

# Cow / Calf Split Sessions

Moderator - Julie Weikel, DVM

## Cell-Mediated Immunity in the Neonatal Calf

**D. Scott McVey, DVM, PhD, Diplomate ACVM**  
**Robert Black, DVM**

*Department of Pathology and Microbiology  
College of Veterinary Medicine  
Kansas State University  
Manhattan, KS 66506*

### Abstract

**Bovine colostrum contains viable leukocytes. The phenotypes and biologic responsiveness as well as clinical significance of colostrum lymphocytes will be reviewed. From the reviewed data, possible functions of these cells will be discussed. In addition, new data that describes absorption, viability, activation states and the repertoire expression of T-lymphocytes will be presented. Information of the kinetics of lymphocyte absorption, positive and negative selection, and function in the immune system of neonatal calves will also be presented. Maternally derived lymphocytes probably have a major role in the initiation, maturation and regulation of immune responses in the newborn calf.**

### Introduction

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 an important role in host defenses in postnatal life. In the bovine fetus, the immune response appears to develop and mature sequentially like in other species. During this development, maternal cells and humoral factors serve as the major protective barrier to infectious agents. However, the newborn calf is essentially agammaglobulinaemic due to 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 suggests these maternally derived cells may have a role in the development of effective cellular immunity.

### Lymphocytic Development

Lymphocytes represent a heterogeneous cell population with respect to their origin, function, and phenotypic characterization. Two major subpopulations of lymphocytes exist, T lymphocytes and B lymphocytes. T lymphocytes develop from stem cells under the influence of the thymus, while B lymphocytes develop in the fetal liver and bone marrow.<sup>19,20</sup> In the fetus, lymphocytes are evident in the thymus at 42 days of gestation with subsequent blood lymphocytes being first observed at day 45.<sup>21</sup> By 55 days of gestation, lymphocytes can be found in the spleen.<sup>21</sup> 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.<sup>20,21</sup>

### 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 the fetal serum. Even though there is evidence of a lymphoid system by mid-gestation, the ability of the bovine fetus to respond sufficiently to antigens and synthesize antibody is usually not acquired until after birth.<sup>21</sup> However, antibody to parainfluenza-3 virus has been detected as early as 120 days of gestation.<sup>34</sup> 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.<sup>6,14</sup> Variations in the ability of the bovine fetus to make antibodies to particular antigens seems to indicate some degree of immunocompetence exists in the bovine fetus and that the fetus may be capable of clearing some infections.

## Ontogeny of Cell-Mediated Immunity

Cell-mediated immunity (CMI) responses include 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. The bovine neonate has the capability to reject skin grafts, similar to an adult, at the time of birth.<sup>5</sup> To determine the approximate age of gestation when a bovine fetus develops the capability to mount a cellular immune response, scientists have used delayed hypersensitivity skin tests and lymphocyte stimulation assays. There have been many studies investigating 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.<sup>22</sup> Few studies have been conducted regarding the ability of the bovine fetus to mount an antigen-specific CMI response. In one study, investigators inoculated fetal calves with *Mycobacterium bovis*, tetanus toxoid, and *Brucella abortus* between 168 to 248 days of gestation. At birth calves had CMI responses to these antigens as measured by delayed-type hypersensitivity skin tests and lymphocyte transformation assays.<sup>29</sup>

## Passive Transfer of Immunity

Although the calf is capable of responding to foreign antigens at birth, it is dependent on passive immunity transferred from the dam. The calf requires maternal, colostrum immunoglobulin for immunity to many pathogens for the first week of life. However, the role of passively acquired CMI is not understood.

A source of natural, passive CMI to the neonatal calf may be through colostrum absorption. T lymphocytes have been found to represent the major subpopulation of lymphocytes in bovine mammary secretions.<sup>8,9,10,11,31</sup> Using the two-fluorochrome method and laser flow cytometry, the mean distribution of cells within the colostrum of five cows revealed 67.8% lymphocytes, 9.0% neutrophils, and 23.3% monocytes.<sup>11</sup> In an analysis using monoclonal antibodies to bovine B and T lymphocytes, data collected from lymphocyte subpopulations from both colostrum and dry secretion yielded a mean percentage of 88.1-89.0% T cells and 2.8-3.5% B cells.<sup>11</sup> The cellular constituents of bovine lacteal secretions have been determined to be immunologically active.<sup>31</sup> The colostrum of humans is also rich in T lymphocytes and has been examined for cellular immunocompetence. Studies have revealed that T cells in human milk express phenotypic markers of recent or previous activa-

tion and that human milk contains cytokines such as tumor necrosis factor-alpha that may play a role in the activation of leukocytes in human milk.<sup>38</sup>

The role of colostrum lymphocytes in the immunity of newborn animals remains unclear. The absorption of colostrum lymphocytes from the digestive tract of the newborn and the entry of these lymphocytes into the lymphatic circulation has been demonstrated in the rat, sheep, and pig.<sup>32,33,36</sup> Recently, it has been postulated that vital colostrum lymphocytes may pass through the intestinal wall of the neonatal calf.<sup>23</sup>

Sheldrake *et al* demonstrated that radiolabelled, syngeneic or allogeneic lymphocytes from colostrum were absorbed by the intestine of neonatal rats and lambs.<sup>33</sup> In lambs, the absorbed lymphocytes were transported by lacteal ducts to mesenteric lymph nodes. Tuboly *et al* observed similar absorption by piglets in the duodenum and jejunum.<sup>36</sup> This neonatal absorption of lymphocytes was observed to be specific for cells of colostrum origin (not from maternal blood). Only viable cells are absorbed in the intestine of the piglet.<sup>35,36</sup> In other studies, absorption of colostrum lymphocytes enhanced blastogenic responses to phytohemagglutinins in fetal peripheral blood mononuclear cells.<sup>36</sup>

More recently, neonatal calves fed colostrum or milk replacers with colostrum leukocytes maintained numbers of peripheral blood lymphocytes, had improved antibody responses to sheep erythrocytes (a T lymphocyte-dependent antigen), and enhanced transformation responses to concanavalin A.<sup>24</sup> In a similar study, intestinal absorption of lymphocytes was associated with improved absorption of colostrum antibody to *Escherichia coli* (especially IgA and IgM).<sup>25</sup> The effects of colostrum leukocytes on phagocytic functions of peripheral blood cells of neonatal calves were not clear as blood neutrophil numbers were decreased but some functional assays were unaffected (tetrazolium blue reduction and phagocytosis).<sup>26</sup> 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.<sup>27</sup> Calves that received colostrum with or without intact leukocytes survived longer than calves not fed colostrum.<sup>28</sup> It is important to note that absorption of colostrum leukocytes alone could not confer immunity to fatal bacterial infection. Colostrum immunoglobulin is required for immunity but inclusion of cells enhanced the protective effects of the colostrum antibodies. A similar pattern was observed in neonatal calves relative to bovine rotavirus infection.<sup>1</sup> Calves fed immune colostrum lymphocytes shed rotavirus after experimental inoculation while calves fed colostrum immunoglobulin with specific neutralizing antibody were protected from viral challenge.<sup>1</sup>

In challenge experiments, feeding colostrum with intact leukocytes was associated with enhanced clear-

ance of intestinal bacterial pathogens as well as higher serum antibody titers to the same microorganisms.<sup>28</sup> These studies demonstrate that colostral leukocytes do influence neonatal immune responses and probably contribute to immunity to infection diseases.

Other immuno-modulating factors have been found in colostrum. Insulin-like growth factors, interleukins, interferons and some inhibitory substances have been identified.<sup>13,16,18</sup> 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 infants acquire T lymphocyte responsiveness to tuberculin by absorption of the specific cells.<sup>30</sup> 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.<sup>2</sup> Also, most of the T lymphocytes in human milk exhibit the CD45 low, CDw29 positive, LFA-1 high phenotype.<sup>3</sup> This phenotype is indicative of memory cell populations and/or recent activation. These same cells are generally more adherent to endothelial cells and responsive to chemotactic stimuli than most peripheral blood lymphocytes. These colostral or milk lymphocytes respond to phytomitogens or lipopolysaccharides by producing interleukins 1 and 2.<sup>4</sup>

The study of colostral leukocytes should be pursued by veterinary immunologists. There is little doubt these cells do influence the immune system of calves. It is likely that these cells may provide T helper cells, effector cells and possible regulation of repertoire development. However, the types of lymphocytes involved may be quite diverse.<sup>15</sup> Clearance of and immunity to respiratory syncytial virus infection in calves was dependent on high numbers of CD8+ lymphocytes.<sup>35</sup> Intraepithelial leukocytes (with high numbers of CD8+ lymphocytes) are important for immunity to bovine coronavirus infection in the intestine.<sup>12</sup> This protection was enhanced markedly by interleukin-2 and tumor necrosis factor. 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 may actually mediate cytotoxic killing of CD4+ helper cells needed for immunity to bovine paratuberculosis.<sup>7</sup> B lymphocytes make up a small portion of all colostral leukocytes. These B lymphocytes do not respond well to mitogens or viral transformation.<sup>17</sup> Also, colostral T lymphocytes can not support differentiation or proliferation of colostral B cells. No specific population of colostral B lymphocytes has been identified as IgA precursors for the mucosal surfaces.<sup>17</sup> One possible function of these cells is to serve as allogeneic, antigen-focussing or

antigen presenting cells in neonatal secondary lymphoid tissue.

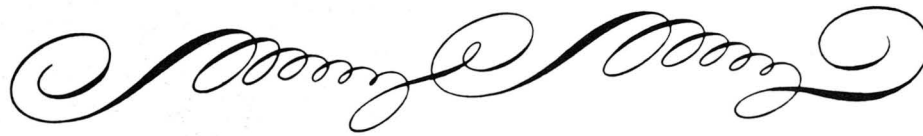
The likely functions of colostral lymphocytes are many and probably mediated by diverse populations of cells. As these colostral lymphocyte populations are characterized, the management and stimulation of maternal cellular populations can be improved to enhance neonatal immunity and better calf survivability. In addition, the influence of colostrum on development of the calf's immune system should be studied to allow optimal development and function of adaptive immune responses.

## References

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## FUTURE MEETINGS

### American Association of Bovine Practitioners

1994	Pittsburgh	September	22-25
1995	San Antonio	September	14-17
1996	San Diego	September	12-15
1997	Montreal	September	18-21
1998	Spokane	September	24-27
1999	Nashville	September	23-26

### World Association for Buiatrics

1994	Bologna, Italy	August 29-September 2
1996	Edinburgh, UK	July 8-12