Some Practical Aspects of Immunity in the Bovine Animal

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The realization of the importance of immune responses in the host to infectious agents has come about in the last 10 to 15 years. Prior to this time the interaction and participation of various cells such as lymphocytes, macrophages and plasma cells; organs, i.e., lymph nodes, bone marrow, thymus and spleen; and the *in vivo* activity of antibodies was not fully appreciated. A summary of some of these advances and an indication of where this knowledge has assisted us in gaining a better understanding of defense against disease processes will be reviewed. Consideration of the antigens associated with the microorganisms, the organs and cells involved in the responses and means of resistance or interaction within the host resulting in disease will be discussed.

Microorganisms whether they be bacteria, viruses or fungi contain myriads of antigens. For instance, bacteria and fungi may possess antigens in exotoxins or endotoxins, in the cell wall and in the various enzymes and euchromatin present within the cell. Similarly, viruses contain numerous antigenic components. Antigenic sites which may consist of as few as eight to ten amino acids are in the protein coat; and the many enzymes and nucleoprotein within each virus. Furthermore as viruses enter cells and begin replication, they cause the cell to produce new enzymes and structural proteins which are released into the extracellular spaces or incorporated into the cell membranes. These substances are antigenic and recognized by the host immune system as foreign substances. There is good evidence today that many neoplasms caused by viruses have new antigenic sites on the cell surface as a result of influence of the virus. The hosts immune system recognizes these new antigens as foreign and responds against the tumor cells.

The bone marrow, thymus, lymph nodes, spleen and gut-associated lymphoid tissue are the organs which harbor most of the cells involved in immunologic responses. Stem cells giving rise to lymphoid and mononuclear cells are located for the most part in the bone marrow. In fetal and embryonic life these cells may be found in the liver, placenta or occasionally in the spleen. Monocytes enter the blood and migrate from the blood into various lymphoid organs and interstitial tissues of the body. Lymphocytes derived from stem cell precursors in the bone marrow may travel via the blood and localize in the cortex or medullary cords of lymph nodes, or in periarterial sheaths and splenic cords of the spleen. These cells are the precursors of the immunoglobulin (antibody) producing cells. Another population of lymphocytes arising from stem cells in the bone marrow migrate to the thymus where they may either proliferate or be destroyed. These lymphocytes, probably through the influence of a thymic hormone secreted by certain cells in this organ, are committed to serve as the carriers of cellular immunity, i.e., delayed hypersensitivity, allograft rejections, and immunologic memory. As these cells leave the thymus they tend to localize in the paracortical areas of lymph nodes and the lymphocytic mantles around germinal centers in lymph nodes and spleen.

Evidence at the present indicates that there are three cell types, namely macrophages, lymphocytes and plasma cells, which are important in immune responses. Macrophages are important because they trap particulate matter which contain antigens (2). The antigenic substance remains closely associated with the macrophage cell membrane allowing other cells such as lymphocytes to come in contact with the antigens. Lymphocytes derived from the bone marrow have immunoglobulins with specific antibody activity present on the cell surface (3). Cells with the specific antibody receptor site then become attached to the complimentary antigenic substance associated with the cell membrane of macrophages. Specific complimentary thymic-derived-lymphocytes become closely associated with the above cells and as a result of this

interaction, both the bone marrow and the thymic derived lymphoid cells undergo a series of cell divisions giving rise to clones of cells (4). These daughter cells are committed to interact only with the specific antigen which triggered the initial response. The bone-marrow-derived-lymphocytes also differentiate into plasma cells; the cells which produce circulating antibodies with activity specifically for the inciting antigen. The daughter population of thymic derived lymphocytes are concerned with delayed hypersensitivity and with controlling the magnitude of the response to the specific antigen inciting the initial response. With few exceptions, immunologic responses result in the above cell-cell interactions and the formation of both humoral (antibody) and cellular (delayed hypersensitivity) responses.

The basic immunoglobulin molecule which contains antibody activity consists of four polypeptide chains with a molecular weight of 160,000. Figure 1 is a representative diagram of such a molecule. Two of the polypeptide chains are

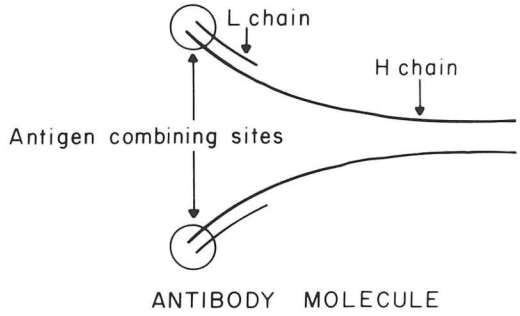

Figure 1: Diagram of an antibody or immunoglobulin molecule with a molecular weight (MW) of 160,000. The molecules consist of two heavy (H) chains with a MW of 60,000 each and two light (L) chains with a MW of 22,000 apiece. There are two antigen combining sites on each molecule.

heavy or H chains and have a molecular weight of approximately 60,000 apiece. The remaining two chains known as light or L chains have a molecular weight of approximately 22,000 apiece. These chains are held together by a series of disulfide bonds. There are two antigen combining sites located at the poles of the heavy and light chains. Each site consists of a portion of a heavy and light chain. The opposite end of the immunoglobulin molecule which consists only of the heavy chains functions as the portion of the molecule to which complement may attach and/or this part of the molecule may be constructed so that the molecule attaches to the surface of cells such as neutrophils, macrophages, basophils or mast cells.

Three different immunoglobulin classes have been recognized to date in the bovine (5). Undoubtedly additional classes, i.e., Immunoglobulin E, will eventually be recognized as has been the case in other species. The three classes of immunoglobulins defined at present are immunoglobulins (Ig) G, M and A. The characteristic differences in immunoglobulin classes are based on the composition of the heavy chains. IgG, the prototype immunoglobulin contains two antigen combining sites and has a molecular weight of 160,000. It is the most prevalent immunoglobulin in the blood and interstitial fluids. In the prepartum cow, this immunoglobulin is selectively transported into the mammary secretions and serves as the major antibody source in the colostrum. IgG is important as a virus neutralizing antibody.

IgM is a polymer of five molecules with ten antigen combining sites and a molecular weight of 900,000. Because of the large size of this immunoglobulin it remains within the intact blood vascular system. The initial antibody response is characteristically IgM which later resides when the IgG levels rise. Certain diseases such as anaplasmosis have unusually high levels of IgM for a prolonged time (6,7).

One of the more recently recognized immunoglobulins in the bovine is IgA or the secretory immunoglobulin (5). The secretory immunoglobulin consists of two molecules with four antigen combining sites and a secretory piece. The molecular weight is approximately 390,000. Secretory IgA is produced by plasma cells in the mucous membranes and glands lining the gastrointestinal, respiratory and genital tracts. The immunoglobulins then move through the epithelial cells lining the lumen of these organs where they acquire the secretory piece. The immunoglobulin then moves into the secretions where it serves an important role in "local immunity." IgA antibodies have virus neutralizing properties and undoubtedly play an important role in regulating other microbial agents.

IgE although not yet characterized in the bovine undoubtedly exists. This immunoglobulin is produced by plasma cells located in the respiratory and gastrointestinal mucous membranes. It consists of a single molecule and has a molecular weight of approximately 200,000. IgE has an affinity for the cytoplasmic membranes of basophils and mast cells. Once the appropriate antigen comes in contact with the IgE molecules on the surface of these cells, the reaction initiates a set of biochemical events in these cells which culminates in the release of vasoactive substances, i.e., serotonin or histamine. In some species, IgE is the immunoglobulin

responsible for acute allergic responses such as anaphylaxis.

Consideration will now be given to immunological conditions which may result in disease. The inability of a foreign substance to initiate an immunologic response when introduced into an animal is called immunologic unresponsiveness. The two principle forms of immunologic unresponsiveness discussed herein are immunologic tolerance and immunologic immaturity. Immunologic tolerance has been defined as the presence of sufficient antigen in the immature animal leading to an inability to respond to the antigen later in life. The best examples of immunologic tolerance are antigens located on cells such as the kidney, heart, etc., in one's own body. These are recognized as self and the immunologic system "tolerates" or does not respond to these organs and tissues. However, if the organs or tissues are transferred to another individual, a vigorous immunologic response rejects the foreign tissue in 10 to 14 days. There is no definitive evidence at present to indicate that replicating microbial agents are capable of causing immunologic unresponsiveness.

Immunologic immaturity is another form of immunologic unresponsiveness. There is good evidence that immunologic responsiveness develops in an orderly sequential manner through fetal and early neonatal life (8). In the fetal lamb, antibodies to a bacteriophage have been detected as early as 41 days of gestation but not to ferritin until 56 days, nor to ovalbumin until 120 days of gestation. Recognition of a similar pattern of maturation to various microbial agents appears to follow that observed with inanimate antigens. Infection of a fetus with viral, bacterial or mycotic agents prior to maturation of the immune response to that agent appears to be an important factor in congenital diseases resulting in anomalies and abortions.

The pregnant uterus of ungulates seems to be unusually susceptible to infection. Most agents causing embryonic and/or fetal infections will not persist for an extended length of time in the nonpregnant uterus. However, the agents readily localize there if a conceptus is present. An important factor in the bovine is the agammaglobulinemic status of the conceptus (9). Since there is no transplacental transfer of immunoglobulins from the maternal to the fetal circulation the fetus lacks passive immunity (10). Furthermore if the fetus is incapable of responding immunologically to the invading microorganism it is vulnerable to the pathogenic properties of the parasite. Invasion of the fetal calf with *Vibrio fetus* var *venerealis* prior to 185 days of gestation results in death and expulsion of an autolyzed calf within four to seven days (11). After 212 days of gestation, the fetal calf appears to resist infection as immunoglobulins were produced. Similar observations have been reported in pregnant cows inoculated with *Leptospira saxkoebing* (12). Congenital viral diseases such as bovine virus diarrhea virus, if administered at the appropriate stage of gestation and prior to the development of immunologic competence to the agent will cause a severe necrotizing encephalitis which manifests itself at birth as cerebellar hypoplasia (13). These examples demonstrate the adverse affects that various microbial agents may have on a developing fetus incapable of defending itself from the pathogenic properties of the invading organism. Furthermore, as the fetus begins to develop immunologic competence to antigens associated with microbial agents the pathological picture changes as maturation progresses. The lesions associated with many fetal infections differs considerably from the pathology observed in mature animals which have well developed immunologic systems.

The unusual susceptibility of the newborn calf to many ubiquitous organisms such as *Eschrichia coli* has been attributed in some instances to an inadequate uptake of colostrum (14,15). In the newborn calf only the colostrum obtained within the first 24 to 36 hours of life is absorbed from the intestine. If no colostrum is offered or as in some cases, interference with absorption occurs the calf has no passive resistance to organisms common to the environment. The consequence is neonatal septicemia caused by organisms of relatively low virulence for immunologically mature animals.

Occasionally immune responses to organisms may be misdirected or the side effects of such a reaction will lead to pathologic changes with adverse effects on the host. Immunohemolytic disease of the newborn calf associated with vaccination of the dam with anaplasmosis vaccine represents a good example of a severe immunopathologic phenomena (16). Anaplasmosis vaccine has been prepared from lypholized extracts of blood of cattle at the height of parasitemia. Administration of this material to cows with a different blood type stimulates an immunologic response to the foreign blood type as well as to the anaplasma organism. If the cow which received the anaplasma vaccine derived from an animal with a different blood type is bred to a bull with a blood type similar to that employed in the vaccine, there

is a 50% chance that the newborn calf will have a blood type similar to that of the sire. The calves are normal at birth. Calves with the same blood type as that of the sire and the vaccine may suffer severe consequences after ingesting the cow's colostrum. Colostral antibodies directed to the calf's red cells then attach to these cells causing agglutination and hemolysis.

There is evidence that the hemolytic crises associated with anaplasmosis in adult animals results from an immunologic phenomena (17). Possibly the anaplasma organism alters the red cells, rendering them foreign or potentially antigenic to the host which then produces antibodies to them. Affected erythrocytes are covered with immunoglobulins, resulting in a hemolytic anemia. A similar mechanism, although not as well documented, has been suggested for the hemolytic anemia in leptospirosis.

Many of the diseases in which delayed hypersensitivity seems to be a prominent feature may have severe pathological changes. The best example is tuberculosis in which a relatively innocuous agent triggers a hyperactive immune response. The hosts attempt to eliminate the organism inadvertently causes severe tissue damage. Fortunately delayed hypersensitivity does not function to the detriment of the host in most cases, but instead serves a very useful purpose in controlling and rejecting tumors and different viral processes which appear in the body from time to time.

The body attempts to control infections by other nonimmunologic means. Two examples which will be touched on briefly here are those concerned with controlling viral infections. Viral interference assists in preventing infection by (1) destroying or digesting the particular receptor sites on cell surfaces to which infectious virus particles attach to before entering a cell, or (2) interfering with viral replication within the cell or (3) by the production of interferons. Probably the most important of these is the latter. Interferons are low molecular weight polypeptides produced by cells which have been infected with certain viruses or foreign nucleic acids (18). Interferons appear to inhibit the replication of viral ribonucleic acids (RNA) and subsequently replication of the virus. Not all viruses stimulate the production of interferons. Viruses most effective in initiating interferon production belong to the encephalovirus, myxovirus and paramyxovirus groups.

The advances in medical technology have made it possible to study means of host defense within an animal. Much of this technology will be applied to diseases of cattle within the next few years, providing information necessary for understanding disease processes. Much of this information will be of considerable value for preventive as well as therapeutic veterinary medicine.

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