. The dimeric IgA and secretory component are then transported to the mucosal surface of the epithelial cell, and this complex is released onto the mucosal surface. Secretory component is important for protecting the IgA molecule from proteolytic enzymes and also serves to anchor the IgA into the mucous layer so that it forms a protective coating on the mucosal surface.

Secretory IgA plays an important role in immunity at mucosal surfaces by agglutinating infectious agents, preventing attachment of infectious agents to epithelial cells, and neutralizing toxins. Other components of the immune response may also be important in protection against various types of infection at mucosal surfaces. For example, in the pig neutrophils can immigrate into the intestinal lumen in large numbers within a 4-hour period in response to antigen-antibody complexes. The recruitment of neutrophils into the intestinal lumen is dependent upon the presence of antibody, which may be circulating IgG antibody (Bellamy and Nielsen 1974), colostral antibody (Sellwood et al. 1986), or locally induced IgA class antibody (Bhogal et al. 1987). Neutrophils in the lumen have been shown to be actively phagocytic (Bhogal et al. 1987). The immigration of neutrophils into the lumen of the gut and their subsequent destruction has been shown to result in an increased concentration of lactoferrin, lysozyme, and cationic proteins. These substances may also contribute to immunity to bacterial infections in the gut.

T lymphocytes may also be important mediators of immunity at mucosal surfaces. This is especially true for respiratory infections with facultative intracellular bacterial pathogens. T lymphocytes may play a role in immunity in the intestinal tract. Salmon (1987) has shown that a high proportion of the intraepithelial lymphocytes in the intestine are of the T_C phenotype. He speculates that these cytotoxic T cells in contact with intestinal epithelial cells may be important in destroying virus-infected epithelial cells.

More detailed information on aspects of immunity at mucosal surfaces may be found in chapters in this book dealing with specific organ systems or specific pathogens.

Fetal and Neonatal Immunity. All components of the native and acquired immune systems develop in utero and are functional at birth. However, they are generally less efficient than in the adult (Hammerberg et al. 1989). Since the normal

newborn piglet 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 sow to the piglet. In the pig there is virtually no transfer of antibody across the placenta. The epitheliochorial placentation of the sow has several epithelial layers between maternal and fetal circulation, which prevents antibody transfer. In the sow, as in other large domestic species, passive transfer of antibody from mother to offspring occurs through the colostrum. The sow 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 piglet. The passive transfer of antibody from sow to piglet in the colostrum and milk is very important for neonatal survival and is discussed in more detail below.

NATIVE DEFENSE MECHANISMS. The newborn piglet has low levels of hemolytic complement activity at birth, which is related to the birth weight with heavier pigs having significantly higher concentrations of serum complement (Rice and Lecuyer 1963). 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 piglets deprived of colostrum 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 (Rice and Lecuyer 1963). The third component of complement (C3) plays a central role in complement activity. Newborn piglet serum has approximately 25% of the C3 levels found in adult swine serum. The C3 concentration increases until it reaches adult levels at 14 days of age. The C3 component of complement is apparently not transferred through the colostrum (Tyler et al. 1988, 1989).

Phagocytic cells are present in newborn animals but generally have reduced phagocytic activity when compared with adult animals (Osburn et al. 1982). The phagocytic activity of neutrophils and macrophages from neonatal piglets has apparently not been evaluated. Since phagocytes depend on complement and/or antibody to opsonize many infectious agents, the overall efficiency of phagocytosis may be reduced due to inadequate levels of complement and antibody. Neutrophils from fetal pigs have been shown to have antibody-dependent cell-mediated cytotoxicity activity against chicken red blood cells that is comparable to that of adult pigs. Neutrophils

from neonatal pigs have also been shown to rapidly emigrate into the lumen of the gut in response to the presence of *E. coli* and colostral antibody (Sellwood et al. 1986; Yang and Schultz 1986).

Natural killer cell activity has been shown to be absent in the peripheral blood of fetal pigs and to be low in pigs of less than 2 weeks of age (Yang and Schultz 1986).

PASSIVE TRANSFER IN THE NEONATE. Pigs are born with almost no serum antibody and absorb IgG, IgM, and IgA from sow colostrum, which is enriched for IgG, IgG₂, and IgA when compared with serum. It has approximately the same concentration of IgM as serum (Table 3.1). When the pig suckles, colostrum is replaced with milk that has a much lower immunoglobulin content. From 3 days of age until the end of lactation, IgA is the predominant antibody found in sow milk. The percentage of immunoglobulin in the mammary gland derived from serum and locally produced in the mammary gland is different in colostrum and milk and varies with the immunoglobulin class (Table 3.2).

All three major classes of Ig (IgG, IgA, and IgM) are absorbed from the colostrum into the circulation of newborn pigs (Porter 1969; Curtis and Bourne 1971). IgA, however, is absorbed less efficiently than the other classes of antibody (Porter 1973; Hill and Porter 1974), apparently because much of the IgA in porcine colostrum is dimeric IgA lacking secretory component (Porter 1973). The neonatal colostrum-deprived piglet has been shown to express secretory component in the gut, which tends to localize in the mucus of the crypt areas (Allen and Porter 1973). Because of the affinity of the dimeric IgA and IgM for secretory component, it has been suggested (Butler et al. 1981) that IgA and IgM are bound in association with secretory component and held in the mucus of the crypt areas and are, therefore, less efficiently absorbed from the colostrum. The IgA present in sow's milk throughout the suckling period may also bind to the secretory component in the crypt areas, thereby providing relatively continuous protection against intestinal pathogens.

Table 3.2. Origin of porcine colostral and milk immunoglobulins

	Plasma Derived (%)	Synthesized Locally (%)
Colostrum		
IgM	85	15
IgG	100	0
IgA	40	60
Milk		
IgM	10	90
IgG	30	70
IgA	10	90

Source: Stokes and Bourne (1989).

Intestinal absorption of immunoglobulin from the colostrum normally ceases by 24–36 hours after birth. If pigs suckle normally, the efficiency of absorption decreases with a half-life of about 3 hours (Speer et al. 1959). Lecce et al. (1961) found that the period of time that the intestine could absorb antibodies was extended up to 5 days in starved pigs that were maintained by parental administration of nutrients. Therefore, piglets that have not had an opportunity to eat during the first 24–36 hours may still benefit from colostrum ingestion.

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

Mechanisms of Immune-mediated Hypersensitivity. Type 1 or immediate-type hypersensitivity involves the synthesis of specific IgE (reaginic or cytotoxic) 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 that are responsible for the clinical signs (e.g., histamine, serotonin, kinins, prostaglandins, and others). Type 1 hypersensitivities may be localized in a particular region or organ or may be systemic (anaphylaxis) (Eyre 1980).

Type 2 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 or viral or bacterial antigens) that have adhered to the cell surface.

Type 3 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 4 hypersensitivity (or delayed-type hypersensitivity) is mediated by sensitized T cells releasing lymphokines and does not involve antibody. The tuberculin skin test is a classic type 4 hypersensitivity reaction.

It is not unusual for clinical hypersensitivity conditions to involve more than one of the four types of hypersensitivity. Hypersensitivity conditions that have been studied in the pig will be briefly reviewed here.

IMMEDIATE-TYPE HYPERSENSITIVITY. Pigs have been shown to develop homocytotropic antibody in response to lungworm (*Metastrongylus* spp.) infection (Barratt 1972). This antibody was demonstrated in the serum of infected pigs using a passive cutaneous anaphylaxis assay. Serum from an infected pig was injected intradermally into a recipient pig. When *Metastrongylus* antigen was injected intravenously 36–48 hours later, an immediate hypersensitivity reaction (wheal and erythema) occurred at the site of serum injection. This reaction was maximal in 30–45 minutes.

Systemic anaphylaxis has been studied experimentally in pigs sensitized to egg albumin (Thomlinson and Buxton 1963). Mild symptoms of anaphylaxis were characterized by heavy and rapid breathing accompanied by coughing and yawning. The animals showed stiffness and incoordination and preferred to lie down. The first signs of severe anaphylactic shock were circling, incoordination, coughing, and intense vascular congestion of the skin, especially of the ears, nose, and periocular area. Convulsions and acute respiratory distress rapidly followed. After a few minutes the pigs began to recover from the severe respiratory distress, then vomiting and defecation occurred; muscular tremors developed 20–40 minutes later. The pigs with severe symptoms of anaphylaxis recovered more rapidly than the pigs with mild symptoms.

It has been suggested that edema disease in swine may be due to an immediate-type hypersensitivity response to *E. coli* antigens (Thomlinson and Buxton 1963).

CYTOTOXIC-TYPE HYPERSENSITIVITY. Type 2 hypersensitivities have been reported in pigs in which autoantibodies have formed against erythrocytes, thrombocytes, or neutrophils (Nordstoga 1965; Lie 1968; Saunders and Kinch 1968; Linklater 1972; Linklater et al. 1973; Dimmock et al. 1982). This results in a depletion of the respective cell type and the associated clinical signs that one would expect (anemia, bleeding diathesis, or increased susceptibility to infection, respectively). These autoantibodies may arise from blood transfusions, from the use of vaccines that contain blood products, or in multiparous sows that develop antibody against the alloantigens shared by the sire and the fetus. In the latter case, one would not expect clinical signs to appear in the sow since she would only produce antibody against cell-surface allotype antigens that are not found on her cells. When a piglet suckles and receives colostrum antibody, the passively transferred antibody causes clinical signs if the pig has inherited the sire's alloantigens. Thrombocytopenic purpura in piglets due to passively transferred antiplatelet antibody seems to be rather common. Pigs appear normal at birth. Death usually occurs between 10–20 days of age. The most striking pathologic feature is the presence of

hemorrhages in the subcutaneous tissues and internal organs. Castration during the period of thrombocytopenia may greatly increase the death rate.

Antibodies against erythrocytes, thrombocytes, and neutrophils may be present in the same piglet. In one report, 50% of the dams of litters affected with thrombocytopenic purpura had erythrocyte isoantibodies in their serum (Linklater et al. 1973). The concurrent presence of hemolytic disease with thrombocytopenic purpura in the piglets will exacerbate the anemia. The degree of severity of these conditions may vary between piglets in one litter depending on the erythrocyte and platelet isoantigens that they have inherited from the sire and the amount of colostrum ingested.

IMMUNE-COMPLEX-TYPE HYPERSENSITIVITY. Immune-complex-mediated glomerulonephritis is a common sequelae to chronic hog cholera virus infection or African swine fever virus infection (Cheville et al. 1970). The lesion associated with these two infections is moderately severe membranoproliferative glomerulonephritis. The immune complexes found in these diseases may also cause periarteritis nodosa, a systemic vasculitis. Immune-complex deposition in swine kidneys is apparently common. One study evaluated 100 kidneys collected at slaughter that had no gross lesions; 97 of the kidneys had IgG deposits and 98 had C3 deposits as demonstrated by immunocytochemistry. The significance of these immune-complex deposits in the kidney is unknown; however, clinical diagnosis of glomerular disease in swine is rare (Shirota et al. 1986).

FOOD HYPERSENSITIVITY. Food hypersensitivity is thought to be responsible for some cases of postweaning diarrhea in piglets (Stokes et al. 1987; Stokes and Bourne 1989; Li et al. 1990). This is apparently a type 4 or delayed-type hypersensitivity. Following the introduction of a new protein antigen to the diet, a small proportion (<0.002%) of that protein is absorbed intact. This may induce an antibody and/or cell-mediated response. The systemic antibody response (IgG) will be subsequently suppressed (oral tolerance) and a local mucosal antibody will persist. The local antibody prevents further absorption of the intact protein. The oral tolerance that develops is a specifically acquired ability to prevent responses to any of the proteins that may be absorbed. Therefore, following the introduction of new dietary antigen, animals pass through a brief phase of hypersensitivity before the development of a protected state of tolerance.

In pigs that were weaned abruptly and placed on a soya-containing diet, soya protein was detected in the sera of all animals for up to 20 days postweaning. A delayed-type hypersensitivity skin test reaction to soya proteins was transiently

positive in the soya-fed group. The changes in gut morphology (crypt hyperplasia and villous atrophy) and the malabsorption associated with early weaning have been characterized. Evidence exists that suggests that these changes occur as a result of a transient hypersensitivity to antigen in the postweaning diet. These intestinal changes can facilitate growth and disease production by *E. coli*. Feeding of large amounts of soya prior to the withdrawal of milk prevented the postweaning malabsorption and diarrhea (Stokes et al. 1987).

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 (a) illness from organisms of normally low pathogenicity or from an attenuated live vaccine, (b) recurrent illnesses that are unusually difficult to control, (c) failure to respond adequately to vaccination, (d) unexplained neonatal illness and death affecting more than one animal in a litter, and (e) a variety of disease syndromes occurring concurrently in a herd. A large number of primary immunodeficiencies have been reported in humans and a few have been reported in other domestic species; however, there are apparently no reports of primary immunodeficiencies in pigs. This is probably due to the relatively low value of the individual piglet and the expense and difficulty associated with diagnosing a primary immunodeficiency. In addition, sows and boars that produce nonvigorous litters are not kept in the breeding herd.

A common cause of secondary immunodeficiency is failure to passively transfer adequate levels of maternal antibody through the colostrum to the piglet, as discussed earlier in this chapter. Other potential causes of secondary immunodeficiency (or immunosuppression) include (a) physical or psychological distress, (b) immunosuppressive infectious agents, (c) inadequate nutrition, and (d) immunotoxic substances. The influence of most of these factors on the porcine immune system has not been adequately studied.

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 vari-

ous species (Kelley 1985). Distress-induced alterations in immune function are mediated by interactions between the neuroendocrine and immune systems. The study of these multisystem 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 (Breazile 1987; Dunn 1988; Kelley 1988).

The neuroendocrine and immune systems communicate in a bidirectional manner via direct neural as well as hormonal signalling systems (Griffin 1989). Neuroendocrine signals that are capable of directly altering the function of cells of the immune system include (a) direct sympathetic innervation to the parenchyma of the thymus, spleen, and bone marrow; (b) glucocorticoids produced by the adrenal cortex after pituitary adrenocorticotrophic hormone (ACTH) stimulation; (c) catecholamines produced by the adrenal medulla; (d) endogenous opiates (endorphin and enkephalins) produced by the pituitary, adrenal medulla, sympathetic terminals, and lymphocytes; (e) vasoactive intestinal peptide released by sympathetic neurons of the intestine and perhaps other sites; and (f) substance P released by sympathetic nerve terminals (Breazile 1987; Dunn 1988; Kelley 1988). Receptors have been detected on lymphocytes and thymocytes for a variety of hormones, including corticosteroids, insulin, testosterone, estrogens, beta-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 (Blalock et al. 1985; Griffin 1989). 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.

Weaning is certainly a stressful event for domestic animals. Piglets are usually separated from their sow, handled extensively, regrouped with unfamiliar pigs, and shifted from a liquid to a

solid diet. Weaning at 2, 3, or 4 weeks of age (but not at 5 weeks of age) has been shown to decrease the *in vivo* and *in vitro* response of porcine lymphocytes to phytohemagglutinin (Blecha et al. 1983). This is considered to be a measure of the pigs ability to mount a cell-mediated immune response. These same parameters were suppressed in artificially reared neonatal piglets compared with their sow-reared littermates (Blecha et al. 1986; Hennessy et al. 1987). Weaning (at 5 weeks of age) 24 hours after the injection of sheep red blood cells (RBCs) decreased the antibody response to the RBCs. Weaning 2 weeks prior to injecting the sheep RBCs did not decrease the antibody response (Blecha and Kelley 1981).

Regrouping of pigs at the time of weaning or at 2 weeks after weaning significantly increased their plasma cortisol concentration. However, there were no measurable changes in lymphocyte blastogenesis or antibody responses at the time of elevated plasma cortisol concentration (Blecha et al. 1985).

Crowding or restraint may also stress pigs sufficiently to decrease their immune responsiveness. Housing eight pigs (11.5–18.0 kg) per group in pens with 0.13 m² of floor space per pig significantly reduced their phytohemagglutinin skin test response as compared with pigs given twice as much space (Yen and Pond 1987). When young pigs were restrained for 2 hours per day over a 3-day period, they had a significantly elevated plasma cortisol concentration, which correlated with a decrease in the size of the thymus gland and with a reduction in the phytohemagglutinin skin test response (Westly and Kelley 1984). Another report indicated that tethering of sows suppressed antibody synthesis to sheep RBCs. It also resulted in a reduction in the amount of antigen-specific antibodies that were transmitted through the colostrum into the blood of the piglets (Kelley 1985).

Immunosuppressive Infectious Agents. Certain infectious agents are capable of suppressing immune function sufficiently to make the animal more susceptible to secondary infections. For example, infection with *Mycoplasma hyopneumoniae*, virulent or vaccine strains of hog cholera virus, or pseudorabies virus increases the susceptibility of pigs to severe *Pasteurella multocida* pneumonia (Smith et al. 1973; Pijoan and Ochoa 1978; Fuentes and Pijoan 1986, 1987). The mechanism of the immunosuppression induced by these agents has not been completely characterized. The pseudorabies virus has been shown to replicate in alveolar macrophages and to impair their bactericidal functions (Iglesias et al. 1989a,b). Porcine parvovirus replicates in alveolar macrophages, as well as lymphocytes, and has been shown to impair macrophage phagocytosis and lymphocyte blastogenesis (Harding and Molitor 1988). Swine influenza virus also replicates in al-

veolar macrophages and kills the macrophages (Charley 1983).

The African swine fever virus causes a peripheral lymphopenia and necrosis of lymphoreticular organs (Wardley and Wilkinson 1980). This virus replicates in both lymphocytes and in the mononuclear phagocytic system and presumably impairs their function (Wardley et al. 1979). It has also been shown to strongly reduce NK cell activity by 2 days after infection (Norley and Wardley 1983a).

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 (Tengerdy 1986).

It is difficult to predict the optimal diet for immune function. There is very little research data in this area for swine. 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.

Dietary and injectable vitamin E and Se have been evaluated for their influence on antibody levels in young pigs. Dietary vitamin E supplementation increased the antibody response to *E. coli* (Ellis and Vorhies 1976). Supplemental (dietary or injectable) vitamin E and/or Se treatment in pigs beginning at 4–5 weeks of age increased their antibody response to sheep RBCs (Peplowski et al. 1981). Dietary vitamin E and Se also increased the blastogenic response of pig lymphocytes to phytohemagglutinin (Larsen and Tollersrud 1981).

Other investigators demonstrated that injection of sows with vitamin E and/or Se at day 100 of gestation resulted in increased serum IgG concentrations in their piglets at 2 weeks postpartum (but not at 20 hours or 4 weeks postpartum) (Hayek et al. 1989). In contrast, other studies found no influence of dietary vitamin E or Se on the im-

mune response of young pigs (Blodgett et al. 1986; Kornegay et al. 1986).

Immunotoxic Substances. In other species, 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 (Koller 1979). Very little immunotoxicology research has been conducted in swine. Aflatoxin in the feed of young pigs has been shown to impair immunity to erysipelas, to enhance the severity of clinical signs due to salmonellosis, and to enhance susceptibility to an oral inoculation with *Serpulina hyodysenteriae* (Cysewski et al. 1978; Miller et al. 1978; Joens et al. 1981).

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 (Blecha 1988). Immunomodulators, also referred to as biologic-response modifiers, can be divided into two categories: endogenous immunomodulators, which are cytokines that are normally produced by the host and are products of the host genome; and 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; however, a cytokine from one species will sometimes work in a different species. The cytokines listed above have all been produced from human and bovine genes. The only porcine recombinant cytokine for which published information is available at the time of this writing is apparently gamma interferon (Charley et al. 1988; Esparza et al. 1988). It has been shown to have antiviral activity in vitro. The technology is readily available to produce the other cytokines if research in other species indicates that they may be useful for therapy or prophylaxis of infectious disease.

Levamisole has been shown to be an immunopotentiating agent in pigs and other species (Blecha 1988). However, its efficacy is dependent on the physiologic status of the animal, the dosage used, and the time of administration. It has had its greatest activity in stressed or immunocompromised animals.

Levamisole (1.5 mg/animal, subcutaneously or intramuscularly) given to 5-week-old or 4-month-old pigs at the time of primary and secondary injection with sheep RBCs increased the secondary antibody response to the RBCs. The primary antibody response was not increased (Reyero et al. 1979).

Levamisole and isoprinosine have been evaluated for their ability to reverse the defects observed in lymphocyte blastogenesis and delayed-type hypersensitivity responses in artificially reared piglets (Hennessy et al. 1987). Levamisole (2 mg, subcutaneously) was administered at 5 and 10 days of age. Isoprinosine (75 mg/kg per day) was administered orally from day 0 to day 10 to a different group of pigs. Both levamisole and isoprinosine enhanced the responses in the artificially reared pigs to values comparable to those of sow-reared controls. It has not been demonstrated that the effects observed with either levamisole or isoprinosine would translate into a clinically observable benefit in pigs.

Polyinosinic/polycytidylic acid (poly IC) has been shown to be an effective interferon inducer in swine (Vengris and Mare 1972; Gainer and Guarnieri 1985; Loewen and Derbyshire 1986). Poly IC complexed with poly-L-lysine and carboxymethylcellulose (poly ICLC) was shown to be a more effective interferon inducer than poly IC alone in newborn piglets (Lesnick and Derbyshire 1988). Poly ICLC (0.5 mg/kg, intravenously) given to 2-day-old piglets induced peak serum interferon levels at 6 hours postinjection and enhanced NK cell activity at 24 hours postinjection to the levels normally found in weaned pigs. The poly ICLC delayed the onset of clinical signs from transmissible gastroenteritis (TGE) virus challenge by 24 hours; however, there was no difference in the eventual outcome of the TGE virus infection (Lesnick and Derbyshire 1988). Recombinant bovine alpha-1 interferon, when administered orally to piglets, also failed to influence the course of TGE virus infection (MacLachlan and Anderson 1986).

Recombinant human IL-2 has been shown to be biologically active in pigs (Charley and Fradelizi 1987; Bhagyam et al. 1988). It has been shown to increase the antibody response when administered at the time of vaccination with either an *Actinobacillus pleuropneumoniae* bacterin or a pseudorabies virus subunit vaccine. Upon subsequent infectious challenge the pigs given IL-2 at the time of vaccination had improved resistance to *A. pleuropneumoniae* infection but not to pseudorabies virus infection (Anderson et al. 1987; Kawashima and Platt 1989).

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 that 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 that induce this type of immunity.

The basic types of immune defense mechanisms against infectious agents (as discussed earlier in this chapter) are (a) native defense mechanisms, the first line of defense and already operational, even in the nonvaccinated animal; (b) humoral immunity, due to the presence of antibodies in the bloodstream; (c) cell-mediated immunity, caused by the action of various types of white blood cells and orchestrated by T lymphocytes; (d) 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. Information regarding protective immunity and vaccination for specific diseases may be found in later chapters of this book.

Selective Induction of Different Types of Immunity.

It is relatively easy to develop a vaccine that will cause the production of IgG and IgM antibodies 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 (a) agglutinating infectious agents, thereby reducing the number of infectious particles (for viruses) and facilitating removal by phagocytosis; (b) binding to and neutralizing toxins; (c) binding to the infectious agent and blocking attachment to cell surfaces; (d) binding to the infectious agent and initiating the classical pathway of complement activation; (e) opsonizing infectious agents and facilitating phagocytosis; (f) 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 that 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 bloodstream and in the tissues to some extent but not on some mucosal surfaces. Therefore, they can help to prevent invasion through the mucosal surface; however, they 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 bloodstream and tissues. Protection on mucosal surfaces is due in large part to secretory IgA, which 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 that have traditionally been used in animal vaccines are not very effective at inducing cell-mediated immunity. New adjuvants are being developed that 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, which are generally diseases that can be controlled by the presence of circulating IgG.

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 it will be inhaled by the animal, or intramammary exposure. If a sow is exposed to an infectious agent in her intestinal tract, she may respond by producing se-

cretory IgA not only in her own intestinal tract, but also in her mammary gland. The sow passes the IgA against the infectious agent to the piglet when it suckles. Therefore, secretory IgA in the sow's milk can protect the piglet from infectious agents present in the sow's intestine. This protection will only last as long as the piglet 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 (a) the animal may have been incubating the disease when it was vaccinated; (b) something may have happened to the vaccine to make it ineffective; (c) the physiologic status of the host may make it unresponsive or hyporesponsive to the vaccine; or (d) 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 reduce 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 effective only 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 dates should not be used. Chemical disinfectant residues on syringes and needles can inactivate modified live vaccines. The use of improper dil-

uent 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 that is appropriate for one vaccine may inactivate a different vaccine. Some vaccines and diluents contain preservatives that 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 piglets' 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 is 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 to minimize the period of vulnerability to infectious diseases. 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.

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 pigs frequently. However, frequent vaccination may be justified in cases of unusually high incidence of disease.

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. This may also occur due to therapy with immunosuppressive drugs (e.g., glucocorticoids).

Another concern is that some modified live vaccines are capable of inducing disease in the immunosuppressed animal. Modified live vaccines are tested for safety in normal, healthy animals and thus 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, including 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 but 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 USDA 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 that 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 be tested under controlled experimental conditions. The vaccinated group must have significantly less disease than the nonvaccinated control group. This testing is typically done on healthy, nonstressed animals under good environmental conditions, 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. However, the vaccine can increase the animals resistance to disease, but this resistance can be overwhelmed if good management practices are not followed.

REFERENCES

- ALLEN, W. D., AND PORTER, P. 1973. Localization by immunofluorescence of secretory component and IgA in the intestinal mucosa of the young pig. *Immunology* 24:365.
- ANDERSON, G.; URBAN, O.; FEDORKA-CRAY, P.; NEWELL, A.; NUNBERG, J.; AND DOYLE, M. 1987. Interleukin-2 and protective immunity in *Haemophilus pleuropneumoniae*: Preliminary studies. *Vaccines* 87:22-25.
- BARRATT, M. E. J. 1972. Immediate hypersensitivity to *Metastrongylus* spp. infection in the pig. *Immunology* 22:601-623.
- BARTA, O., AND HUBBERT, N. L. 1978. Testing of hemolytic complement components in domestic animals. *Am J Vet Res* 39(8):1303-1308.
- BELLAMY, J. E. C., AND NIELSEN, N. O. 1974. Immune-mediated emigration of neutrophils into the lumen of the small intestine. *Infect Immun* 9(4):615-619.
- BHAGYAM, R. C.; JARRETT-ZACZEK, D.; AND FERGUSON, F. G. 1988. Activation of swine peripheral blood lymphocytes with human recombinant interleukin-2. *Immunology* 64:607-613.
- BHOGAL, B. S.; NAGY, L. K.; AND WALKER, P. D. 1987. Neutrophil mediated and IgA dependent antibacterial immunity against enteropathogenic *Escherichia coli* in the porcine intestinal mucosa. *Vet Immunol Immunopathol* 14:23-44.
- BINNS, R. M.; PABST, R.; AND LICENCE, S. T. 1986. The behavior of pig lymphocyte populations in vivo. *Swine Biomed Res* 3:1837-1853.
- BLALOCK, J. E.; HARBOUR-McMENAMIN, D.; AND SMITH, E. M. 1985. Peptide hormones shared by the neuroendocrine and immunologic systems. *J Immunol* 135:858-861s.
- BLECHA, F. 1988. Immunomodulation: A means of disease prevention in stressed livestock. *J Anim Sci* 66:2084-2090.
- BLECHA, F., AND KELLEY, K. W. 1981. Effects of cold and weaning stressors on the antibody-mediated immune response of pigs. *J Anim Sci* 53(2):439-447.
- BLECHA, F.; POLLMANN, D. S.; AND NICHOLS, D. A. 1983. Weaning pigs at an early age decreases cellular immunity. *J Anim Sci* 56(2):396-400.
- _____. 1985. Immunologic reactions of pigs regrouped at or near weaning. *Am J Vet Res* 46(9):1934-1937.
- BLECHA, F.; POLLMANN, D. S.; AND KLUBER, III, E. F. 1986. Decreased mononuclear cell response to mitogens in artificially reared neonatal pigs. *Can J Vet Res* 50:522-525.
- BLODGETT, D. J.; SCHURIG, G. G.; AND KORNEGAY, E. T. 1986. Immunomodulation in weanling swine with dietary selenium. *Am J Vet Res* 47:1517-1519.
- BREAZILE, J. E. 1987. Physiologic basis and consequences of distress in animals. *J Am Vet Med Assoc* 191:1212-1215.
- BUTLER, J. E.; KLOBASA, F.; AND WERHAHN, E. 1981. The differential localizations of IgA, IgM, and IgG in the gut of suckled neonatal piglets. *Vet Immunol Immunopathol* 2:53-65.
- CHARLEY, B. 1983. Interaction of influenza virus with swine alveolar macrophages: Influence of anti-virus antibodies and cytochalasin B. *Ann Virol* 134(E):51-59.
- CHARLEY, B., AND FRADELIZI, D. 1987. Differential effects of human and porcine interleukin 2 on natural killing (NK) activity of newborn piglets and adult pigs lymphocytes. *Ann Rech Vet* 18:227-232.
- CHARLEY, B.; McCULLOUGH, K.; AND MARTINOD, S. 1988. Antiviral and antigenic properties of recombinant porcine interferon gamma. *Vet Immunol Immunopathol* 19:95-103.
- CHEVILLE, N. F.; MENGELING, W. L.; AND ZINOBER, M. R. 1970. Ultrastructural and immunofluorescent studies of glomerulonephritis in chronic hog cholera. *Lab Invest* 22(5):458-467.
- CURTIS, J., AND BOURNE, F. J. 1971. Immunoglobulin quantitation in sow serum, colostrum and milk and the serum of young pigs. *Biochim Biophys Acta* 236:319-332.
- CYSEWSKI, S. J.; WOOD, R. L.; PIER, A. C.; AND BAETZ, A. L. 1978. Effects of aflatoxin on the development of acquired immunity to swine erysipelas. *Am J Vet Res* 39(3):445-448.
- DIMMOCK, C. K.; WEBSTER, W. R.; SHIELS, I. A.; AND EDWARDS, C. L. 1982. Isoimmune thrombocytopenic purpura in piglets. *Aust Vet J* 59:157-159.
- DUNCAN, I. A.; BINNS, R. M.; AND DUFFUS, W. P. H. 1989. The null T cell in pig blood is not an NK cell. *Immunol* 68:392-395.
- DUNN, A. J. 1988. Nervous system-immune system interactions: An overview. *J Receptor Res* 8:589-607.
- ELLIS, R. P., AND VORHIES, M. W. 1976. Effect of supplemental dietary vitamin E on the serologic response of swine to an *Escherichia coli* bacterin. *J Am Vet Med Assoc* 168(3):231-232.
- ESPARZA, I.; GONZALEZ, J. C.; AND VINUELA, E. 1988. Effect of interferon alpha, interferon gamma and tumor necrosis factor on African swine fever virus replication in porcine monocytes and macrophages. *J Gen Virol* 69:2973-2980.
- EYRE, P. 1980. Pharmacological aspects of hypersensitivity in domestic animals: A review. *Vet Res Commun* 4:83-98.
- FERGUSON, F. G.; PINTO, A. J.; CONFER, F. L.; AND BOTTICELLI, G. 1986. Characteristics of Yorkshire swine natural killer cells. *Swine Biomed Res* 3:1915-1924.
- FRANEK, F., AND RIHA, I. 1964. Purification and structural characterization of 5S gamma globulin in newborn pigs. *Immunochemistry* 1:49.
- FUENTES, M., AND PIJOAN, C. 1986. Phagocytosis and intracellular killing of *Pasteurella multocida* by porcine alveolar macrophages after infection with pseudorabies virus. *Vet Immunol Immunopathol* 13:165-172.
- _____. 1987. Pneumonia in pigs induced by intranasal challenge exposure with pseudorabies virus and *Pasteurella multocida*. *Am J Vet Res* 48(10):1446-1448.
- GAINER, J. H., AND GUARNIERI, J. 1985. Effects of poly I:C in porcine iron-deficient neutropenia. *Cornell Vet* 75:454-465.
- GRIFFIN, J. F. T. 1989. Stress and immunity: A unifying concept. *Vet Immunol Immunopathol* 20:263-312.
- HALPERN, M. S., AND KOSHLAND, M. E. 1970. Novel subunit in secretory IgA. *Nature* 228:1276.
- HAMMERBERG, C.; SCHURIG, G. G.; AND OCHS, D. L. 1989. Immunodeficiency in young pigs. *Am J Vet Res* 50(6):868-874.
- HARDING, M. J., AND MOLITOR, T. W. 1988. Porcine parvovirus: Replication in and inhibition of selected cellular functions of swine alveolar macrophages and peripheral blood lymphocytes. *Arch Virol* 101:105-117.
- HAYEK, M. G.; MITCHELL, G. E., JR.; HARMON, R. J.; STAHLY, T. S.; CROMWELL, G. L.; TUCKER, R. E.; AND BARKER, K. B. 1989. Porcine immunoglobulin transfer after prepartum treatment with selenium or vitamin E. *J Anim Sci* 67:1299-1306.

- HENNESSY, K. J.; BLECHA, F.; POLLMANN, D. S.; AND KLUBER, E. F. 1987. Isoprinosine and levamisole immunomodulation in artificially reared neonatal pigs. *Am J Vet Res* 48:477-480.
- HILL, I. R., AND PORTER, P. 1974. Studies of bactericidal activity to *Escherichia coli* of porcine serum and colostral immunoglobulins and the role of lysozyme with secretory IgA. *Immunol* 26:1239-1250.
- IGLESIAS, G.; PIJOAN, C.; AND MOLITOR, T. 1989a. Interactions of pseudorabies virus with swine alveolar macrophages I: Virus replication. *Arch Virol* 104:107-115.
- _____. 1989b. Interactions of pseudorabies virus with swine alveolar macrophages: Effects of virus infection on cell functions. *J Leuk Biol* 45:410-415.
- JOENS, L. A.; PIER, A. C.; AND CUTLIP, R. C. 1981. Effects of aflatoxin consumption on the clinical course of swine dysentery. *Am J Vet Res* 42(7):1170-1172.
- KAWASHIMA, K., AND PLATT, K. B. 1989. The effect of human recombinant interleukin-2 on the porcine immune response to a pseudorabies virus subunit vaccine. *Vet Immunol Immunopathol* 22:345-353.
- KELLEY, K. W. 1985. Immunological consequences of changing environmental stimuli. In *Animal Stress*. Ed. G. P. Moberg. Bethesda, Md.: American Physiological Society, pp. 193-223.
- _____. 1988. Cross-talk between the immune and endocrine systems. *J Anim Sci* 66:2095-2108.
- KIM, Y. B., AND ICHIMURA, O. 1986. Porcine natural killer (NK)/killer (K) cell system. *Swine Biomed Res* 3:1811-1819.
- KIM, Y. B.; BRADLEY, S. G.; AND WATSON, D. W. 1966. Ontogeny of the immune response. I. Development of immunoglobulins in germfree and conventional colostrum-deprived piglets. *J Immunol* 97:52-63.
- KOLLER, L. D. 1979. Effects of environmental contaminants on the immune system. *Adv Vet Sci Comp Med* 23:267-295.
- KORNEGAY, E. T.; MELDRUM, J. B.; SCHURIG, G.; LINDEMANN, M. D.; AND GWAZDAUSKAS, F. C. 1986. Lack of influence of nursery temperature on the response of weanling pigs to supplemental vitamins C and E. *J Anim Sci* 63:484-491.
- LARSEN, H. J., AND TOLLERSRUD, S. 1981. Effect of dietary vitamin E and selenium on the phytohaemagglutinin response of pig lymphocytes. *Res Vet Sci* 31:301-305.
- LECCE, J. G.; MATRONE, G.; AND MORGAN, D. O. 1961. Porcine neonatal nutrition: Absorption of unaltered porcine proteins and polyvinyl pyrrolidone from the gut of piglets. *J Nutr* 73:158.
- LESNICK, C. E., AND DERBYSHIRE, J. B. 1988. Activation of natural killer cells in newborn piglets by interferon induction. *Vet Immunol Immunopathol* 18:109-117.
- LI, D. F.; NELSEN, J. L.; REDDY, P. G.; BLECHA, F.; HANCOCK, J. D.; ALLEE, G. L.; GOODBAND, R. D.; AND KLEMM, R. D. 1990. Transient hypersensitivity to soybean meal in the early-weaned pig. *J Anim Sci* 68:1790-1799.
- LIE, H. 1968. Thrombocytopenic purpura in baby pigs. *Acta Vet Scand* 9:285-301.
- LINKLATER, K. A. 1972. Iso-antibodies to red cell antigens in pigs' sera 3. The effect of maternal red cell iso-antibodies on the red cells of piglets. *Anim Blood Groups Biochem Genet* 3:77-84.
- LINKLATER, K. A.; McTAGGART, H. S.; and IMLAH, P. 1973. Haemolytic disease of the newborn, thrombocytopenic purpura and neutropenia occurring concurrently in a litter of piglets. *Br Vet J* 129:36-46.
- LOEWEN, K. G., AND DERBYSHIRE, J. B. 1986. Interferon induction with polyinosinic:polycytidylic acid in the newborn piglet. *Can J Vet Res* 50:232-237.
- LOFSTEDT, J.; ROTH, J. A.; ROSS, R. F.; AND WAGNER, W. C. 1983. Depression of polymorphonuclear leukocyte function associated with experimentally induced *Escherichia coli* mastitis in sows. *Am J Vet Res* 44(7):1224-1228.
- LUNNEY, J. K., AND PESCOVITZ, M. D. 1987. Phenotypic and functional characterization of pig lymphocyte populations. *Vet Immunol Immunopathol* 17:135-144.
- MACLACHLAN, N. J., AND ANDERSON, K. P. 1986. Effect of recombinant DNA-derived bovine alpha-1 interferon on transmissible gastroenteritis virus infection in swine. *Am J Vet Res* 47(5):1149-1152.
- MESTECKY, J.; ZIKAN, J.; AND BUTLER, W. T. 1971. Immunoglobulin M and secretory immunoglobulin A: Presence of a common polypeptide chain different from light chains. *Science* 171:1163.
- METZGER, J. J., AND FOUGEREAU, M. 1968. Characterisations biochimiques des immunoglobulines gamma-G et gamma-M porcines. *Rech Vet* 1:37.
- MILLER, D. M.; STUART, B. P.; AND CROWELL, W. A. 1978. Aflatoxicosis in swine: Its effect on immunity and relationship to salmonellosis. *Proc Annu Meet Am Assoc Vet Lab Diagn* pp. 135-146.
- NIELSEN, K. H. 1977. Bovine reagenic antibody III. Cross-reaction of antihuman IgE and antibody reagenic immunoglobulin antisera with sera from several species of mammals. *Can J Comp Med* 41:345-348.
- NORDSTOGA, K. 1965. Thrombocytopenic purpura in baby pigs caused by maternal isoimmunization. *Pathol Vet* 2:601-610.
- NORLEY, S. G., AND WARDLEY, R. C. 1983a. Investigation of porcine natural-killer cell activity with reference to African swine fever virus infection. *Immunol* 49:593-597.
- _____. 1983b. Effector mechanisms in the pig. Antibody-dependent cellular cytolysis of African swine fever virus infected cells. *Res Vet Sci* 35:75-79.
- OHGAMI, M.; DOERSCHUK, C. M.; ENGLISH, D.; DODEK, P. M.; AND HOGG, J. C. 1989. Kinetics of radiolabeled neutrophils in swine. *J Appl Physiol* 66:1881-1885.
- OSBURN, B. I.; MACLACHLAN, N. J.; AND TERRELL, T. G. 1982. Ontogeny of the immune system. *J Am Vet Med Assoc* 181(10):1049-1052.
- PABST, R., AND BINNS, R. M. 1986. Comparison of lymphocyte production and migration in pig lymph nodes, tonsils, spleen, bone marrow and thymus. *Swine Biomed Res* 3:1865-1871.
- PEPŁOWSKI, M. A.; MAHAN, D. C.; MURRAY, F. A.; MOXON, A. L.; CANTOR, A. H.; AND EKSTROM, K. E. 1981. Effect of dietary and injectable vitamin E and selenium in weanling swine antigenically challenged with sheep red blood cells. *J Anim Sci* 51:344-351.
- PIJOAN, C., AND OCHOA, G. 1978. Interaction between a hog cholera vaccine strain and *Pasteurella multocida* in the production of porcine pneumonia. *J Comp Pathol* 88:167-170.
- PORTER, P. 1969. Transfer of immunoglobulins IgG, IgA and IgM to lacteal secretions in the parturient sow and their absorption by the neonatal piglet. *Biochim Biophys Acta* 181:381-392.
- _____. 1973. Studies of porcine secretory IgA and its component chains in relation to intestinal absorption of colostral immunoglobulins by the neonatal pig. *Immunol* 24:163-176.
- _____. 1986. Immune System. In *Diseases of Swine*. Ed. A. D. Leman, B. Straw, R. D. Glock, W. L. Mengeling, R. H. C. Penny, and E. Scholl. Ames: The Iowa State Univ Press, p. 44.
- PORTER, P., AND ALLEN, W. D. 1972. Classes of immunoglobulins related to immunity in the pig. *J Am Vet Med Assoc* 160:511.
- REYERO, C.; STÖCKL, W.; AND THALHAMMER, J. G.

1979. Stimulation of the antibody response to sheep red blood cells in piglets and young pigs by levamisole. *Br Vet J* 135:17-24.
- RICE, C. E., AND L'ECUYER, C. 1963. Complement titres of naturally and artificially raised piglets. *Can J Comp Med Vet Sci* 27:157-161.
- SALMON, H. 1986. Surface markers of swine lymphocytes: Application to the study of local immune system of mammary gland and transplanted gut. *Swine Biomed Res* 3:1855-1864.
- _____. 1987. The intestinal and mammary immune system in pigs. *Vet Immunol Immunopathol* 17:367-388.
- SAUNDERS, C. N., AND KINCH, D. A. 1968. Thrombocytopenic purpura of pigs. *J Comp Pathol* 78:513-523.
- SELLWOOD, R.; HALL, G.; AND ANGER, H. 1986. Emigration of polymorphonuclear leukocytes into the intestinal lumen of the neonatal piglet in response to challenge with K88-positive *Escherichia coli*. *Res Vet Sci* 40:128-135.
- SHIROTA, K.; KOYAMA, R.; AND NOMURA, Y. 1986. Glomerulopathy in swine: Microscopic lesions and IgG or C3 deposition in 100 pigs. *Jpn J Vet Sci* 48(1):15-21.
- SMITH, I. M.; HODGES, R. T.; BETTS, A. O.; AND HAYWARD, A. H. S. 1973. Experimental infections of gnotobiotic piglets with *Pasteurella septica* (sero-group A) alone or with *Mycoplasma hyopneumoniae*. *J Comp Pathol* 83:307-321.
- SPEER, V. C.; BROWN, H.; QUINN, L.; AND CATRON, D. V. 1959. The cessation of antibody absorption in the young pig. *Immunol* 83:632.
- STERTZL, J.; KOSTKA, J.; RIHA, I.; AND MANDEL, I. 1960. Attempts to determine the formation and character of globulin and of natural and immune antibodies in young pigs reared without colostrum. *Folia Microbiol* 5:29.
- STOKES, C., AND BOURNE, J. F. 1989. Mucosal immunity. In *Veterinary Clinical Immunology*. Ed. R. E. W. Halliwell and N. T. Gorman. Philadelphia: W. B. Saunders, p. 164.
- STOKES, C. R.; MILLER, B. G.; BAILEY, M.; WILSON, A. D.; AND BOURNE, F. J. 1987. The immune response to dietary antigens and its influence on disease susceptibility in farm animals. *Vet Immunol Immunopathol* 17:413-423.
- TENGERDY, R. P. 1986. Nutrition, immunity and disease resistance. (Proc 6th Int Conf Prod Dis in Farm Anim, Sept. 1986, Belfast, Northern Ireland), p. 175.
- THOMLINSON, J. R., AND BUXTON, A. 1963. Anaphylaxis in pigs and its relationship to the pathogenesis of oedema disease and gastro-enteritis associated with *Escherichia coli*. *Immunology* 6:126-139.
- TYLER, J. W.; CULLOR, J. S.; OSBURN, B. I.; AND PARKER, K. 1988. Age-related variations in serum concentrations of the third component of complement in swine. *Am J Vet Res* 49(7):1104-1106.
- TYLER, J. W.; CULLOR, J. S.; DOUGLAS, V. L.; PARKER, K. M.; AND SMITH, W. L. 1989. Ontogeny of the third component of complement in neonatal swine. *Am J Vet Res* 50(7):1141-1144.
- VENGRIS, V. E., AND MARE, C. J. 1972. Swine interferon. II. Induction in pigs with viral and synthetic inducers. *Can J Comp Med* 36:288-293.
- WARDLEY, R. C., AND WILKINSON, P. J. 1980. Lymphocyte responses to African swine fever virus infection. *Res Vet Sci* 28:185-189.
- WARDLEY, R. C.; HAMILTON, F.; AND WILKINSON, P. J. 1979. The replication of virulent and attenuated strains of African swine fever virus in porcine macrophages. *Arch Virol* 61:217-225.
- WESTLY, H. J., AND KELLEY, K. W. 1984. Physiologic concentrations of cortisol suppress cell-mediated immune events in the domestic pig (41926). *Proc Soc Exp Biol Med* 177:156-164.
- YANG, W. C., AND SCHULTZ, R. D. 1986. Ontogeny of natural killer cell activity and antibody-dependent cell-mediated cytotoxicity in pigs. *Dev Comp Immunol* 10:405-418.
- YEN, J. T., AND POND, W. G. 1987. Effect of dietary supplementation with vitamin C or carbadox on weanling pigs subjected to crowding stress. *J Anim Sci* 64:1672-1681.
- ZARKOWER, A.; ESKEW, M. L.; SCHEUCHENZUBER, W. J.; FERGUSON, F. G.; AND CONFER, F. 1982. Antibody-dependent cell-mediated cytotoxicity in pigs. *Am J Vet Res* 43(9):1590-1593.