Effect of maternal cells transferred with colostrum on cellular responses to pathogen antigens in neonatal calves

Douglas C. Donovan, PhD; Adrian J. Reber, PhD; Jon D. Gabbard, BS; Maria Aceves-Avila, DVM; Kimberly L. Galland, BS; Katheryn A. Holbert; Lane O. Ely, PhD; David J. Hurley, PhD

Objective—To assess the effect of maternal cells or cellular components on neonatal immune responses to intracellular pathogens in calves.

Animals—15 Holstein calves.

Procedures—Calves were fed whole colostrum, frozen colostrum, or cell-free colostrum within 4 hours after birth. Leukocytes were obtained from calves before feeding colostrum and 1, 2, 7, 14, 21, and 28 days after ingestion. Proliferative responses against bovine viral diarrhea virus (BVDV) and mycobacterial purified protein derivatives were evaluated. Dams received a vaccine containing inactivated BVDV, but were not vaccinated against mycobacterial antigens.

Results—All calves had essentially no IgG in circulation at birth, but comparable and substantial concentrations by day 1. Calves that received whole colostrum had enhanced responses to BVDV antigen 1 and 2 days after ingestion of colostrum. In contrast, calves that received frozen colostrum or cell-free colostrum did not respond to BVDV. No differences were identified among the 3 groups in response to mycobacterial antigens.

Conclusions and Clinical Relevance—Results indicated that transfer of live maternal cells from colostrum to neonatal calves enhanced responses to antigens against which the dams had previously responded (BVDV), but not to antigens to which the dams were naïve (mycobacterial purified protein derivatives). Results suggested that cell-mediated immune transfer to neonates can be enhanced by maternal vaccination. (*Am J Vet Res* 2007;68:778–782)

The thickness and density of the bovine placenta prevent transfer of cells and immunoglobulin from the cow to the calf before birth. As a result, the calf is immunologically naïve at birth, and maternal transfer of passive immunity and immune priming by the cow must occur via ingestion of colostrum. Failure to transfer colostral components from the cow to the calf within a few hours of birth has important negative consequences on the health and development of the calf.[1] Furthermore, insufficient transport of immunologic components across the neonatal intestine, characterized by low serum concentrations of maternal IgG in the calf after suckling, is also detrimental.[2]

Producers routinely feed frozen colostrum collected from cows on their premises to aid in passive transfer of antibodies against pathogens in the local environment. Extra colostrum is routinely frozen and stored for up to 1 year.[3] The benefits of frozen colostrum over the use of milk replacer or cheese-whey–derived antibody replacement products have been documented.[4,5]

There is emerging evidence that antibody transfer to newborn animals, although important and relatively easy to assess, is not the sole constituent of transferred maternal immunity. Cells, cytokines, and proteins (such as lactoferrin) directed against microbial pathogens also appear to have an effect on neonatal immunity and possibly on immune development. It is clear that viable cells are present in colostrum, and that maternal cells from colostrum enter into the neonatal circulation,[6-9] peaking 24 hours after birth.[10] However, few studies have assessed the capacity of those cells to affect antigenic responses of the neonate. To date, we are aware of only the studies of Reber et al[11] and Riedel-Caspari et al[12] that document that maternal cells enhance mixed leukocyte culture stimulation and responses of circulating leukocytes from neonates and the response of neonatal calves to *Escherichia coli*, respectively. The purpose of the study reported here was to assess the effect of maternal cells or cellular components on neonatal immune responses to intracellular pathogens in calves.

Abbreviations

BVDV	Bovine viral diarrhea virus
PPD	Purified protein derivative
MAP	*Mycobacterium avium* subsp *paratuberculosis*

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From the Departments of Population Health (Donovan, Reber, Gabbard, Aceves-Avila, Galland, Holbert, Hurley) and Large Animal Medicine (Donovan, Reber, Hurley), College of Veterinary Medicine, and the Department of Animal and Dairy Science, College of Agricultural and Environmental Sciences (Ely), University of Georgia, Athens, GA 30602.
Supported by a Veterinary Medical Experiment Station Grant and an unrestricted gift from Pfizer Animal Health.
Address correspondence to Dr. Hurley.

Materials and Methods

Animals and treatments—Cattle used in this study were obtained from the University of Georgia Dairy Facility. All studies and procedures were reviewed and approved by the Institutional Animal Care and Use Committee of the University of Georgia under approval No. A2005-10027-c2.

Cows were vaccinated with a 5-way respiratory vaccine[a] that contains inactivated BVDV, chemically altered modified-live infectious bovine rhinotracheitis virus and parainfluenza-3 virus, and modified-live bovine respiratory syncytial virus. Cows delivered calves during 4 periods: October and November of 2004, mid-January through May of 2005, late September through November of 2005, and late January through mid-May of 2006. Cows were selected for participation in the study on the basis of parity, expected calving date, and not being involved in other studies that might alter colostrum quality. Attempts were made to space calves in the study at least 1 week apart. Thirty days prior to expected parturition, 30 mL of blood was obtained from cows to determine the cellular proliferation response to BVDVs (NADL and NY-1).

Fifteen Holstein dairy calves were used. To obtain presuckle blood samples, an electronic monitoring system[b] was used to alert investigators at the initiation of parturition. Monitors were sutured into the vulva of dams 7 days prior to the anticipated calving date. After collection of blood samples, each calf received colostrum from its dam. Five calves were fed whole colostrum, 5 were fed frozen colostrum, and 5 received cell-free colostrum. Each calf consumed at least 4 pints of colostrum within 4 hours of birth. All calves received an additional 4 pints of their dam's colostrum approximately 12 hours later. All calves received a milk replacer diet after colostrum treatment.

Forty-five milliliters of heparinized[c] blood and 5 mL of blood for serum[d] were obtained via jugular venipuncture from calves at birth (prior to feeding colostrum), 1 day after birth, 2 days after birth, and at weekly intervals for the next 4 weeks. Peripheral blood mononuclear cells were isolated from heparinized blood by single-step buoyant density floatation with a separation agent[e] as described.[11] Effector function was evaluated by measurement of proliferation in response to BVDV and mycobacterial PPD antigens by use of stimulation with the superantigen *Staphylococcus* enterotoxin B as a positive proliferation control. Serum IgG concentrations were determined at all sampling times.

Colostrum—Whole colostrum was collected from each cow in this study. Calves that received whole colostrum were bottle-fed colostrum from their dams. Colostrum to be frozen was double bagged in ziplock storage bags and placed between stainless steel plates precooled to –80°C to rapidly freeze the colostrum. Once frozen, the bags containing the colostrum were placed in a water bath at 50°C until the colostrum was slushy, at which time the bags were placed in a water bath at 37°C until the colostrum was completely thawed. This colostrum preparation was bottle-fed to calves in the frozen colostrum treatment group. To assess the effectiveness of the frozen colostrum treatment, colostrum samples from the frozen colostrum treatment group were examined for cell viability. Frozen colostrum samples were thawed, diluted 1:4 in PBS solution to reduce viscosity, and diluted 1:2 in trypan blue and examined microscopically. The frozen colostrum treatment effectively destroyed colostral leukocytes. The few intact cells that remained were nonviable as indicated by trypan blue uptake.

Cell-free colostrum was prepared by centrifugation for 40 minutes at 1,500 × *g* at 26°C in 1-L bottles. A soft lipid layer formed at the top of the supernatant. The lipid layer was cut away from the tube wall and carefully transferred along with the supernatant fluid to sterile bottles. The cell pellet was discarded. The lipid layer and supernatant were mixed together and placed in double ziplock storage bags. The cell-free colostrum was quick frozen at –80°C as described, thawed, and bottle-fed to the calves.

Serum antibody ELISA—Serum IgG concentrations were measured by use of a sandwich ELISA. Rabbit anti-bovine IgG antibody[f] diluted 1:400 in sodium carbonate buffer at pH 9.0 was used to coat plates[g] at 4°C overnight. The plates were washed 3 times with PBS solution containing 0.5% Tween 20. Samples were diluted in wash buffer at 1:10 for screening, and samples with positive results for IgG were further diluted to determine titer endpoint. Samples were placed in quadruplicate wells for analysis. Samples were incubated for 1 hour at 22°C, after which the plates were washed 3 times. Bound IgG was detected by use of a rabbit anti-bovine IgG conjugated to horseradish peroxidase.[h] The detection antibody was diluted 1:1,000 in wash buffer, and the wells were incubated with detection antibody for 30 minutes. The plates were washed 3 times with wash buffer, and bound detection antibody was detected by use of 2,2′-azino-di-3 ethylbenzthiazoline sulfonic acid substrate. The plates were incubated for 30 minutes to allow color development and measured with a plate reader with a 405-nm filter. The IgG concentration was determined relative to a 6-point curve made with serial dilutions of bovine gamma globulin standard.[i]

Proliferation—Cellular proliferation responses to 2 families of intracellular antigens, BVDV and mycobacterial PPD, both dependent on cell-mediated immune mechanisms, were assessed over the first 4 weeks after birth in these 3 groups of calves. The cows used in this study were vaccinated against bovine respiratory disease with vaccines containing killed BVDV, but not mycobacterial antigens. In addition, all cows were skin tested and were serologically negative for antibodies against *Mycobacterium bovis* and MAP antigens. Peripheral blood mononuclear cells were suspended at a concentration of 5×10^6 cells/mL in RPMI 1640[j] that contained 2mM L-glutamine,[k] 1mM sodium pyruvate,[k] and 50 µg of gentamicin sulfate/mL.[k] Peripheral blood mononuclear cells stimulated with PPDs were supplemented with 10% gamma-irradiated fetal bovine serum,[l] whereas peripheral blood mononuclear cells stimulated with live BVDV were supplemented with 10% donor horse serum[l] (to avoid any effect of antibodies against BVDV in fetal bovine serum). Cells were stimulated in

quadruplicate wells of 96-well round-bottom plates[m] (5×10^5 cells/well) with 1 µg of *Staphylococcus* enterotoxin/mL, 1 µg of MAP/mL, 1 µg of *M bovis*–PPD/mL, 1 µg of MAP–PPD/mL, and live NY-1 or NADL BVDV at approximately 1.0 and 0.5 multiplicity of infection. Peripheral blood mononuclear cells stimulated with PPDs were incubated for 3 days at 37°C and 5% CO_2; peripheral blood mononuclear cells stimulated with virus were incubated for 5 days under the same conditions. Six hours prior to harvesting, the wells were pulsed with 10 µL of ^{3}H-thymidine (20 µCi/mL; specific activity, 6.7 Ci/mM).[n] Cells were harvested on a cell harvester[o] after 6 hours of incubation with ^{3}H-thymidine. The cells were lysed with distilled water, and the DNA was collected on glass fiber filters. Cellular proliferation was determined by incorporation of hydrogen 3 thymidine into cellular DNA by use of a scintillation counter.[p] Results are expressed as specific incorporation according to the following formula: specific incorporation = (counts/min of stimulated cells in RPMI – counts/min of unstimulated cells in RPMI).

Statistical analysis—Internal clustering of proliferation data was first evaluated for outlier data by use of a Q test. Data points were rejected when the Q value exceeded 0.76.[13] An ANOVA was conducted to determine overall treatment differences. Post hoc Bonferroni multiple mean comparisons were conducted to compare effects of the different colostrum preparations on adaptive immune responses. Statistical analysis was performed with commercial software.[q] Values of $P < 0.05$ were considered significant.

Results

Transfer of maternal antibody—Serum concentrations of IgG before suckling were low for all calves and had increased to similar concentrations (approx 4,000 mg/dL) by day 1. There were no significant differences among the 3 groups of calves at any sample time.

Proliferative responses of maternal leukocytes to BVDV before calving—Peripheral blood mononuclear cells from all cows responded similarly to BVDV 30 days prior to the expected calving date (from 2,000 to 7,000 counts/min), and there were no significant differences among the cows when their calves were assigned to the colostrum treatment groups.

Proliferative responses of neonatal leukocytes to cytopathic or noncytopathic strains of BVDV—Peripheral blood mononuclear cells obtained from calves before suckling did not proliferate in response to BVDV. In contrast, leukocytes collected from calves 1 day after ingestion of whole colostrum had significantly ($P < 0.001$) enhanced proliferative responses to 0.5 multiplicity of infection for the NADL strain of BVDV, compared with responses before ingestion of whole colostrum treatment (**Figure 1**). The response to the NADL strain of BVDV then steadily declined over the next week, until the response returned to background value and remained at that value for the rest of the study.

Peripheral blood mononuclear cells from calves in the frozen colostrum and cell-free colostrum groups did not proliferate in response to a multiplicity of infection of 0.5 for the NADL strain of BVDV prior to receiving colostrum (Figure 1). One day after ingestion of the colostrum preparations, proliferative responses of peripheral blood mononuclear cells from calves fed whole colostrum were significantly ($P < 0.01$) greater than responses of peripheral blood mononuclear cells from calves fed either frozen colostrum or cell-free colostrum. The proliferative responses of peripheral blood mononuclear cells incubated with a multiplicity of infection of 1 for NADL followed the same patterns as those to the 0.5 multiplicity of infection.

NY-1–specific proliferation—Proliferative responses to BVDV antigens were also assessed by use of

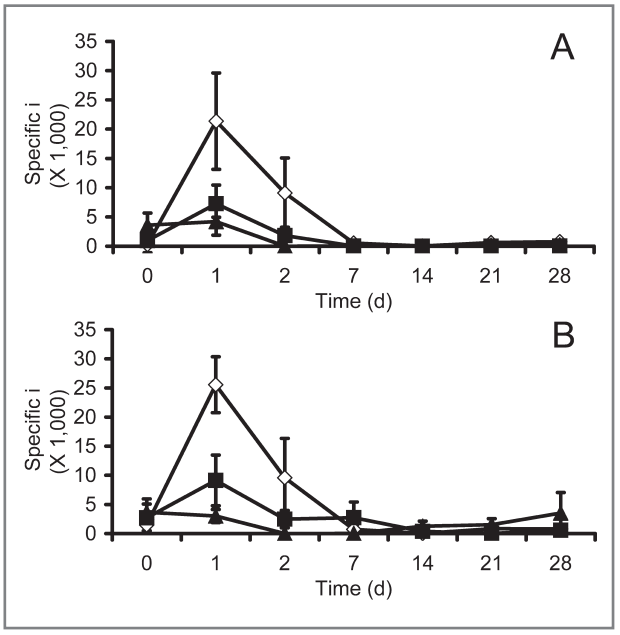

Figure 1—Illustration of the effect of transferred maternal colostral leukocytes from vaccinated dams on the proliferative response to BVDV in vitro in calves. Calves were fed whole colostrum (diamonds), frozen colostrum (squares), and cell-free colostrum (triangles) from vaccinated dams at birth. Data (mean ± SEM) indicate cellular proliferation in response to the (A) cytopathic NADL strain and (B) noncytopathic NY-1 strain, examined before suckling (time 0) through 28 days of age. Specific i = Scintillation count per minute of stimulated cells minus counts per minute of unstimulated cells.

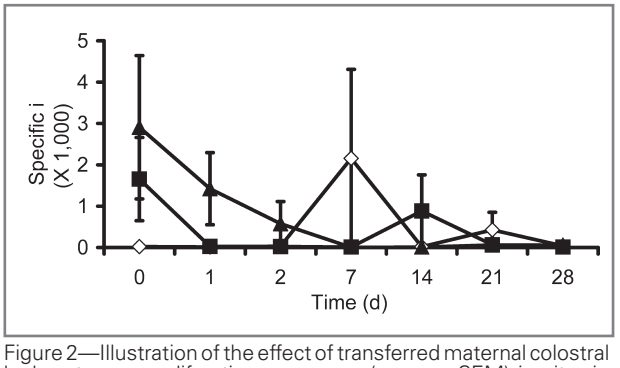

Figure 2—Illustration of the effect of transferred maternal colostral leukocytes on proliferative responses (mean ± SEM) in vitro in calves. Calves were fed whole colostrum, frozen colostrum, and cell-free colostrum from dams at birth, and cellular proliferation was evaluated in response to MAP, an antigen to which the dams were not vaccinated. *See* Figure 1 for key.

a multiplicity of infection of 0.5 for the noncytopathic BVDV strain, NY-1, as a stimulus. There were no significant proliferative responses of peripheral blood mononuclear cells from any of the calves to this strain of BVDV before suckling (Figure 1). Additionally, proliferative responses of peripheral blood mononuclear cells from calves that received frozen colostrum and cell-free colostrum were not significantly different from the presuckle value at any point in the study. However, 1 day after ingestion of whole colostrum, the proliferative responses to NY-1 were significantly ($P = 0.01$) increased, compared with responses before suckling. Furthermore, proliferative responses of peripheral blood mononuclear cells from calves fed whole colostrum to the 0.5 multiplicity of infection of the NY-1 virus 1 day after ingestion of the colostrum preparations were significantly greater than responses obtained with peripheral blood mononuclear cells from calves fed frozen colostrum or cell-free colostrum ($P < 0.05$ and $P = 0.01$, respectively) 1 day after ingestion of colostrum. The enhanced proliferative responses to NY-1 returned to baseline value by the first week after colostrum ingestion. Responses to the 1.0 multiplicity of infection of NY-1 virus followed similar patterns.

Mycobacterial-specific proliferation—Proliferative responses of neonatal peripheral blood mononuclear cells were also evaluated for antigens (PPD preparations from *M bovis, M avium,* and MAP) to which their dams had no measurable response. Because the responses of the peripheral blood mononuclear cells from the calves to the 3 PPD preparations were similar, only the proliferative response to MAP is provided as a representative of the pattern of response (**Figure 2**); MAP is associated with paratuberculosis that often is acquired as a neonatal infection. Because of the variability in the responses of the individual calves to PPD, there were no significant differences observed among the 3 groups to any of the PPD antigens. There also were no significant differences in the responses to PPDs starting 7 days after ingestion of the colostrum preparations and continuing through the end of the study.

Discussion

Colostrum contains high concentrations of nutrients, hormones, and immunologic components. These immunologic components include immunoglobulin, cytokines, and substantial numbers of maternal leukocytes.[14-16] Results of the present study suggest that specific responsiveness against antigens to which the dam has mounted an immune response can be transferred to a neonatal calf by live maternal cells in colostrum. In this study, a significant in vitro response to the NADL and NY-1 strains of BVDV was observed 1 day after ingestion of whole colostrum that contained live maternal cells. In contrast, the responses to PPD preparations, antigens to which the cows were naïve, were unaffected by the transfer of live maternal cells. Although an in vitro response to a virus does not necessarily predict an in vivo response, it is a reasonable indicator that is widely used to monitor vaccine-induced responses.[17]

The peripheral blood mononuclear cells from the dams had similar proliferative responses 30 days before calving to the noncytopathic NY-1 and cytopathic NADL BVDVs. It is possible that the transfer of memory T cells via colostrum provided the observed enhanced responsiveness to these viruses in the neonates on the first and second day after suckling. We are not able to comment on their potential to protect across genotypes because no type 2 viruses were evaluated in this study.

It has been reported that circulating maternal mononuclear cells that had been exposed to the mammary environment in the form of filtered acellular colostrum (but not tissue culture medium) entered the circulation of neonatal calves with a peak concentration of 2% to 3% of total circulating cells 24 hours after ingestion.[10] This temporal sequence coincided with peak enhancement of BVDV-specific proliferative responses of circulating leukocytes observed in calves in the present study. Furthermore, enhanced capacity to stimulate a 1-way mixed leukocyte reaction (antigen presenting capacity) and to respond in 1-way mixed leukocyte reaction in calves the day after receiving colostrum containing live maternal cells, relative to those receiving acellular colostrum, has been reported.[11] Collectively, these findings suggest that transfer of live maternal cells may be an important factor in colostrum that provides immune protection. These maternal cells may contribute to the protection of the neonate within the first 24 hours after ingestion and appear to have no further direct effect on antigen-specific responses in the blood by 7 days after ingestion.

Results indicated that all the calves received equivalent transfer of IgG from the dam without regard to treatment. These results are consistent with those of Holloway et al,[18] who reported that serum IgG concentrations were not different between calves fed fresh versus frozen colostrum. Neither the cellular components of lysed maternal cells (transferred with frozen colostrum) nor the humoral portion of colostrum was sufficient to transfer the observed enhancement in response to BVDV antigens. Thus, the antibodies found in colostrum did not contribute to the observed enhancement of virus-specific proliferation. Results of the present study indicated the capacity of maternal leukocytes to enhance the initial protection of the neonate from intracellular infection. Maternal vaccination may represent the best path to maximizing the transfer of responsive maternal leukocytes in colostrum. Therefore, it appears that simply measuring the concentration of transferred antibodies may not be sufficient to fully comprehend the extent of immunity transferred from dam to neonate.

a. CattleMaster 5, Pfizer Animal Health, Exton, Pa.
b. Foalert Inc, Acworth, Ga.
c. Heparin sodium salt, grade 1-A from porcine intestinal mucosa, Sigma, St Louis, Mo.
d. Vacutainer, Becton-Dickinson, Franklin Lakes, NJ.
e. Histopaque 1083, Sigma, St Louis, Mo.
f. Catalogue No. B5645, Sigma, St Louis, Mo.
g. Immulon 4 HBX, Catalog #3855, Thermo, Milford, Mass.
h. Catalogue No. A5295, Sigma, St Louis, Mo.
i. Catalogue No. I5506, Sigma, St Louis, Mo.
j. Mediatech Inc, Herndon, Va.
k. Sigma, St Louis, Mo.
l. Atlanta Biological, Lawrenceville, Ga.
m. Falcon, Becton Dickson, Franklin Lakes, NJ.

n. ICN, Irvine, Calif.
o. PHD cell harvester, Brandel, Gathersberg, Md.
p. Model LS6500 scintillation counter, Beckman-Coulter, Fullerton, Calif.
q. Prism, version 4, Graph Pad Software Inc, San Diego, Calif.

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