Cell-Mediated Immunity in the Neonatal Calf

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To understand the immunological capabilities of the bovine neonate, the complete ontogeny of the bovine immune system must be understood. As in most species, the developing bovine fetus is deficient in a number of different cellular and humoral factors that play important roles in host defenses during postnatal life. In the bovine fetus, as in other species, the immune response appears to develop and mature sequentially. During this development, maternal cells and humoral factors serve as the major protective barriers to infectious agents. However, the newborn calf is essentially agammaglobulinaemic because of the syndesmochorial placentation that does not permit the transfer of maternal immunoglobulins to the fetal circulation. The newborn calf is dependent on the absorption of immunoglobulins from colostrum to provide adequate humoral immunity. Colostrum also contains numerous leukocytes. The role and relative importance of these cells are not clear. However, recent data suggest that these maternally derived cells may influence the development of effective cellular immunity.

Lymphocytic Development

Lymphocytes represent a heterogeneous cell population with respect to their origin, function, and phenotypic characteristics. Two major subpopulations of lymphocytes are T lymphocytes and B lymphocytes. T lymphocytes develop from stem cells under the influence of the thymus, whereas B lymphocytes develop in the fetal liver and bone marrow.[19,20] In the bovine fetus, lymphocytes are evident in the thymus at 42 days of gestation, with subsequent blood lymphocytes being first observed at day 45.[21] By 55 days of gestation, lymphocytes can be found in the spleen.[21] Peripheral lymph nodes appear to be populated at 60 days of gestation, with mesenteric lymph nodes following at around 100 days, and gastrointestinal tract lymphoid tissue being populated at 175 days.[20,21]

Ontogeny of the Humoral Response

The maturation of the humoral immune system in the bovine fetus has been evaluated primarily by quantifying immunoglobulins or specific antibody in fetal serum. Even though evidence indicates lymphoid development by mid-gestation, the ability of the bovine fetus to respond sufficiently to antigens and synthesize antibody usually is not acquired until after birth.[21] However, antibody to parainfluenza-3 virus has been detected as early as 120 days of gestation.[34] In contrast, antibody to the bovine viral diarrhea virus has not been detected until 190 days of gestation, and antibody to *Trichomonas foetus* has not been detected until 30 days after birth.[6,14] The ability of the bovine fetus to make antibodies to particular antigens indicates a degree of early immunocompetence and perhaps the capability to clear some infections.

Ontogeny of Cell-Mediated Immunity

Cell-mediated immunity (CMI) includes such responses as graft rejection, delayed-type hypersensitivity and resistance to intracellular microorganisms (viruses and bacteria). T lymphocytes produce lymphokines or act as cytotoxic cells to mediate and/or regulate these responses. At birth, the bovine neonate can reject skin grafts like an adult.[5] The approximate age of gestation when a bovine fetus develops the capability to mount a cellular immune response has been studied using delayed hypersensitivity skin tests and lymphocyte stimulation assays. Studies have investigated the response of fetal bovine lymphocytes to nonspecific mitogenic stimulation with lectins such as concanavalin A, pokeweed mitogen, and phytohemagglutinin. Mitogenic-induced blastogenesis of bovine fetal lymphocytes has been demonstrated as early in gestation as day 78.[22] Few studies have investigated the ability of the bovine fetus to mount an antigen-specific, CMI response. However,

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in one study, investigators inoculated fetal calves with *Mycobacterium bovis*, tetanus toxoid, and *Brucella abortus* between days 168 to 248 of gestation. At birth, calves had CMI responses to these antigens as measured by delayed-type hypersensitivity skin tests and lymphocyte transformation assays.[29]

Passive Transfer of Immunity

Although the calf is capable of responding to most foreign antigens at birth, resistance to disease is dependent on passive immunity transferred from the dam. The calf requires maternal, colostral immunoglobulin for immunity to many pathogens during the first weeks of life. However, the role of passively acquired CMI is not known. Colostral absorption of leukocytes may be a source of natural, passive CMI for the neonatal calf. T lymphocytes represent the major subpopulation of lymphocytes in bovine mammary secretions.[8,9,10,11,31] Using the two-fluorochrome method and laser flow cytometry to determine the mean distribution of cells within the colostrum of five cows revealed 67.8% lymphocytes, 9.0% neutrophils, and 23.3% monocytes.[11] In an analysis using monoclonal antibodies to bovine B and T lymphocytes, data collected from lymphocyte subpopulations from both colostrum and dry-cow secretions yielded mean percentages of 88.1-89.0% T cells and 2.8-3.5% B cells.[11] The lymphocytes in bovine lacteal secretions are immunologically active.[31]

The importance of immunocytes in bovine colostrum and milk is suggested by human studies. The colostrum of humans is also rich in T lymphocytes that are immunocompetent. T cells in human milk express phenotypic markers characteristic of activation. Human milk contains cytokines such as tumor necrosis factor-alpha, interleukin(IL)-1 and IL-6. These cytokines play a role in the regulation of the leukocytes contained in the human milk.[38]

The role of colostral lymphocytes in the immunity of newborn animals remains unclear. Studies in the rat, sheep and pig showed that colostral lymphocytes are absorbed through the digestive tract of the newborn and they enter the lymphatic circulation.[32,33,36] It has been postulated recently that vital colostral lymphocytes also pass through the intestinal wall of the neonatal calf.[23] Sheldrake *et al.* demonstrated that radiolabelled, syngeneic or allogeneic lymphocytes from colostrum were absorbed by the intestine of neonatal rats and lambs.[33] In lambs, the absorbed lymphocytes were transported by lacteal ducts to mesenteric lymph nodes. Tuboly *et al.* observed similar absorption in the duodenum and jejunum in piglets.[36] This neonatal absorption of lymphocytes was observed to be specific for cells of colostral origin (not from maternal blood). Only viable cells are absorbed in the intestine of the piglet.[35,36] In other studies, absorption of colostral lymphocytes enhanced blastogenic responses to phytomitogens in fetal peripheral blood mononuclear cells.[36]

More recently, neonatal calves fed colostrum or milk replacers with colostral leukocytes maintained numbers of peripheral blood lymphocytes, had improved antibody responses to sheep erythrocytes (a T lymphocyte-dependent antigen), and had enhanced transformation responses to the T cell mitogen concanavalin A.[24] In a similar study, intestinal absorption of lymphocytes was associated with improved absorption of colostral antibody against *Escherichia coli* (especially IgA and IgM).[25] Neonatal calves that did not receive colostral leukocytes had reduced numbers of neutrophils in their blood, but these neutrophils appeared to function normally.[26] Further, the bactericidal properties of whole blood were enhanced if calves received intact colostrum compared to blood from calves that received colostrum depleted of cells.[27] Calves that received colostrum with or without intact leukocytes survived longer than calves not fed colostrum.[28] It is important to note that absorption of colostral leukocytes alone could not confer immunity to fatal bacterial infection. Colostral immunoglobulin is required for immunity, but inclusion of cells enhances the protective effects of the colostral antibodies. A similar pattern was observed in neonatal calves experimentally infected with bovine rotavirus.[1] Calves fed rotavirus-immune colostral lymphocytes alone continued to shed rotavirus after experimental inoculation. Calves fed only colostral immunoglobulin with specific neutralizing antibody were protected from viral challenge.[1] These studies demonstrate that colostral leukocytes do influence neonatal immune responses and probably contribute to immunity to infectious diseases.

Other immuno-modulating factors have been found in colostrum. Insulin-like growth factors, interleukins, interferons, and some blastogenic inhibitors have been identified.[13,16,18] These factors may influence the neonatal immune system and especially gut-associated lymphoid tissue for the first few days of life.

Colostral lymphocytes may be very important for immunity to intracellular infections. Breast-fed human infants acquire T lymphocyte responsiveness to tuberculin by absorption of the specific cells.[30] Further, human milk and colostrum have two fold higher concentrations of lymphocytes expressing non-covalently bound gamma/delta T-cell receptors when compared to blood lymphocyte populations.[2] These gamma/delta T cells generally are thought to be important for immunity at mucosal surfaces to intracellular pathogens. Also, most of the T lymphocytes in human milk exhibit the CD45 low, CDw29 positive, LFA-1 high phenotype.[3] This phenotype is indicative of presently activated or memory T lymphocytes. These same cells are generally more

adherent to endothelial cells and more responsive to chemotactic stimuli than most peripheral blood lymphocytes. These colostral or milk lymphocytes respond to phytomitogens or lipopolysaccharides by producing the immunostimulatory cytokines IL-1 and IL-2.[4]

Colostral leukocytes should be studied further by veterinary immunologists. There is little doubt that these cells do influence the immune system of calves. These cells may provide T helper cells, effector cells and regulation of repertoire development. However, the types of lymphocytes involved may be quite diverse.[15] Clearance of and immunity to respiratory syncytial virus infection in calves was dependent on high numbers of CD8+ lymphocytes.[35] Intraepithelial leukocytes of the gut that contain many CD8+ lymphocytes were important for immunity to bovine coronavirus infection in the intestine.[12] This protection was enhanced markedly by systemic delivery of IL-2 and tumor necrosis factor.

Other lymphocyte populations are important for immunity at mucosal surfaces. Gamma/delta T lymphocytes may represent a set of broadly reactive lymphocytes that act as a first line of defense for many microbial infections. However, the protective effect may block activity of CD4+ lymphocytes. In fact, gamma/delta T lymphocytes actually may mediate cytotoxic killing of CD4+ helper cells needed for immunity to bovine paratuberculosis.[7] B lymphocytes make up a small portion of all colostral leukocytes and do not respond well to mitogens or viral transformation.[17] Also, colostral T lymphocytes cannot support differentiation or proliferation of colostral B cells. Colostral B lymphocytes have not been identified as the precursors for IgA-secreting plasma cells at mucosal surfaces.[17] However, they may serve as allogeneic, antigen-focussing or antigen-presenting cells in neonatal secondary lymphoid tissue.

The likely functions of colostral lymphocytes are many and probably mediated by diverse populations of cells. Better characterization of colostral lymphocyte populations will permit improved stimulation of maternal cellular populations that can lead to enhanced neonatal immunity and better calf survivability. An improved understanding of the calf's developing immune system should also help to optimize life-long adaptive immune responses in cattle.

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