Review Article

Bovine colostral cells—the often forgotten component of colostrum

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At birth, neonatal calves have all the essential immune components they need as adult cattle; however, it takes at least 2 to 4 weeks for those components to become fully functional.[1,2] The immune system does not become completely mature until the animal is 5 to 8 months old; for example, it takes 8 months for T cells (CD4+, CD8+, and T-cell receptor γδ+ cells) to reach peak numbers.[3] Thus, the immature immune system of young calves likely moderates rather than prevents disease. This does not imply that young (< 8 months old) calves cannot respond to antigens; it simply means that the response to those antigens is weaker, slower, and easier to overcome than it will be when the immune system is mature.[4]

Cattle and other ruminants have a syndesmochorial placenta, which forms a syncytium between the maternal endometrium and the fetal trophectoderm, separating the maternal and fetal blood supplies and preventing the transmission of immunologic components from the dam to the fetus in utero. During the immediate preparturient period, pregnant cows initiate colostrogenesis, and blood cells and other colostral components migrate to the mammary gland[5–7] where their phenotype and function are altered.[8–11]

Because bovine maternal and fetal blood supplies are kept separate in utero, calves are immunologically naïve immediately after birth, which puts them at high risk for disease. The acquisition of passive immunity, generally through the ingestion of colostrum, helps protect calves against disease during this critical period. Cattle producers routinely feed newborn calves fresh (ie, never frozen) or frozen colostrum, which contains antibodies against pathogens within the local environment; however, antibodies are not the sole constituents of colostrum and passively acquired immunity.[12]

In addition to maternally derived antibodies, colostrum contains immunologically important cytokines and a large number of maternally derived (maternal) leukocytes, which collectively contribute to the immunoprotection of the neonate.[13–16] Although the role of colostral antibodies in the protection of neonates against disease has been well documented,[17–20] the role of colostral cellular components in passive immunity is less well understood.[21–28] In the early 1970s, researchers began to suggest that maternal lymphocytes in colostrum were primed antigen-responsive cells that, when ingested soon after birth, penetrate the permeable intestinal wall and provide transient local or systemic cell-mediated immunity, the breadth of which reflects the antigenic exposure of the cow from which the colostrum was acquired.[28,29]

ABBREVIATIONS

BVDV	Bovine viral diarrhea virus
CMC	Colostral mononuclear cell
PBMC	Peripheral blood mononuclear cell
PPD	Purified protein derivative

Migration, trafficking, and homing of colostral cells

The development of immunologic tolerance subsequent to passive transfer of maternal leukocytes via ingestion of colostrum was first described in suckling rats in 1975.[30] The crossing of colostral cells across the neonatal intestinal barrier has since been described in many other species including mice,[31] sheep,[32,33] baboons,[34] and pigs.[35–38] Colostral cells pass through the intestinal wall, migrate to the lymphatic system, enter the systemic blood circulation, and are recirculated to important immune system organs such as the liver and spleen.[31–38] Results of a study[35] with both in vivo and ex vivo experiments involving piglets indicate that only colostral cells derived from the dam of a piglet can pass through the neonatal intestinal barrier; peripheral blood leukocytes from that dam or colostral cells from another sow cannot pass through the neonatal intestinal barrier.

In a study[21] involving neonatal calves, fluorescein isothiocyanate–labeled colostral leukocytes were identified in the Peyer patches of the jejunum and ileum 1.5 to 2 hours after experimental injection of the cells into the intestine, which indicates that colostral leukocytes are absorbed through the intestinal barrier by the follicle-associated epithelium of Peyer patches. Results of a subsequent study[12] suggest that colostral cells are trafficked from the small intestine into the neonatal circulation and trafficking peaks approximately 24 hours after birth. For that to occur,

colostral leukocytes must undergo functional and phenotypic changes so that they can enter the systemic circulation and tissues of the neonate. It has been postulated that L-selectin, a cell adhesion molecule found on lymphocytes, has an important role in the migration of lymphocytes across the walls of high endothelial venules into the bloodstream but is less necessary for the transport of colostral lymphocytes across the intestinal barrier. That is because the shear rate between the intestinal mucosa and contents is much less than that between the blood vessel mucosa and blood circulation; therefore, factors that improve cell adhesion, such as L-selectin, are necessary to increase cell contact time with the blood vessel mucosa and allow the lymphocytes to enter the bloodstream. The loss of L-selectin from the surface of lymphocytes is associated with the development of memory cells.[38–42]

Results of another study[8] indicate that exposure of PBMCs to a colostral environment induced phenotypic changes that facilitate the trafficking of those cells from the neonatal intestine into the systemic circulation. In that study,[8] PBMCs were labeled with a fluorescent tracer, inoculated into culture medium with or without 25% acellular colostral secretions, and fed to calves within 6 hours after birth. Fluorescent-labeled cells were subsequently identified in the peripheral blood circulation of calves that were fed the medium with the colostral secretions, but not in calves that were fed the medium without colostral secretions.[8] In those calves, the concentration of fluorescent-labeled cells peaked in the peripheral blood circulation between 12 and 24 hours after ingestion and disappeared from the peripheral blood circulation by 36 hours after ingestion.[8]

The bovine lymphocyte antigen DRB3 gene encodes cell surface glycoproteins that present antigenic peptides to CD4+ T helper cells to initiate an immune response. This gene is commonly used as a marker for particular immunologic traits. Results of a study[41] in which nucleotide sequencing methods were used to identify noninherited maternal bovine lymphocyte antigen DRB3 in neonatal calves indicate that colostral leukocytes migrate to the mesenteric lymph nodes, Peyer patches, and spleen after ingestion. Those results support the findings of another study,[8] which indicate that maternal colostral cells disappear from the peripheral circulation of neonatal calves within 24 hours after colostrum ingestion. If colostral cells are upregulated and expressing homing and trafficking markers when they are incorporated into the colostrum, it will aid the migration of those cells across the intestinal barrier and into the peripheral circulation of neonatal calves and promote the expression of a memory phenotype.[8,42–44] Thus, maternal lymphocytes transferred to neonatal calves through ingestion of colostrum may provide a level of immune surveillance in nonlymphoid tissues during the first hours of life.

Quantity and phenotype of colostral cells

Colostrogenesis is the transfer of cellular and noncellular components from the maternal circulation into colostrum within the mammary gland. In domestic ruminants, colostrogenesis begins several weeks prior to parturition and abruptly concludes near the time of parturition.[45] The regulation of colostrogenesis is complex and involves both local[46] and systemic[47] hormonal signaling. In cattle, 1 IgG subclass in particular, IgG1, is actively transferred to colostrum in high concentrations.[48,49]

Identification of cells within colostrum is difficult because of its high fat content.[50,51] Macrophages phagocytize fat globules, which alters their morphology and makes them difficult to differentiate from other cell types[52]; however, different colostral cell subtypes can be successfully identified. Bovine colostrum contains approximately 10^6 leukocytes/mL,[28,53] although that value varies depending on the age, breed, health, and immune status of individual cows. In clinically normal bovine mammary glands, macrophages are the predominant cell type, representing 35% to 79% of all cells.[54] In bovine colostrum, CD11b+ macrophages are the predominant cell type and account for 50% to 90% of all colostral cells,[53] and it is likely that a substantial number of those cells migrate to the peripheral blood of neonatal calves following colostrum ingestion.[55]

The role of colostral cells in the transfer of cell-mediated immunity between dam and offspring was first investigated in the 1970s.[56] Colostral cells were first investigated in lambs[32] and then calves.[21,42] Calf studies involved the analysis of bovine cellular phenotypes[21] and transport of colostral cells.[42] Following parturition, CD8+ and γδ+ T cells are the predominant T cells in the mammary glands of lactating cows.[43] CD8+ T cells are also the predominant cells among mucosal lymphocytes such as bronchoalveolar and ileal intraepithelial lymphocytes.[57,58] Conversely, the proportion of CD4+ T cells is greater than that of CD8+ T cells in the mammary gland of nonlactating cows and decreases to < 20% during lactation.[59] Moreover, the percentages of CD4+ T cells and CD4+CD26+ T cells (ie, activated CD4+ T cells) and the ratio of CD4+ to CD8+ T cells in the colostrum of primiparous cows are significantly less than those of multiparous (> 3 lactations) cows, whereas the percentages of CD8+ T cells and CD8+A2+ T cells (ie, activated CD8+ cells) in colostrum did not differ significantly between primiparous and multiparous cows.[60] The investigators of that study[60] presumed that the lack of exposure to pathogens was the reason primiparous cows had a lower percentage of CD4+ T cells in their colostrum than multiparous cows. In the peripheral blood of neonatal calves, the proportion of γδ+ T cells is greater than the proportion of B cells, but those proportions become reversed approximately 6 to 9 weeks after birth,[60] which suggests the presence of passively acquired cellular immunity. Lymphocyte

subpopulations present in the peripheral blood of cattle change with age,[61] and those changes and the associated alterations in cytokine production may affect the susceptibility of individual cows to diseases such as mastitis during the periparturient period.[60]

In addition to macrophages, milk and colostrum contain lymphocytes and polymorphonuclear and epithelial cells. In cattle, the proportion of T lymphocytes varies from 16% in colostrum to 62% in milk, and the ratio of CD4+ to CD8+ T cells is 0.85 in milk, compared with 1.5 in peripheral blood.[62] The predominate subpopulation of lymphocytes in milk is T cells, whereas B cells account for < 5% of the lymphocyte population,[22,62] and polymorphonuclear cells account for 3% to 26% and epithelial cells account for 2% to 15% of the total cell population.[62–67] Results of a study[51] in which flow cytometric analysis was used to analyze isolated CMCs indicate that 25.4 ± 17.1% of CMCs express CD2 (ie, are T lymphocytes or natural killer cells), 19.5 ± 13.6% of CMCs express CD3 (a pan T-cell marker), 2.9 ± 3.0% of CMCs express CD21 (ie, are B lymphocytes), and 32.7 ± 13.7% of CMCs express CD14 (ie, are macrophages). Those cells are viable in colostrum and have varying cytokine transcript expression patterns.[24,27,68–71]

Immunologic function of colostral cells

Following colostrum ingestion, maternal cells, which have already processed foreign antigens, can cross the intestinal barrier of neonatal calves and induce immune responses against those processed antigens that might not otherwise be induced because of antigen capture and inactivation by IgA, local immune response failure associated with rapid division of or lack of antigen receptors on neonatal intestinal cells, or the presence of antigenic epitopes that differ from those processed by neonatal antigen-presenting cells. Thus, in neonatal calves, antigen presentation by CMCs directs and modulates the immune response to those antigens and promotes the balance between immune tolerance and allergy.[22]

Ingestion of colostrum containing CMCs immediately after birth is critical for stimulation of the naïve immune system of neonatal calves.[72] Results of a study[23] in which neonatal calves were fed CMC-free colostrum or colostrum supplemented with CMCs immediately after experimental inoculation with 10^9 colony forming units of enteropathogenic *Escherichia coli* (within 3 hours after birth) indicate that the blood lymphocyte response to nonspecific mitogens and serum titer of antibodies against enteropathogenic *E coli* were greater for calves fed colostrum with CMCs than for calves fed CMC-free colostrum.

In neonatal calves, blood lymphocyte count increases markedly between 6 and 12 hours after ingestion of colostrum, but a similar increase in blood lymphocyte count is not observed for calves deprived of colostrum.[73] Phagocytosis is also more efficient in colostrum-fed calves than in colostrum-deprived calves.[73] Freezing colostrum results in lysis of colostral leukocytes.[74] Although neonatal calves fed either fresh or frozen colostrum had functional phagocytic leukocytes, neutrophils, and monocytes present in the blood at both 2 and 21 days of age, calves fed frozen colostrum had a higher percentage of activated neutrophils at 2 days of age than calves fed fresh colostrum.[74] The investigators of that study[74] suggested that products of leukocyte destruction such as cytokines and transfer factor may have an important role in early cellular immune function.

Responses to mitogens

The reported response of CMCs to mitogens varies among experimental studies.[75–77] In 1 study,[75] CMCs did not respond to mitogens and antigens as strongly as did PBMCs, possibly because colostrum has a higher proportion of macrophages than does peripheral blood.[74] Similarly, in another study,[76] although incubation of CMCs with concanavalin A (a plant mitogen) resulted in significant in vitro lymphoblastic transformation, the mitogenic response for the CMCs was less than that observed for autologous blood lymphocytes. However, the mitogenic response did not differ between CMCs and PBMCs in yet another study.[77]

The response of CMCs to nonspecific mitogens is believed to be dependent on multiple factors including the existence of nonviable cells that generate toxic factors for other cells in culture, the presence of soluble components in mammary gland secretions capable of blocking receptors involved in mitogen-induced blastogenesis,[77] and the possible existence of suppressive cells or molecules in lymphocyte cultures of lacteal secretions.[9] Some investigators[53] have proposed that macrophage depletion may facilitate detection of lymphocyte proliferation in response to a mitogen in a laboratory setting. Milk of periparturient cows has a preponderance of macrophages and a paucity of lymphocytes, particularly T lymphocytes, and those macrophages may inactivate mitogens before they can stimulate proliferation of colostral lymphocytes capable of clonal expansion.[53,55]

Responses to specific antigens

The ability of specific antigens to elicit an immunologic response in milk or colostral lymphocytes varies.[76,78–83] Significant in vitro blastogenic activity is observed in milk and colostral lymphocytes obtained from cows following natural infection with *Brucella abortus* soluble antigen,[78] intramammary immunization with killed *Mycobacterium bovis*,[84] and parenteral immunization with an adjuvanted modified-live rotavirus vaccine during gestation.[85]

Results of a study[33] involving sheep indicate that maternal lymphocytes in colostrum from ewes exposed to tetanus antitoxin (antigen) remain immunologically active following absorption across the intestinal mucosa of neonatal lambs and are able to transfer immunologic memory to those lambs. Memory T cells transferred from colostrum to the systemic blood circulation of neonatal ruminates likely contribute to the enhanced antigen responsiveness of those animals.

The transfer of live maternal cells from colostrum to neonatal calves enhances the immune responses of those calves to antigens against which the dams had previously responded within the first 24 hours after colostrum ingestion but appear to have no effect on the immune response 7 days after colostrum ingestion.[12] In another study,[53] colostral lymphocytes had low or undetectable virus-specific proliferative responses, a finding that was consistent with results of yet another study.[84] Collectively, the data from those 2 studies[53,84] suggest that responsiveness of milk or colostral lymphocytes to mitogenic and antigenic stimulation is much less than that of blood lymphocytes.

The difference in the proliferative abilities between blood lymphocytes and milk or colostral lymphocytes is likely associated with multiple factors such as differences in the lymphocyte subpopulations in mammary gland secretions and blood,[86,87] the presence of inhibitory factors in mammary gland secretions that may decrease lymphocyte metabolism or induce lymphocyte toxicosis,[88,89] and varying proportions of lymphocytes, macrophages, and other cell constituents in cell suspensions after the isolation procedure. Methods more sensitive than those currently available need to be developed to better elucidate the activity of the low number of maternal antigen-specific T lymphocytes that are transferred via colostrum to neonatal calves.[53]

In a study[72] conducted to evaluate the effects of colostral cells on the immune responsiveness of blood leukocytes in neonatal calves, calves that were fed whole colostrum from their dams developed immune tolerance to maternal cells but retained immune responsiveness to cells from unrelated cows. In a subsequent study,[90] the lymphocytes of calves fed whole colostrum had a higher density of major histocompatibility complex I receptors on their surface during the first week of life, compared with the lymphocytes of calves fed colostrum without colostral cells. Those results indicate that ingestion of colostral cells by neonatal calves facilitates the development of blood lymphocytes, which in turn enhances immune system activation in those calves.[90] For example, calves that received colostrum containing leukocytes and then were experimentally infected with *E coli* shed substantially less bacteria and developed significantly higher serum concentrations of IgA and IgM, compared with similar calves that were fed colostrum from which the leukocytes had been removed.[23]

In another study,[12] neonatal calves were fed whole fresh or frozen colostrum or CMC-free colostrum within 4 hours after birth, and the PBMC responses against BVDV and mycobacterial PPD were assessed in those calves immediately before (baseline) and at 1, 2, 7, 13, 21, and 28 days after colostrum ingestion. The colostrum fed to the calves was obtained from cows that had been exposed to (vaccinated against) BVDV but had no measurable response to PPDs from *M bovis*, *Mycobacterium avium*, and *Mycobacterium avium* subsp *paratuberculosis*. For calves fed whole fresh colostrum, the PBMC response against BVDV was significantly greater than that at baseline 1 day after colostrum ingestion, then steadily declined back to the baseline response by 7 days after ingestion and remained fairly stable for the remainder of the observation period.[12] Conversely, a proliferative PBMC response against BVDV was not identified in calves fed whole frozen or CMC-free colostrum.[12] The PBMC response against the mycobacterial PPDs did not vary among the 3 groups of calves.[12] The investigators of that study[12] concluded that the transfer of live maternal cells from colostrum to neonatal calves enhances the subsequent immune responses of those calves against antigens to which the dam had been exposed but not against antigens to which the dam had not been exposed; thus, passive transfer of cell-mediated immunity to neonatal calves can be enhanced by dam vaccination.

Cellular immune responses to vaccines in calves following ingestion of viable colostral cells

The effect of maternal antibodies in the serum of young calves on the ability of those calves to respond to various vaccines is a subject of ongoing debate and investigation. In 1 study,[53] calves that received colostrum at birth (ie, had maternal antibodies present in their serum) and were parenterally administered a multivalent modified-live virus vaccine at 10 days of age developed virus-specific T lymphocyte responses against the viruses in the vaccine, whereas similar calves that were not vaccinated did not. That finding indicates the cellular immune system of young calves can be primed by parenteral vaccination despite the presence of detectable serum concentrations of maternal antibodies[53] and lack of substantial vaccine-induced antibody production.

The investigators of that study[53] also reported that lymphoid proliferation in response to stimulation by bovine respiratory syncytial virus, bovine herpesvirus type 1, and concanavalin A and expression of interferon γ in the blood of adult cattle and weaned calves were generally lower than those of preweaned calves. This low level lymphoid proliferation in response to mitogens in young weaned calves may be the result of immature immune cell function or a predominance of γδ T lymphocytes, which have a much lower or undetectable proliferative response to mitogens and some specific antigens than αβ T lymphocytes that are the predominate T cells in the blood of adult cattle.[91]

Methods used to obtain and purify bovine colostral cells

Recovering CMCs from a fat-rich environment is not easy, and numerous protocols have been developed to obtain and isolate CMCs for in vitro experiments; however, the repeatability of many of those protocols has not been validated. Therefore, the methodology will be described in general terms. Initially, colostrum was placed in centrifuge tubes and directly centrifuged to obtain a cell pellet.[85] Because

colostrum has a high viscosity, that was a fairly inefficient method for CMC recovery, and subsequent protocols included diluting the colostrum 1:2 or 1:4 with PBS solution prior to centrifugation.[21,23] A more recent protocol requires pouring the colostrum through a gauze pad and then diluting it at a ratio of 3:5 with Dulbecco PBS solution containing 1% fetal calf serum prior to centrifugation.[51] Following centrifugation, the fluid layer is decanted and the cell pellets are washed.[21,51,63,85] Then the cell pellets are resuspended and layered onto various substances for cell isolation by density-gradient centrifugation. The viability of colostrum and milk lymphocytes as determined by a trypan dye exclusion test ranges from 80% to 90% for protocols that use Ficoll-diatrizoate (specific gravity, 1.084 g/mL) for gradient-density centrifugation.[53,81] Cell isolation by use of Ficoll-Conray (specific gravity, 1.085 g/mL) for gradient-density centrifugation has also been described.[69] A protocol for isolating bovine CMCs for phenotyping and functional studies involves recovery of the cellular fraction from a reduced volume of colostrum and centrifugation with a commercial column, which increases the feasibility of removing the lipid layer.[51] In our laboratory, we have achieved a 17% increase in the yield of viable lymphocytes and macrophages from colostrum and milk by use of a Percoll method, compared with a Ficoll-Hypaque method,[71] and our yields of viable cells are consistent with those reported for colostrum and milk by investigators of other studies.[92–94] The Percoll protocol uses 2 gradients (43% and 70%), which likely aids in the recovery of purified cells from the fatty colostral environment and helps preserve cell viability.

For many protocols, the mean viability of cells isolated from colostrum and milk is approximately 85%. However, only 1% to 2% of colostral lymphocytes are typically recovered, perhaps because many lymphocytes become entrapped in the fatty component of colostrum and are discarded with the supernatant after the first centrifugation step.[75,76] The low recovery rate of colostral lymphocytes may also be associated with the altered buoyant density of lipid-layer colostral leukocytes, which is responsible for the differential migration of colostral lymphocytes relative to blood leukocytes.[90]

Most CMC isolation protocols have successfully yielded viable cells for use in proliferation experiments[12,53,72,85]; however, protocols for isolating CMCs for use in flow cytometry have not been described. To our knowledge, only the CMC isolation protocol described by Meganck et al[51] has been successfully used to phenotype those cells. Nevertheless, many aspects regarding optimal methods for isolating and purifying bovine colostral cells remain unclear.

Conclusion

The importance of passive transfer of maternal antibodies from colostrum to the circulation of neonatal calves in the protection of those calves against disease during the first weeks of life is well recognized, but evidence is mounting that the cellular fraction of colostrum also has an important role in supporting the neonatal immune system. Thus, simple measurement of the serum immunoglobulin concentration of neonatal calves may not accurately reflect the extent of passive immunity transferred from the dam to the calf via colostrum. Viable maternal leukocytes that are absorbed from colostrum into the neonatal circulation are immunocompetent[94,95] and can prime immune responses and enhance T-cell activation, which facilitate antibody production.[96]

Investigation of the cellular fraction of colostrum is challenging because sufficient amounts of colostrum are not always available for study and isolation of cells from colostrum is labor intensive and time-consuming.[51] Although multiple studies have been performed to determine the proportion of lymphocytes and macrophages within the CMC population, the results of those studies vary, likely because of differences among studies in regard to the definition of colostrum,[85] health status of the mammary glands from which milk or colostrum specimens were obtained,[97] stage of lactation,[98] techniques used to identify CMCs,[76] and gating strategy used.[50]

In 2006, Reber et al[8] proposed a model for the trafficking and function of maternal cells in neonatal calves. In that model, after colostrum is ingested, maternal leukocytes are absorbed across the intestinal wall, enter the neonatal circulation, and home to peripheral nonlymphoid and secondary lymphoid tissues. That model was consistent with results of an earlier study[99] in which maternal leukocytes disappeared from the peripheral blood circulation and were detected in neonatal mesenteric lymph nodes, Peyer patches, and spleen within 24 hours after colostrum ingestion. The investigators of that study[99] suggest that the reason maternal leukocytes disappear from the peripheral circulation is because they are sequestered in neonatal tissues and secondary lymphoid tissues where they secrete cytokines that promote the development of the immune system.[23,71,100] Results of another study[72] indicate that the immune system development for calves fed cell-free colostrum is 1 to 2 weeks slower than that of calves fed whole fresh colostrum, possibly because the cell-free colostrum did not contain any maternal leukocytes. Compared with maternal leukocytes, neonatal leukocytes are naïve and have low receptor expression, have suboptimal cytokine production, and may be unresponsive to antigen stimulation, all of which are associated with physiologic stress. Calves fed cell-free colostrum also have more CD11a+ lymphocytes in the peripheral blood during the first 2 weeks of life than do calves fed whole colostrum.[42] An α-chain integrin, CD11a is involved in leukocyte adhesion and costimulatory signaling. Upregulation of CD11a is an indication of systemic inflammation or subclinical disease; therefore, calves deprived of maternal leukocytes are more susceptible to environmental stress

and infectious agents than calves not deprived of maternal leukocytes.[42] It is possible that the transfer of memory T cells (ie, maternal leukocytes) via colostrum enhances the immune system responsiveness of neonatal calves against viruses and other pathogens during the first couple days of life; thus, vaccination of the dam may represent a promising strategy to improve the passive transfer of antibodies and memory T cells against specific pathogens.

The purpose of this report was to review the function of bovine colostral cells to enhance understanding of its role in neonatal calf immunity. Currently available scientific literature provides unequivocal evidence that the cellular component of colostrum has a role in immune system development of neonatal calves; however, it has not been studied as extensively as the humoral component. Further studies are necessary to determine the quantity and phenotype of CMCs to better elucidate the role of whole colostrum on immune system function of neonatal calves.

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