REVIEW OF BOVINE IMMUNOLOGY FOR THE VETERINARY PRACTITIONER

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Introduction

Immune response has been defined as "all of the phenomena that result from specific interaction of cells of the immune system with antigen" (14). This definition emphasizes the cardinal features of the immune response. It is a specific response to a foreign entity, antigen. It is the response of a particular organ system which has only in recent years come to be well understood.

The survival advantage of specific acquired immunity is considerable since the phenomenon has persisted throughout evolution for some 500 million years and, within the lifespan of the individual, inherited or induced deficiency of immune response is associated with both infectious and neoplastic disease which is invariably fatal.

Immunology owes must to cattle since the first truly safe vaccine was produced by Jenner (1798) who realized that vaccinia or cowpox produced a harmless infection in man but rendered him immune to infection by the small pox agent. At a much later date (1949) Sir McFarlane Burnet drew upon knowledge of blood cell chimerism in bovine freemartins to formulate his theories of immunity which have provided us with current concepts of "self and non-self" and clonal selection. That is that individuals do not normally mount an immune response to their own tissue and that immune response to a specific antigen is the function of a single family of lymphocytes triggered by interaction with that antigen only. For each antigen or group of closely related antigens there exists a separate population or clone of lymphocytes (7).

The nature of immunity was recognized by Roux and Yersin (1888) who found that specific resistance to diphtheria could be transferred with serum, a finding which led to widespread use of serotherapy with antitoxin prepared in horses. The word antibody was coined at about this time to describe the active component of sera capable of imparting protection. In attempts to immunize against tuberculosis Koch in the 1890's recognized "bacterial allergy" or delayed hypersensitivity. These two phenomena, antitoxic immunity and delayed hypersensitivity represent two components of immune response, humoral and cellular immunity and these two components have definite morphological as well as functional correlates in the immune system.

The Immune System

Lymphocytes are the basic components of the immune system and its organs are all of those which produce or contain lymphocytes. These organs are of two orders, primary and secondary. Primary organs have a controlling or regulatory function and include the thymus and bursa of Fabricius in birds and the thymus and bone marrow in mammals (2). Secondary lymphoid organs are those in which the actual effectors of immune response are produced. Antibody is the effector of humoral immunity and the sensitized small lymphocyte the mediator of cellular immunity. The spleen and lymph nodes are the principal secondary organs but individual lymphocytes or lymph follicles in any tissue can function in immune response.

The relationship between primary and secondary organs is one of conditioning uncommitted lymphocytes within primary organs for export to secondary organs as immunologically functional cells. Primitive marrow stem cells give rise to lymphocytes which circulate to the thymus or bursa of Fabricius in which locations they undergo transformation to blast or dividing cells the progeny of which leave the primary organs to locate in secondary organs as mature immunocompetent lymphocytes able to take part in immune response. In general thymic derived or T lymphocytes function in cellular immune response and bursa or bone marrow dependent lymphocytes (B lymphocytes) generate humoral immune response.

T and B lymphocytes have a characteristic distribution within lymph tissue. In lymph nodes the outer cortex including the lymph follicles and the cords of the lymph node medulla are populated by B lymphocytes which are functional in antibody synthesis. The deep cortical or paracortical area is

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thymus-dependent and contains lymphocytes which are active in cellular immune response. Similar relationships exist in the spleen in which the lymph follicles and red pulp are populated by B lymphocytes and the white pulp other than the follicles is thymus-dependent.

B lymphocytes upon interaction with antigen begin to synthesize antibody and differentiate morphologically to become plasma cells. T lymphocytes do not release antibody but upon interaction synthesise and release factors known as lymphokines. These include the following: *a*) leucocyte chemotactic factor, *b*) macrophage activation factor which enhances killing of phagocytosed organisms, *c*) cytotoxic factor capable of destroying target cells, *d*) a factor which inhibits macrophage motility, and *e*) transfer factor which can transfer delayed hypersensitivity to a non-sensitized host. This latter has been identified only in man, monkey and guinea pig (6). The end result of T cell activities is the destruction of such targets as tumour cells, tissue grafts or cells which contain intracellular parasites including bacteria, fungi and viruses.

Antibody may be assayed by any of several serological reactions including bacterial agglutination, complement fixation, virus neutralization, precipitation and opsonization. Indirect fluorescent antibody tests which detect antibody bound directly to antigen without the need for any of the above secondary phenomena for visualization and which can be performed with ease and rapidity at minimal cost hold considerable promise for the simplification of serology. Radioisotopic techniques offer yet another degree of sophistication and sensitivity which shall undoubtedly be universally exploited in the future (19).

Cellular immune response is not so readily assayed. The *in vivo* detection of delayed skin reactions following injection of tuberculin or Johnin are measures of cellular immune response but these do not lend themselves to precise quantitation which is desirable for the evaluation for the course of disease or of immunization. The macrophage migration inhibition and lymphocyte transformation tests have come into use for *in vitro* evaluation of cellular immune response and through their application the importance of cell mediated immunity has come into focus. The macrophage migration inhibition test utilizes the release of migration inhibitory factor from antigen exposed sensitized lymphocytes. In appropriate systems the presence of this factor can be shown to inhibit the normal migration of monocytes on glass surfaces and the degree of inhibition may be correlated with the degree of immunization in the cellular as opposed to the humoral system (4, 36).

In summary, the immune system responds to antigen in two ways. It synthesizes antibody and it produces specifically sensitized lymphocytes. Antibody is specific for the antigen which stimulated its production and it has a protective function through virus or toxin neutralization, lysis of bacterial cells in the presence of complement and opsonization of bacterial cells to assist in their phagocytosis. The presence of antibody is detected by the classic serological reactions. Sensitized lymphocytes are of primary importance in the destruction of tumour or graft cells and of cells containing intracellular parasites including many bacteria and viruses.

The Humoral Immune Response of Cattle

When stimulated parenterally by a suitable amount of a properly antigenic material, humoral immune response, or antibody synthesis, proceeds after a lag period which is frequently about five days in cattle. After this time antibody can be detected in the serum and continues to increase in amount to reach a peak at four to five weeks. It then gradually declines to low or undetectable levels if antigenic stimulation is terminated. This first response to antigen is known as the primary response. A second injection of antigen results in further antibody synthesis following a shortened lag period. In the secondary response a higher maximum titer is attained more quickly than the maximum in primary response. Because the amount of antibody produced is greater than in primary response the duration of the titer is greater. This is because antibody is catabolized at a fixed rate.

The secondary response is a manifestation of immunological memory; the result of expansion of a population of small lymphocytes capable of initial interaction with antigen. Immunological memory may persist for years but active immune response may proceed only if antigen is present.

The above is a description of an ideal immune response. It is possible by manipulation of antigen dose to stimulate immunological memory without recognizable antibody synthesis in the primary response or to induce marked primary antibody synthesis without generating memory (39). It is commonly observed that injection of a second dose of antigen when titer is still detectable from the primary response results in reduction rather

than increase in serum antibody titer due to removal of antibody in complex with antigen.

Immune response depends upon immunogenicity of the injected material; that is upon the ability of the antigen to trigger immune response. Antigens of low immunogenicity may fail completely to trigger antibody synthesis at usual dose levels but may do so at very high dose or when combined with such agents as peanut oil or alum. The latter are known as immunological adjuvants and have a nonspecific enhancing effect upon immune response. Some are capable of altering the nature of the response to accentuate either humoral or cellular response or to stimulate synthesis of a particular immunoglobulin in greater than usual amounts. These attributes are commonly utilized in programs of protective immunization of cattle.

Route of injection is also an important consideration. The subcutaneous route appears to be a good one in cattle if the objective is the production of high serum titers. In species in which it has been examined, intradermal injection is a good route for the stimulation of cellular immune responses. Intravenous injection has a high attendant risk of untoward reactions and is best avoided. Where a disease producing agent can be shown to be restricted to a local site, or to have its principal effect there, attempts to immunize in that specific location may be of more benefit than immunization by a systemic route.

Killed preparations are often less immunogenic than are living because of their inability to multiply and increase total antigen, to disseminate and widely stimulate the immune system and to synthesize metabolites which may be important antigens for stimulation of protective immunity. The bovine fetus has been shown to be capable of immune response as early as 164 days of gestation (15) and there are indications that much earlier response is possible (38). The fetus is also able to synthesize antibody in response to infectious agents such as the virus of bovine virus diarrhea and may have measurable titers at birth as a result of intrauterine infection (9). The neonate is clearly immunologically competent but may not be able to mount an active immune response following vaccination or infection if it has a high antibody titer obtained passively from its mother via colostrum since specific antibody inhibits autibody synthesis. This inability may persist for several weeks depending upon the magnitude of the passive titer and delay in immunoglobulin synthesis until four weeks post partum has been observed with serious implications for protection of the calf at that time or until endogenous antibody synthesis is significant (24).

Bovine Immunoglobulins

Antibody function is a property of proteins known as immunoglobulins. This is a heterogeneous group made up of several distinct classes and subclasses of molecules which differ in physicochemical, antigenic and biological properties. There are presently recognized five bovine immunoglobulins: IgG_1, IgG_2, IgM, IgA and IgE (13, 16).

Differences exist between breeds of cattle in relation to immunoglobulins. Enhanced ability of Brown Swiss calves to synthesize antibody to certain antigens has been reported (37). A selective deficiency of IgG_2 in Danish Reds was associated with increased susceptibility to pyrogenic infection (29) and a tendency exists in calves of the Jersey breed to have higher serum globulin levels than Holstein calves following ingestion of colostrum (40).

Immunoglobulins IgG_1 and IgG_2 are quantitatively and functionally highly important in bovine serum and certain secretions (13). They are abundant in the serum of hyperimmunized animals and are not restricted to the blood vascular space, having free access to interstitial fluids. IgG_1 is selectively concentrated in bovine colostrum in which secretion it is found in greater quantity than immunoglobulins of other classes (25). In addition when IgG_1 is present in secretions such as those of the respiratory tract it generally exceeds IgG_2 in concentration again indicating selective secretion or possibly local synthesis (13). IgG is associated with virus neutralization, toxin neutralization, bacterial agglutination and opsonization. Only IgG_1 can fix complement and as a consequence is more active in bacterial lysis and opsonization than IgG_2 (3).

Immunoglobulin IgM is a large molecule which is efficient in bacterial agglutination, complement fixation and opsonization (33). Antibody to Gram negative bacteria tends to be of this class and there is approximately twice as much IgM in bovine colostrum as in serum on a volume basis (8, 13). Because of its large size IgM is more restricted to the intravascular space than is IgG (18, 43). Purified bovine IgM is apparently more efficacious in preventing calf Colisepticemia than is purified IgG but neither immunoglobulin alone is as effective as the combination of both (22). In addition to prevention of Colisepti-

cemia both milk whey and purified IgM appear to exert a local effect in the gastrointestinal tract which can significantly delay the onset of enteric Colibacillosis (23). A selective deficiency of IgM has been reported in leukotic cattle apparently the result of damage to IgM-producing lymphocytes (42).

Immunoglobulin IgA is the predominant immunoglobulin in most but not all external secretions and has been shown in many species to have great significance in protection against viral respiratory tract infections. IgA mediated protection against bacterial infection of the intestinal tract is accepted in man and appears to be aided by resistance of IgA to degradation by proteolytic enzymes. Since IgA neither opsonizes nor fixes complement (51) perhaps it functions by preventing close adherence of bacterial cells to epithelial surfaces, a relationship which is important for pathogenicity of many bacteria including *E. coli* (48).

Local Immunity

Local immunity or immune response active at the surface of organs which have access to the external environment is important in protection against infection at these sites. It has been rather well established in several species that protection of these organs is associated with local synthesis of antibody which is predominantly of the IgA class arising in plasma cells which are abundant immediately beneath epithelia (26, 41). Local production of IgA antibody probably also provides the relatively small amount of this immunoglobulin found in bovine serum by retrograde drainage via lymphatics (8).

Local antibody is best stimulated by topical infection or vaccination with viable agents but it can be induced by agents inoculated parenterally which may be able to spread to local sites. Killed agents may also induce local antibody synthesis but require larger and more numerous doses to accomplish this (27). It has recently been found possible to stimulate antibody production in the bovine mammary gland by instillation of attenuated *Escherichia coli*. The resultant antibody is capable of inhibiting growth of the immunizing serotype *in vitro* and is potentially important in protection of the neonate from enteric infection (49, 50).

Local stimulation can be very effective in some cases in eliciting systemic antibody response which may be equivalent to that produced following parenteral immunization with living agents. In the respiratory tract attempts at local immunization must provide droplets of a size that can reach the area of the respiratory tree to be protected since it is possible to induce nasal antibody without bronchiolar and vice versa (45). For bronchoalveolar deposition particles must be in the order of one to two microns in size.

The reproductive tract is unique in that systemic immunization results in a high degree of transfer of IgG into its secretions (13, 46). This may be true of both male and female cattle (11). Local infection does provide stimulus for formation of antibody predominantly of the IgA class but it is interesting that serum derived antibody appears to be more effective in protection against *V. fetus* than is the local antibody (46).

The significance of local concentrations of IgG in bovine external secretions may be considerable since, if extrapolation is permitted from other species the ability of IgG to opsonize in the complement-free environment of the respiratory tract could provide it with an advantage over the more abundant but non-opsonizing IgA (35).

Cellular Immune Response

Investigation of cellular immune response is in all species far behind that of the humoral system, however, it is an established fact that the sensitized lymphocyte plays a role in protective immunity in a variety of viral and bacterial infections and that such lymphocytes are present within the respiratory passages as well as the serum of guinea pigs and dogs (17, 21, 30, 44, 45). As in the case of local antibody this type of local response is best stimulated by local immunization or infection.

The feasibility of applying the macrophage migration inhibition test to bovine leucocytes as an assay for cellular immune response has been shown by Aalund who demonstrated positive reactions in cattle with Johne's disease (1). That cell-mediated immunity may be active in protection of cattle against infectious bovine rhinotracheitis virus has recently been proposed (12) and the impairment of both cellular and humoral immunity has been reported in bovine viral diarrhea virus infected cattle (20, 28). Cell-mediated immune response has also been recognized in bovine anaplasmosis (5) and tuberculin sensitive lymphocytes have been studied by means of their antigen-stimulated synthesis of nucleoprotein (32).

The tendency has been to relate efficacy of immunization to the presence of serum or local antibody; however these may be mere indices of a response the functional component

of which could be shared equally or disproportionately with sensitized lymphocytes.

Immunopathology

Disease is the result of interaction between agent and host. The host response can contribute in large measure to tissue damage and in certain situations may produce greater damage than intrinsic virulence mechanisms of the agent itself. This type of host response must be considered in attempts to induce protective immunity. Certain human respiratory pathogens produce more severe disease in immunized hosts than in non-immune as a result of local pulmonary hypersensitivity. Enigmatically this type of reaction is not recognized in individuals with high levels of antibody in local secretions but occurs when serum antibody is high in the absence of high secretory titers (10). Hypersensitivity pneumonitis can readily be induced in guinea pigs by systemic immunization followed by aerosol exposure of the lung to the antigens used for immunization. The observed pneumonia is the result of both antibody and sensitized lymphocytes combining with antigen in the lung (47). A similar mechanism is most probably operational in the pathogenesis of at least some naturally occurring bovine interstitial pneumonias (31, 34).

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