Effects of the ingestion of whole colostrum or cell-free colostrum on the capacity of leukocytes in newborn calves to stimulate or respond in one-way mixed leukocyte cultures

Adrian J. Reber, PhD; Arnold R. Hippen, PhD; David J. Hurley, PhD

Objective—To evaluate effects of colostral cells on the ability of neonatal leukocytes to respond in a mixed leukocyte response (MLR) as a means of evaluating specific immune responsiveness.

Animals—10 Holstein calves, their respective dams, and 10 unrelated adult Holstein cows.

Procedure—Soon after birth, their calves were fed maternal whole colostrum or colostrum after cells were removed by centrifugation. Responses for leukocytes obtained from calves during the first 5 weeks after birth, their dams, and unrelated cows were measured by use of 1-way MLR as an indicator of immune development. An internal control treatment, proliferation of lymphocytes stimulated with *Staphylococcus* enterotoxin B (SEB), was also measured.

Results—Transfer of colostral leukocytes had a significant effect on the MLR and SEB-induced response in calves. Calves receiving whole colostrum had enhanced responses to maternal and unrelated leukocytes 24 hours after ingestion of colostrum. These responses decreased quickly, indicating direct modulation of the neonatal immune response. Calves receiving whole colostrum effectively stimulated the MLR by 24 hours after ingestion of colostrum. In contrast, calves receiving acellular colostrum did not effectively stimulate the MLR until 2 to 3 weeks after birth.

Conclusions and Clinical Relevance—Ingestion of maternal colostral leukocytes immediately after birth stimulates development of the neonatal immune system. These maternal leukocytes enhance development of antigen-presenting capacity as indicated by their ability to stimulate the MLR and SEB response. The influence of ingested maternal cells on neonatal immunity was also indicated by a reduction in reactivity of neonatal cells to maternal alloantigens. (*Am J Vet Res* 2005;66:1854–1860)

Although the immune system of neonatal calves is immature, it is capable of mounting immune responses at the time of birth.[1,2] The immune system develops during the fetal period[3] inside the protected environment of the placenta without external antigenic stimulation.[4,5] At birth, newborn calves are thrust into an environment filled with pathogens without the benefit of an immune system developed to specifically respond to the local environment because the specific cues for immune development in the fetus are from the normally sterile environment of the uterus. Fortunately, the dam continuously mounts specific immune responses to all types of organisms found in the local environment and provides transfer of that immunity to the calf in the form of colostrum. Transfer of colostrum takes on an important role in cattle because there is not a substantial transplacental transfer of immune components during fetal development. Colostrum contains high amounts of nutrients and developmentally important hormones. In addition, colostrum also contains important immunologic components including cytokines, maternal antibodies, and substantial numbers of maternal leukocytes. The antibody component of bovine colostrum is dominated by IgG (approx 75 mg/mL), which is almost exclusively IgG_1.[6] Additionally, much lower concentrations of IgA and IgM (approx 4.4 and 4.9 mg/mL, respectively) are found in the antibody component of colostrum.[6] The leukocyte component of colostrum is composed primarily of macrophages, which comprise 40% to 50% of the colostral leukocytes.[7-9] The remaining colostral leukocytes consist of lymphocytes (22% to 25%) and neutrophils (25% to 37%).[7-9] The colostral lymphocytes consist of B cells (2.5% to 3.5%), T cells (88% to 89%), and natural killer cells (5% to 15%).[9] The benefits of feeding maternal colostrum are recognized. However, protection of neonates has traditionally been ascribed only to transfer of maternal antibody without regard to the potential benefits of colostral leukocytes or other colostral components.

Individuals of the same species differ in that they have variable sets of genes that regulate the immune system. These genes are the primary drivers of the recognition of foreign tissue during transplantation. Thus, the complex of these genes has been named the **major histocompatibility complex (MHC)**, and MHC antigens are often referred to as alloantigens. The transfer of cells from an individual into an incompatible recipient results in the stimulation and expansion of lymphocytes to nonself alloantigens. In a typical host-versus-graft reaction, host lymphocytes migrate to the grafted organs, where they respond to the MHC antigens on the cells of the graft. These lymphocytes proliferate and lyse cells bearing the allogenic MHC, which results in destruction of the graft.

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From the Departments of Biology and Microbiology (Reber, Hurley) and Dairy Science (Hippen), College of Agriculture and Biological Sciences, South Dakota State University, Brookings, SD 57007. Dr. Reber's and Dr. Hurley's present address is Food Animal Health and Management Program, Department of Large Animal Medicine, College of Veterinary Medicine, University of Georgia, Athens, GA 30602.
Supported by the USDA Hatch Project SD00208-H and USDA Animal Health Formula Funds Project GEOV-0320.
Address correspondence to Dr. Hurley.

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A study[10] published in 1975 offers the first evidence that colostral cells influence neonatal immune responses; those investigators reported differences in the rejection of skin allografts by rats fed maternal colostrum, compared with results for cross-fostered rats. Since that initial report, the physical transfer of colostral leukocytes into neonatal circulation has been documented in several species, including pigs,[11] sheep,[12,13] cattle (unpublished data), and baboons.[14]

In that original study,[10] investigators also reported that newborn Fischer × Dark Agouti, F_1-generation rats nursed by their Fischer dams rejected allografts of ear skin from Lewis rats. In contrast, the newborn rats did not reject the allografts of ear skin from Lewis rats when they suckled Lewis or Fischer × Lewis dams immediately after birth. Other investigators conducted a study[15] on the effects of transferred colostral leukocytes by use of congenitally athymic nude mice that are naturally deficient in T lymphocytes. In that study,[15] BALB/c nude mice that suckled on C57BL/6 dams developed a wasting syndrome (consistent with a graft-vs-host response) indicative of transferred colostral leukocytes. These nude mice succumbed to the wasting syndrome because they lacked the T cells necessary to eliminate transferred alloreactive colostral leukocytes. None of the mice survived beyond 35 days of age, whereas BALB/c nude mice nursed by their BALB/c dams survived significantly longer with no significant mortality prior to weaning. Furthermore, investigators in that study[15] documented that resistance to a transplantable interstitial cell (ie, Leydig cell) tumor could be transferred to susceptible C57BL/6 mice by suckling on resistant A-strain mice.

To investigate the effects of transferred cellular immunity on neonatal immune responses, we examined the effects of maternal colostral leukocytes on a **mixed leukocyte response** (**MLR**; ie, response to alloantigens) test conducted by use of leukocytes obtained from neonatal calves. Immediately after birth, calves were fed whole colostrum containing maternal leukocytes or colostrum from which the cells had been removed (**cell-free colostrum [CFC]**). The response of calves to maternal and unrelated leukocytes was measured periodically during the first 5 weeks after birth by use of 1-way mixed leukocyte cultures. Polyclonal stimulation of T-lymphocyte proliferation was also monitored by use of the superantigen ***Staphylococcus* enterotoxin B (SEB)** as a proliferation-specific control sample for the culture system and an indicator of the development of the response capacity of T lymphocytes in the neonatal calves.

Materials and Methods

Animals—Ten neonatal Holstein calves and their respective dams were used in this study. The calves and dams were part of a university dairy research herd located at South Dakota State University. An additional 10 unrelated adult Holstein cows from the same herd were also used in the study. All studies and procedures were reviewed and approved by the Institutional Animal Care and Use Committee of South Dakota State University (approval No. 98-A024).

Soon after birth, 5 neonatal calves were bottle-fed fresh, whole colostrum obtained from their dams. The other 5 neonatal calves were bottle-fed CFC derived from colostrum obtained from their dams; the CFC was derived by use of centrifugation to remove cells. Briefly, colostrum was collected from each dam of the calves that were to receive CFC. The colostrum was centrifuged at 1,500 × *g* at 26°C for 40 minutes. The soft lipid layer at the top of each tube and supernatant were carefully removed, and the cell pellet was discarded. The lipid layer and supernatant were mixed and double bagged in closable plastic storage bags.[a] The bags were placed between frozen stainless-steel plates, and the mixture was snap-frozen at –80°C to destroy any remaining cells. The frozen CFC was initially thawed in a water bath at 50°C until it attained a slushy consistency. It was then placed in a water bath at 37°C until completely thawed; it was subsequently fed to the 5 neonatal calves. Each calf consumed at least 2 L of colostrum or CFC within 6 hours after birth. All calves were subsequently fed milk replacer after the initial feeding of colostrum or CFC.

The 10 unrelated adult Holstein cows from the same herd were selected prior to birth of the calves. Unrelated adult cows were selected on the basis of high values for an MLR test between the dams and unrelated cows (leukocytes from each cow had substantial proliferation in an MLR test). These high MLR values were considered to be an indication of differences in MHC class II antigens between the dams and unrelated cows and, by extrapolation, an indication of probable differences in MHC antigens between the calves and unrelated cows.

Procedure—Mononuclear cells were used to evaluate the MLR between the neonatal calves and their dams. The 10 unrelated adult Holstein cows from the same herd were used to determine the responses of the calves to MHC antigens of unrelated cows. Blood samples (10 mL) were obtained from the jugular vein of each calf and cow at the time of parturition (before feeding colostrum), 24 hours after birth, and at weekly intervals thereafter for the next 5 weeks to enable us to measure proliferative responses and the capacity of the cells as MLR stimulators.

Isolation of leukocytes—Peripheral blood mononuclear leukocytes were isolated from venous blood samples by use of single-step density separation. Needles (18 gauge) were attached to sterile 60-mL syringes for use as a controlled layering device. These were placed at a slant into the mouth of 50-mL centrifuge tubes containing 10 mL of a single-density gradient (1.083 g/mL).[b] Heparinized blood was diluted (1:4) in PBS solution, then placed slowly in syringes in 40-mL aliquots, which allowed the blood to form layers over the density gradient. Tubes were centrifuged at 800 × *g* for 45 minutes. The mononuclear cell layer was removed from the interface between the diluted plasma and density gradient; we were careful to avoid disturbing the RBC layer. Mononuclear cells were pelleted by centrifugation at 800 × *g* for 15 minutes. Supernatant was decanted, and the cells were washed with PBS solution containing 0.5% bovine serum albumin.[c] The cells were centrifuged twice at 400 × *g* for 5 minutes and washed in sterile PBS solution containing 0.5% bovine serum albumin. Cells were counted by use of a hemacytometer[d] and suspended at a concentration of 2×10^7 cells/mL.

Evaluation of 1-way MLR tests—Peripheral blood mononuclear cells from the calves, dams, and unrelated cows were used in the MLR test. Samples obtained from each animal were divided into 2 equal portions. One portion of each sample was diluted to the working concentration (2×10^6 cells/mL) in standard medium (RPMI medium[e] containing 10% fetal bovine serum,[f] 2mM L-glutamine,[g] 1mM sodium pyruvate,[h] and gentamicin sulfate[i] [50 µg/mL]). These cells were used as responder cells. The other portion of

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each sample was treated by incubation with mitomycin C[j] for 1 hour to inhibit cellular proliferation; these cells were used as stimulator cells.

A solution of mitomycin C (0.1 mg/mL) was prepared in RPMI medium[c] containing 5×10^{-5}M β-mercaptoethanol. A volume of the mitomycin C solution equal to the sample volume was added to each of the samples of stimulator cells and mixed thoroughly. Cells were incubated for 1 hour at 37°C. Stimulator cells were then centrifuged ($400 \times g$) and washed 3 times with PBS solution containing 5% bovine serum albumin. Stimulator cells were adjusted to a concentration of 2×10^6 cells/mL in standard media.

Combinations of responder cells (100 µL) and stimulator cells (100 µL) were prepared in quadruplicate wells of a round-bottom 96-well plate and incubated at 37°C and 5% carbon dioxide (**Appendix**). Cells were harvested onto filter paper on day 5 of incubation; wells were pulsed with 10 µL of ^{3}H-thymidine[k] (20 µCi/mL; specific activity, 6.7 Ci/mM) 6 hours before harvesting, and specific incorporation was determined by use of a scintillation counter.

Proliferation in response to SEB—A superantigen (ie, SEB) was used as a broad-spectrum stimulator to monitor the proliferative capacity of neonatal T lymphocytes and as a control test for the effectiveness of the mitomycin C treatment. The SEB was used at a concentration of 1 µg/mL.

Data analysis—All calf data were adjusted relative to the proliferation measured in assays of the adult cows as 1-way MLR values or in response to SEB. Mean of the responses for the dam and an unrelated cow was calculated for each set of measurements as a reference response used to adjust the data for each calf in each set of measurements. Treatment differences were analyzed at each time point by use of the Student *t* test. Values were considered significant at $P < 0.05$. Outlying data points were determined by use of the Q test[16] and rejected from further statistical analysis.

Results

MLR and SEB responses of adult cells—The mean of the responses for the dam and an unrelated cow was calculated for each set of measurements and used to adjust the data for each calf in each set of measurements. The mean response of the dams and unrelated cows for the MLR was 7,083 ± 605 and for stimulation with SEB was 24,634 ± 1,268.

MLR of adult cells to neonatal cells—Cells obtained from adult cows did not mount a substantial MLR against cells obtained from neonatal calves at the time of birth (**Figure 1**). The responses of dams and unrelated cows to leukocytes from calves fed whole colostrum increased steadily during the first 3 weeks after birth and reached values similar to the adult reference MLR of 100%. The responses by dams decreased at the fifth week after calves were fed colostrum. There was little difference observed between the responses of the dams and unrelated cows to neonatal cells obtained from calves that received whole colostrum, except at the 5-week time point. In contrast, there was a prolonged lack of response by cells from the dams and unrelated cows to the cells from calves that received CFC. This failure to respond persisted for 1 to 2 weeks. The response of unrelated cows to neonatal cells increased to 122% of the reference response at 3 weeks, and the response was maintained at or near 100% through the fifth week. The responses of maternal cells to cells from calves receiving CFC increased to 21% of the reference MLR at 2 weeks after birth. The responses remained at this value throughout the remainder of the study period. In comparison to cells from calves that received CFC, cells from calves that received whole colostrum were significantly better stimulators of the MLR by peripheral blood mononuclear leukocytes from dams or unrelated cows at 1 week after feeding of colostrum. The response also developed more quickly (25% to 35% of the reference response at 1 week after birth) in calves fed whole colostrum.

Response of neonatal cells to SEB—Cells obtained from all calves immediately after birth were highly responsive to SEB (**Figure 2**). This increase in responsiveness persisted through 1 week after birth but then decreased over time. The response of leukocytes obtained from neonatal calves to SEB reached values similar to those for the response of leukocytes from adult cattle at 2 weeks after feeding of colostrum and was maintained through the fifth week. Leukocytes from calves receiving whole colostrum had an increase in proliferative response to SEB for a brief period at 3 weeks after feeding of colostrum.

MLR of neonatal cells to cells from dams—The initial MLR for cells of calves obtained before colostral feeding to cells from dams was similar for all calves test-

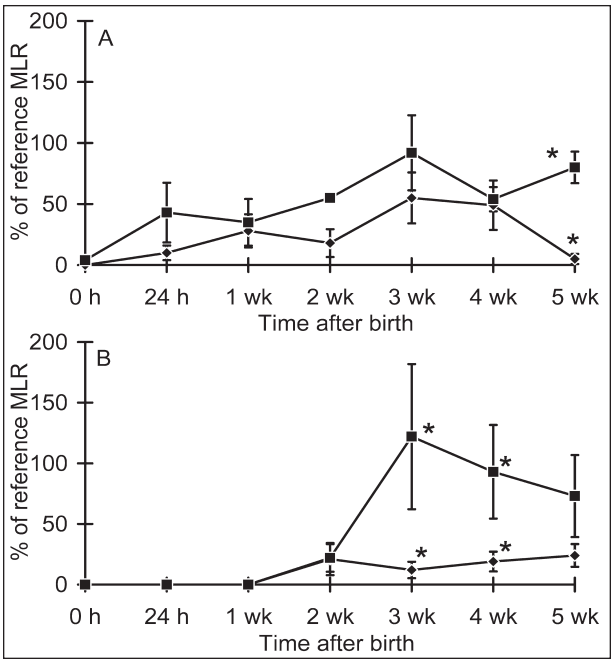

Figure 1—Mean ± SEM results of a 1-way mixed leukocyte response (MLR) for cells obtained from adult cows and stimulated with leukocytes obtained from 5 calves that received whole colostrum (A) or 5 calves that received cell-free colostrum (CFC; B) within 6 hours after birth. Values reported represent the percentage of a reference MLR (mean for 2 adult cows) induced by stimulation of cells from the 10 dams (diamonds) or cells from 10 unrelated cows (squares) with stimulator cells obtained from neonatal calves at various time points during the first 5 weeks after birth. Time of birth of a calf was designated as time 0. Notice that leukocytes from calves that were deprived of maternal colostral leukocytes (ie, fed CFC) failed to stimulate the MLR during the first 1 to 2 weeks after birth. *Within a time point, values differ significantly ($P < 0.05$) between leukocytes from dams and unrelated cows.

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ed (mean, 5% of the reference MLR; **Figures 3 and 4**). The MLR of leukocytes from neonatal calves receiving whole colostrum to cells obtained from the dams peaked (100% of reference MLR) at 24 hours after feeding of colostrum. The MLR for leukocytes from calves receiving whole colostrum decreased at 1 week after feeding and remained near 0 throughout the rest of the 5-week study period. Mean ± SEM MLR for these calves during weeks 1 through 5 was 11 ± 4% of the reference MLR. The MLR of leukocytes obtained 24 hours after birth from calves receiving CFC was not significantly different from the response for leukocytes obtained at birth from those same calves, but it increased steadily during the 5-week period. Mean MLR of the leukocytes for these calves was 39 ± 14% of the reference MLR for weeks 2 through 5. Maternal cells in the colostrum appeared to induce a state of tolerance to maternal antigen in calves fed whole colostrum; however, differences in responses between leukocytes from calves fed whole colostrum or CFC were not significantly different over time ($P = 0.2$ and $P = 0.1$ at weeks 3 and 4 after feeding, respectively). A significant ($P = 0.02$) difference in the response of leukocytes from calves fed whole colostrum or CFC was observed only at 24 hours and 5 weeks after feeding.

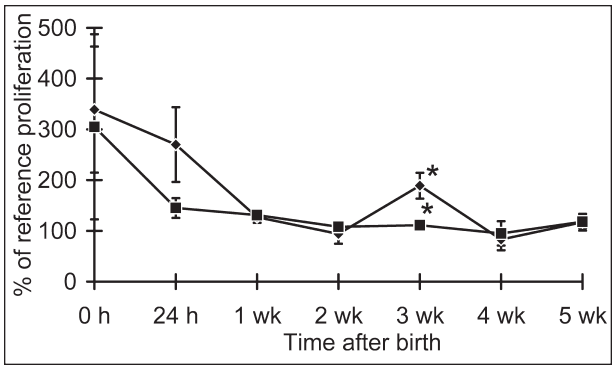

Figure 2—Mean ± SEM results for the ability of leukocytes obtained from 5 calves that received whole colostrum (diamonds) or 5 calves that received CFC (squares) within 6 hours after birth to respond to the T-cell superantigen *Staphylococcus* enterotoxin B (SEB). Time of birth of a calf was designated as time 0. Values reported represent the percentage of a reference SEB response (mean for 2 adult cows) that was used as an internal reference for each experiment. Notice that leukocytes from all calves were highly responsive to SEB immediately after birth but that the responsiveness decreased over time and was similar to values for leukocytes from adult cows (ie, 100%) by 2 weeks after birth. *Within a time point, values differ significantly ($P < 0.05$) between leukocytes from calves receiving whole colostrum and calves receiving CFC.

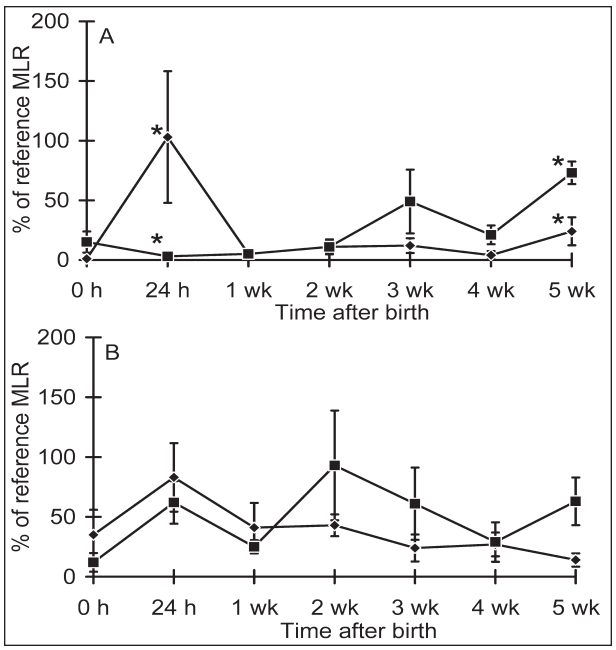

Figure 3—Mean ± SEM results for the ability of leukocytes obtained from 5 calves that received whole colostrum (diamonds) or 5 calves that received CFC (squares) within 6 hours after birth to proliferate in response to stimulator cells obtained from the dams (A) or unrelated cows (B). Time of birth of a calf was designated as time 0. Values reported represent the percentage of a reference MLR (mean for 2 adult cows). Notice that the MLR of leukocytes from neonatal calves fed whole colostrum mounted peak responses against maternal alloantigens at 24 hours and 5 weeks after birth that were significantly ($P < 0.05$) higher than for calves receiving CFC. A similar difference was observed at 5 weeks after feeding.

MLR of cells from neonatal calves to cells from unrelated cows—The peak MLR for leukocytes obtained from neonatal calves receiving whole colostrum to leukocytes obtained from unrelated cows was detected at 24 hours after feeding (Figures 3 and 4). The MLR at 24 hours was 83% of the reference MLR. The MLR for leukocytes obtained from calves receiving whole colostrum to leukocytes obtained from unrelated cows decreased to 41% of the reference MLR by 1 week after feeding and continued to decrease through

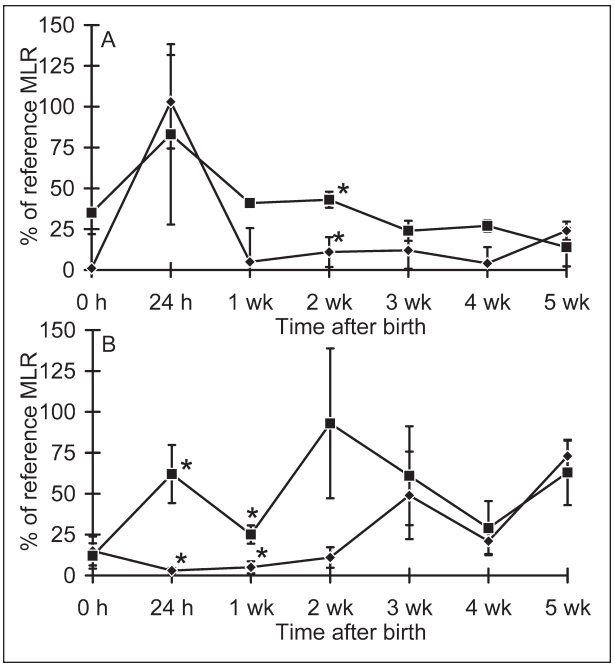

Figure 4—Mean ± SEM results of a 1-way MLR for leukocytes obtained from 5 calves that received whole colostrum (A) or 5 calves that received CFC (B) within 6 hours after birth; leukocytes were stimulated with cells obtained from the dams (diamonds) or from unrelated cows (squares). Time of birth of a calf was designated as time 0. Values reported represent the percentage of a reference MLR (mean for 2 adult cows). The differences in results for the 1-way MLR revealed differing patterns during the first 5 weeks after birth for leukocytes from the calves receiving whole colostrum and calves receiving CFC.

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week 5. Mean ± SEM MLR of leukocytes obtained from these calves to leukocytes obtained from unrelated cows was 30 ± 5% of the reference MLR for weeks 1 through 5. Leukocytes obtained from calves receiving CFC had a nonsignificant increase in responsiveness to leukocytes obtained from unrelated cows during the first 2 weeks after birth, including a peak in response to leukocytes from unrelated cows at 24 hours after birth. A peak response of 100% of the reference MLR was observed at 2 weeks. This response decreased to approximately 50% of the reference MLR between weeks 3 through 5, with the lowest response observed at week 4. This pattern appears to be characteristic of the pattern of lymphocyte recirculation in neonates.[17-19]

Discussion

The transfer of maternal leukocytes into the circulation and tissues of neonates via colostrum has been documented in several species. However, the role these leukocytes play and their effects on the development and function of the naïve immune system of neonates are poorly understood.

Calves receiving CFC developed the ability to stimulate an MLR at a much slower rate than did calves receiving whole colostrum. Leukocytes from calves fed CFC did not appear to be good stimulators of an MLR during the first 2 weeks after birth. Cells from calves fed CFC were not capable of stimulating an MLR until the second week after feeding. This indicated an important physiologic role of colostral cells in the development of neonatal responses to antigens, specifically the development of **antigen-presenting cells (APCs)** responsible for the initiation of specific immune responses.

The poor response of cells from the adult cows to cells obtained from the calves at birth suggested that the neonates were deficient in circulating APCs that could express sufficient amounts of MHC class II antigen to stimulate a substantial MLR. The primary target for an MLR is allogenic MHC class II antigens. This receptor is typically found in high concentrations on the surface of APCs, with the highest expression found on dendritic cells. Leukocytes from calves receiving whole colostrum (ie, maternal colostral cells) appeared to be better stimulators of adult leukocytes by 24 hours after feeding, with increasing activity through the second week after feeding. This increased response to neonatal leukocytes indicated that maternal cells influenced the development of mature circulating APCs in the neonatal calves.

On the surface, this finding would seem to be somewhat of a paradox in that leukocytes from all the neonatal calves mounted a substantial proliferative response to SEB, which is also an MHC class II–dependent response.[20] In combination, analysis of these 2 findings suggests that it would be prudent to measure the differences in MHC class II expression at birth and after colostrum ingestion in calves fed whole colostrum or CFC to establish the role of maternal cells in modulation of neonatal MHC class II expression. In another study,[21] investigators indicated that calves have an extremely low degree of MHC class II expression at birth, and the fraction of cells positive when tested for MHC class II expression increases rapidly after ingestion of colostrum (within 1 to 4 hours), but this response was delayed in colostrum-deprived calves. This observation provides insight into a possible mechanism for the difference in MLR stimulator activity of cells from calves fed CFC being correlated with delayed induction of a critical number of cells with MHC class II expression. It is also possible that maternal cells provide sufficient MHC class II alloantigen to facilitate SEB responses but do not provide sufficient MHC class II alloantigen to drive an MLR. The MLR is quantitatively related to the formation of an MLR mismatch conjugate, but MHC class II binding of SEB is almost catalytic in the initiation of proliferation.[22]

In unpublished experiments conducted by our laboratory group, we observed that maternal colostral leukocytes began to enter the neonatal peripheral blood at approximately 12 hours after feeding of colostrum and peaked in the neonatal circulation at approximately 24 hours after feeding. This time period was associated with improved alloantigen and SEB responses and the capacity to stimulate an MLR for all calves receiving whole colostrum in the study reported here (Figure 4). This suggests an important role for maternal leukocytes in development of neonatal immune responses. Leukocytes of neonatal calves had enhanced responses to MLR and superantigen stimulation at 24 hours after feeding of colostral leukocytes.

The peak response of neonatal leukocytes to maternal MHC antigens was observed at 24 hours after calves were fed colostrum. This indicated an important interaction between the maternal cells and neonatal immune system. At that time, maternal cells exerted their maximal immunomodulating effects on the neonatal immune system. The maternal cells exerted developmentally important effects on the neonatal immune responses and appeared to induce tolerance to maternal alloantigens. This tolerance to maternal alloantigens was observed by 1 week after feeding as indicated in the reduced responsiveness to maternal cells for leukocytes from calves fed whole colostrum. This reduced responsiveness persisted throughout the remainder of the study. Calves receiving CFC had a slowly increasing response to maternal cells from weeks 1 through 5. These calves were deprived of immediate stimulation by maternal cells. Therefore, the increasing response was indicative of development of the neonatal immune system without the modulating effects of transferred maternal colostral leukocytes.

Leukocytes of neonatal calves obtained during the first week after birth were highly responsive to superantigen stimulation. This response to SEB decreased to values for adult leukocytes by 2 weeks after feeding of colostrum. Stimulation with SEB indicated that maternal colostral cells had little impact on the overall responsiveness of neonatal T cells to a nonspecific stimulator. However, analysis of our data supports a role for maternal colostral leukocytes in the development of the antigen-presenting capacity in neonates. Effective antigen presentation is critical for the initiation of an effective antigen-specific response. Therefore, even if maternal colostral cells have little

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direct impact on the responsiveness of neonatal T cells, the enhanced antigen-presenting capacity of these calves would still be reflected in an improved capacity to initiate antigen-specific T-cell responses.

A partial explanation of patterns seen in the results of the study reported here is evident in the parallels between the reported distribution patterns of leukocytes in the peripheral blood of neonates and the pattern of proliferative responses we observed. Similar patterns of leukocyte circulation in the bloodstream of neonatal calves have been documented by several researchers,[17-19] who observed a depletion of circulating leukocytes from the bloodstream of neonatal calves at 1 week of age. The number of circulating cells peaked between 2 and 3 weeks after birth, followed by a slight decrease during the fourth week after birth. Similar patterns were observed in the number of circulating neutrophils and mononuclear cells.[18,19] The same pattern of circulation was detected in neonatal pigs by use of monoclonal antibodies for pan T lymphocytes and T-cytotoxic and T-helper subpopulations.[23] Our results reflect similar changes in the pattern of circulating leukocytes as determined on the basis of changes observed in cell function. The responses of leukocytes from dams and unrelated cows to leukocytes from calves fed whole colostrum revealed an increased response at 2 and 3 weeks after colostral feeding (Figure 1). These results reflected the distribution of good stimulator cells (mature APCs) in the blood of calves. This pattern was also seen in the response to SEB for leukocytes obtained from neonates fed whole colostrum (Figure 2). This pattern of leukocyte distribution strongly supports our interpretation of the role of colostral leukocytes in development of neonatal immune responses. These observations provide substantial support for the role of maternal colostral leukocytes in stimulating the development of neonatal immune responses.

The role of maternal colostral leukocytes in protection of neonatal animals has been ignored for some time. Analysis of our results indicates colostral leukocytes are in the neonatal circulation and exert a developmental influence on neonatal immune responses. The APCs in calves fed whole colostrum appeared to develop more quickly, compared with the development of APCs in calves fed CFC. Responses to MHC antigens of dams and unrelated cows were enhanced at 24 hours in leukocytes of calves receiving whole colostrum. This was followed by a state of tolerance to maternal MHC antigens in calves receiving whole colostrum.

Although neonatal calves are born with an immune system that is poorly developed, the dams are able to provide for the development of immune protection. The role of maternal antibodies in protection of neonates cannot be denied. However, preformed antibodies are not adaptable after transfer to the neonates. The effects exerted by maternal colostral leukocytes are important for the health of neonates and in development of neonatal immune responses. By stimulating the development of the neonatal immune system, colostral leukocytes enhance the ability of neonates to provide protection at a time when they are most vulnerable to infectious agents.

a. Ziploc bags, SC Johnson & Son Inc, Racine, Wis.
b. Histopaque 1083, Sigma Chemical Co, St Louis, Mo.
c. Albumin, bovine fraction V solution (7.5%), Sigma Chemical Co, St Louis, Mo.
d. Bright-Line hemacytometer, Hausser Scientific, Horsham, Pa.
e. RPMI 1640, Mediatech Inc, Herndon, Va.
f. Premium Select, Atlanta Biological, Norcross, Ga.
g. L-glutamine, Sigma Chemical Co, St Louis, Mo.
h. Sodium pyruvate solution, Sigma Chemical Co, St Louis, Mo.
i. Gentamicin sulfate, Sigma Chemical Co, St Louis, Mo.
j. Mitomycin C from *Streptomyces caespitosus*, Sigma Chemical Co, St Louis, Mo.
k. Thymidine (methyl-^{3}H), ICN, Irvine, Calif.

Appendix

Combinations of leukocytes used to measure the mixed leukocyte response (MLR) and response to stimulation with the superantigen *Staphylococcus* enterotoxin B (SEB) for cells obtained from 10 neonatal Holstein calves during the first 5 weeks after birth, dams of those calves, and 10 unrelated adult Holstein cows.

Test	Neonatal calves	Dams	Unrelated cows
MLR	C × C′	M × M′	U × U′
	C × M′	M × C′	U × C′
	C × U′	M × U′	U × M′
Superantigen stimulation	C + SEB	M + SEB	U + SEB
	C′+ SEB	M′+ SEB	U′+ SEB

C = Leukocytes from neonatal calves. C′ = Leukocytes from neonatal calves treated by use of mitomycin C to block proliferation in the assay. M = Maternal leukocytes (ie, leukocytes from the dams). M′ = Maternal leukocytes treated by use of mitomycin C to block proliferation in the assay. U = Leukocytes from unrelated cows. U′ = Leukocytes from unrelated cows treated by use of mitomycin C to block proliferation in the assay.

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