Effect of feeding whole compared with cell-free colostrum on calf immune status: The neonatal period

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ABSTRACT

Mortality and decreased weight gain resulting from infection and disease in dairy calves are problems within the dairy industry. The bovine neonate relies solely on colostrum to acquire antibodies through passive transfer. To date, colostrum quality is determined by the concentration of antibodies. However, proteins and cells in the colostrum might also enhance immune development in the neonate. To determine the effect of maternal colostral immune cells on calf health and immune status, maternal colostrum was fed either fresh or after lysis of cells by flash-freezing in liquid nitrogen. Thirty-seven female Holstein and Jersey dairy calves were fed 4 quarts total of whole colostrum (WC) or cell-free colostrum (CFC) at birth. Respiratory and fecal scores were measured from birth to d 45 of life. Calf peripheral blood samples were obtained before and after feeding colostrum as well as on d 1, 3, 7, 14, 21, and 28 of life. Peripheral blood mononuclear cells were collected and analyzed for cellular parameters by flow cytometry. Total respiratory scores were greater in CFC-fed calves compared with WC-fed calves on d 38 of life. There were fewer CD4⁺ T cells and CD4⁺CD62L⁺CD45RO[−] T cells on d 1 and fewer CD4⁺CD62L⁺CD45RO⁺ T cells on d 1 and 3 in CFC-fed calves compared with WC-fed calves. Compared with WC-fed calves, CFC-fed calves had a greater percentage of CD4⁺CD62L[−]CD45RO⁺ T cells on d 0.25, 1, 3, and 7, and a greater percentage of monocytes on d 7. Our data suggest that colostral cells adoptively transfer and enhance neonatal immunity during the first month of life.

Key words: colostrum, adoptive transfer, immunity, dairy calf

INTRODUCTION

The neonatal immune system is immunologically naive, making the dairy calf highly susceptible to bacterial and viral pathogens. The National Animal Health Monitoring System reported that 56.5% of all unweaned heifer deaths were due to scours or diarrhea and 22.25% were due to respiratory problems (USDA-APHIS, 2007). Disease incidence in calves negatively affects the profitability of the dairy operation and decreases animal well-being. To abrogate illness and disease in calves, colostrum is required for passive transfer of nutrients and antibodies (Quigley and Drewry, 1998). To date, colostrum quality is determined by antibody content alone. However, colostrum also contains leukocytes, cytokines, antimicrobial proteins, and hormones (Reiter and Brock, 1975; Hagiwara et al., 2000; Blum and Baumrucker, 2002). These colostrum components may have a positive effect on neonatal status and long-term development of immune function.

Similar to passive transfer of antibodies, adoptive transfer delivers colostral leukocytes through the intestinal epithelium and into circulation (Schnorr and Pearson, 1984; Williams, 1993; Liebler-Tenorio et al., 2002). Adoptively transferred colostral cells may be antigen-specific, aiding in immediate pathogen clearance. Antigen-specific colostral cells have memory characteristics and may reside in calf lymphoid tissue until activated by exposure to a future pathogen (Donovan et al., 2007). Previous literature supports a role for maternal colostral cells in modification of adhesion marker expression on T lymphocytes and monocytes. Calves fed cell-free colostrum (COL−) had greater numbers and percentages of circulating monocytes expressing CD11a on d 2 and CD11c on d 14 compared with calves fed whole colostrum (COL+; Reber et al., 2008a). Calves fed COL− had a greater percentage of T cells expressing CD11a on d 2 and d 14 compared with calves fed COL+. An increase in adhesion markers on immune cells in calves fed COL− may reflect an increase in pathogen insult or stress response compared with calves fed COL+ (Reber et al., 2008a,b).

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The feeding of maternal colostrum cells correlates with increased bacterial clearance in the neonate. Riedel-Caspari (1993) experimentally infected calves with *Escherichia coli* and immediately fed either COL+ or COL-. Calves fed COL+ excreted fewer colony-forming units of *E. coli* during the first week of infection. In addition, levels of fecal bacteria in calves fed COL+ reached the lower limit of detectability sooner and concentrations of *E. coli*-specific antibodies during the first 48 h postinfection were higher than in calves fed COL- (Riedel-Caspari, 1993). Similarly, newborn rats fed fresh rat milk, frozen rat milk plus rat milk leukocytes, or commercial formula plus rat milk leukocytes had increased survival following an oral dose of *Klebsiella pneumoniae* compared with rats fed frozen rat milk or commercial formula alone (Pitt et al., 1977). These data suggest a role for adoptively transferred leukocytes through colostrum ingestion on pathogen clearance, neonatal immune function, or both.

In this study, we examined the effect of maternal colostrum cells on health and immune cell profiles in dairy calves. We hypothesized that feeding whole colostrum (WC) containing intact, viable cells at birth would enhance levels of cellular blood parameters within the first month of life compared with feeding cell-free colostrum (CFC) in which cells were lysed.

MATERIALS AND METHODS

Feeding Colostrum Containing Intact Versus Lysed Immune Cells

Twenty-nine Holstein and 8 Jersey cows from the Virginia Tech Dairy Center (Blacksburg) were equipped with a birth monitoring system (FoAlert, Acworth, GA). The FoAlert birth monitoring system was used for immediate notification of parturition. In brief, a veterinarian sutured a transmitter to the outside of the vulva on each cow at ≥ 2 wk before calving. During parturition, physical separation of the vulva triggered the transmitter, resulting in an audible alarm and phone calls to preprogrammed numbers on an automated dialer. This system ensured that staff arrived before parturition and prevented suckling of the dam by the calf. All animal handling and sampling protocols were in accordance with the Virginia Tech's Institutional Animal Care and Use Committee.

After parturition was complete, the calf was cleaned by the cow but not permitted to suckle. The calf was then removed, weighed, and administered one dose of TSV-2 nasal vaccine (Zoetis, Madison, NJ). The dam was moved into a chute where colostrum was collected aseptically from each quarter using a portable milking

unit. If clumps or blood was observed in colostrum, mastitis was assumed and the calf was not enrolled. Colostrum was tested with a digital Brix refractometer to assess total colostrum protein and samples saved for subsequent standard bacteriology analyses (NMC, 2004). Only quality colostrum with a Brix score of 23 or higher (Deelen et al., 2014) was fed to calves.

Calves were fed quality WC or CFC from their respective dams, according to the following protocols. Following collection and testing, 1.9 L of WC was used to fill two 2-quart bottles. One bottle was fed to the calf within 3 h of birth, and the second bottle was refrigerated (4°C) and, immediately before the second feeding at 5 to 8 h after birth, slowly warmed to 37°C. Refrigeration had no significant effect on cell viability in WC (data not shown).

To lyse immune cells for the CFC treatment, 4 perfluoroalkoxy (PFA) bags (Welch Fluorocarbon Inc., Dover, NH) were filled each with 1 L (1.06 quarts) of colostrum per bag. Each PFA bag was placed in a Styrofoam box and covered with liquid nitrogen. Precautions were taken to ensure that liquid nitrogen did not come into direct contact with colostrum. The PFA bag was turned approximately every 3 min until the colostrum was completely frozen. Following complete freezing of colostrum, the first 2 quarts of colostrum were slowly warmed to 37°C and fed to the calf within 3 h of birth. The second 2 quarts of colostrum were thawed at 37°C, transferred to a bottle, refrigerated (4°C), and then slowly warmed to 37°C immediately before the second feeding at 5 to 8 h after birth.

An esophageal tube feeder was used only in cases when calves did not suckle from the bottle. In this study, 5 calves required feeding by esophageal feeder (2 in fresh and 3 in frozen treatment), precluding statistical analyses and any likely effect on our results. Animals fed by esophageal tube feeder received colostrum in the same volume and at the same time points as bottle-fed calves. All calves entered the Virginia Tech dairy herd, and routine farm management protocols were applied.

Blood Collection for Plasma and Isolation of Peripheral Blood Mononuclear Cells

Calves were bled at birth (before colostrum feeding), 6 h after birth (following second feeding of colostrum), and on d 1, 3, 7, 14, 21 and 28. Peripheral blood mononuclear cells (PBMC) were isolated on a Ficoll-Paque density gradient (1.077 g/mL; BD Falcon, Franklin Lakes, NJ), from whole blood collected using 40 mM EDTA (10% vol/vol) as described previously (Shafer-Weaver et al., 1999). Following initial centrifugation, plasma was removed, aliquoted, and stored at -20°C

until further analysis. Mononuclear cells were resuspended at 1×10^7 cells/mL in PBS containing 0.05% sodium azide.

Plasma Antibody ELISA

Bovine ELISA quantitation sets (Bethyl Laboratories Inc., Montgomery, TX) were used in the assessment of IgG₁ (E10-116), IgG₂ (E10-117), IgM (E10-101), and IgA (E10-121) in plasma samples. Each sample and standard was run in duplicate.

Nunc-Immuno microwells (Sigma-Aldrich, St. Louis, MO) were coated with 100 μ L of bovine IgG₁, IgG₂, IgM, or IgA antibody and diluted in sample/conjugate diluent (50 mM Tris, 0.14 M NaCl, 0.05% Tween 20, pH 8.0). The plates were incubated at 25°C for 1 h and subsequently washed 5 times in wash solution (50 mM Tris, 0.14 M NaCl, 0.05% Tween 20, pH 8.0). Two hundred microliters of blocking solution (50 mM Tris, 0.14 M NaCl, 0.05% Tween 20, pH 8.0) was added and the plates were incubated at 25°C for 30 min. Plates were washed 5 times, and 100 μ L of the standard or diluted sample was added into the corresponding well. Plasma samples were diluted 1:100 in the sample/conjugate diluent, and colostrum samples were diluted 1:50,000 in the sample/conjugate diluent. Plates were then incubated for 1 h at 25°C and washed 5 times. One hundred microliters of diluted horseradish peroxidase (HRP) detection antibody (1:20,000 in the sample/conjugate diluent) was then added to each well. After 1 h of incubation at 25°C, the plate was washed 5 times and 100 μ L of TMB Substrate Solution (3,3',5,5'-tetramethylbenzidine; BD Biosciences, San Diego, CA) was added to each well. To stop development, 100 μ L of stop solution (0.18 M H₂SO₄) was added. The absorbance of each well was read with an ELISA plate reader (μ Quant Universal Microplate Spectrophotometer; BioTek, Winooski, VT) at 450 nm; data are presented as micrograms per milliliter.

Health Scores

Observational health data were collected from birth to d 45. Coughing, nasal discharge, ear disposition, eye discharge, and fecal consistency were scored using the Calf Health Scoring Chart (University of Madison-Wisconsin School of Veterinary Medicine; www.vetmed.wisc.edu/dms/fapm/fapmtools/calves.htm). Fecal consistency was scored on a scale from 0 to 3, where 0 represented normal consistency and 3 represented severe scours. Coughing, nasal discharge, ear disposition, and eye discharge were also scored on a scale from 0 to 3, where 0 represented a healthy animal and 3 represented

a very ill animal. Cough, nasal discharge, ear disposition, and eye discharge scores were combined to create a total respiratory score for each day. Total respiratory score data exhibited a strong positive skew in distribution of the sample means. For this reason, data were collapsed to represent a binomial distribution, in which 0 represented healthy animals and any score above zero received a 1, signifying a nonhealthy animal.

Isolation of Colostral Immune Cells

Colostrum was pelleted by centrifugation at $1,500 \times g$ and 15°C for 30 min. Casein and free fat were carefully removed and colostrum whey was discarded. If colostrum was contaminated with red blood cells, the pellet was resuspended briefly in 10 μ L of RNase/DNase-free H₂O. Immediately, 2 $\times$ modified Eagle's medium was added and the tube centrifuged at $200 \times g$ at 4°C for 10 min. Cells were resuspended in 10 mL of PBS, and a 10- μ L aliquot was diluted 1:100 in 0.04% Trypan Blue solution (Life Technologies, Carlsbad, CA) for counting. Colostral mononuclear cells were observed under an Axiovert 40 microscope (Zeiss, Göttingen, Germany). No cells were detectable in colostrum that had undergone flash freezing with liquid nitrogen and slow thawing. In addition, the types of immune cells in colostrum were analyzed by flow cytometry (Supplemental Figure S1; <http://dx.doi.org/10.3168/jds.2014-8422>).

Leukocyte Analysis

Flow cytometric analysis of cell surface markers was performed using bovine-specific monoclonal antibodies (Table 1). Markers were chosen based on cellular type identified. In brief, CD4⁺ T lymphocytes stimulate antibody production from B cells (CD21⁺) and induce macrophage development from monocytes (CD14⁺), and granulocytes. The $\gamma\delta$ T cell can respond directly to a pathogen, similar to innate immune cells, or can be activated by phagocytes, similar to adaptive immune cells. The distribution of cell types in bovine fetal blood compared with adult blood is reviewed by Wilson et al. (1996). T-Lymphocyte memory subpopulations are distinguished by cell surface marker expression, including CD62L (L-selectin), CD45RO, and CCR7. Marker CD62L is an adhesion surface protein on T lymphocytes, which enhances cell migration from blood to the lymph node; CD45RO is a cell surface glycoprotein, which is upregulated after activation by a pathogen or other stimulus; and CCR7 is a chemokine receptor ligand, which is responsible for lymphocyte migration through endothelial venules into lymph nodes (Sallusto et al., 1999). Because of limited cell numbers following

Table 1. Primary and secondary antibodies used for flow cytometric analysis of peripheral blood mononuclear cell populations

Primary antibody target ¹	Clone	Isotype	Primary antibody dilution	Secondary antibody
CD4 ⁺ T cell	IL-A11	IgG2a	1:400	IgG2a-FITC ²
CD62L	BAQ-92A	IgG1	1:400	IgG1-PE-Texas Red ²
CD45RO	IL-A1116	IgG3	1:400	IgG3-PE ³
B cell (CD21 ⁺)	BAQ-15A	IgM	1:400	IgM-APC ²
Monocyte (CD14 ⁺)	MM61	IgG1	1:400	IgG1-PE-Texas Red ²
$\gamma\delta$ TCR ⁺ T cell	CACTB32A	IgG1	1:800	IgG1-PE-Texas Red ²

¹Primary mouse-generated monoclonal antibodies purchased from VMRD (Pullman, WA).

²Secondary goat anti-mouse monoclonal antibodies purchased from Life Technologies (Carlsbad, CA).

³Secondary goat anti-mouse monoclonal antibodies purchased from Southern Biotechnologies (Birmingham, AL).

isolation of PBMC from calves, we restricted our analysis of memory cells to CD45RO and CD62L cell types.

Mononuclear cells were resuspended at 1×10^7 cells/mL in PBS containing 0.05% sodium azide, and 10^6 cells were aliquoted per well in 96-well plates. Cells were incubated with primary antibody for 30 min at 4°C. Cells were washed 2 times with PBS and subsequently incubated with isotype-specific secondary antibodies conjugated to fluorescein isothiocyanate (FITC), phycoerythrin (PE), PE-Texas Red, or allophycocyanin (APC) for 30 min at 4°C. Cells were washed 2 times and fixed with 1% formaldehyde in PBS for 20 min at room temperature. Cells were washed again and resuspended in 150 μ L of PBS containing 0.05% sodium azide.

Percentages of cells were determined by flow cytometry (Accuri C6, BD, San Jose, CA) and analyzed using FlowJo Software (TreeStar, Ashland, OR).

Statistical Analysis

All statistical tests were performed using the PROC GLIMMIX procedure of SAS 9.3 (SAS Institute Inc. Cary, NC). Non-normally distributed data, as determined by the studentized *t* residuals, were natural log-transformed. A natural log-transformation was applied to the number and percentage of CD4⁺ T cells and numbers of CD4⁺CD62L⁺CD45RO⁻, CD4⁺CD62L⁻CD45RO⁺, and CD4⁺CD62L⁺CD45RO⁺ T cells. For transformed data sets, significance was declared using transformed data, whereas nontransformed least squares means and standard errors of the mean (LSM $\pm$ SEM) are reported in text and figures. Each statistical model included the fixed effects of treatment, time, breed, temperature at birth, and the associated interactions. Each calf was designated a temperature code based on the minimum and maximum ambient temperature the day of birth: cold (**C**)-born (minimum day temperature $\leq -30^\circ\text{C}$); mild (**M**)-born (minimum day temperature $> -30^\circ\text{C}$ and maximum day temperature $\leq 80^\circ\text{C}$); and hot (**H**)-born (maximum day temperature

$> 80^\circ\text{C}$). The repeated statement was applied to the day of blood collection using calf nested within treatment as the subject. If pretreatment levels were significantly different from each other, a covariate was assigned for h 0. Error structures were tested and the one giving the lowest Akaike information criterion was chosen for analysis. An analysis of simple effects was completed, regardless of the associated interaction significance, using the SLICE function and a subsequent Tukey adjustment in SAS. Because maturation of the calf's immune system overtime is directly related to age (Adkins et al., 2004; Levy, 2007), we compared treatment (WC or CFC) within day by analysis of simple effects (i.e., SLICE option in SAS), regardless of significance of the omnibus *F* test (Keppel, 1991; Winer et al., 1991). Significance was declared at $P \leq 0.05$.

RESULTS

Antibody Titers

Concentrations of plasma IgG₁, IgG₂, IgM, and IgA were analyzed from blood samples collected before colostrum ingestion and on d 1 of life. Treatment did not affect concentrations ($\mu\text{g/mL}$) of IgG₁, IgG₂, IgM, and IgA antibodies (Figure 1). Although we detected no significant treatment $\times$ temperature effect, an analysis of simple effects using the SLICE function indicated a lower concentration of IgA in WC-fed, C-born calves ($3.17 \times 10^3 \pm 2.14 \times 10^2 \mu\text{g/mL}$) compared with H-born calves fed the same treatment ($4.58 \times 10^3 \pm 2.98 \times 10^2 \mu\text{g/mL}$; $P = 0.0017$). When analyzing the concentration of IgG₂ antibodies, no significant treatment $\times$ temperature or treatment $\times$ breed effects were found. However, an analysis of simple effects indicated a decreased concentration of IgG₂ in WC-fed, C-born calves ($1.50 \times 10^3 \pm 1.57 \times 10^2 \mu\text{g/mL}$) compared with H-born calves fed the same treatment ($2.03 \times 10^3 \pm 1.51 \times 10^2 \mu\text{g/mL}$; $P = 0.033$). Jersey calves fed CFC had a decreased concentration of IgG₂ compared with

Figure 1. Antibody concentrations in calves fed whole ($n = 14$) or cell-free ($n = 16$) colostrum before and 1 d after feeding.

Holstein calves fed the same treatment ($1.27 \times 10^3 \pm 1.58 \times 10^2$ µg/mL and $1.80 \times 10^3 \pm 1.03 \times 10^2$ µg/mL, respectively; $P = 0.012$).

Health Scores

Fecal consistency scores were lowest on d 1 in CFC- and WC-fed calves (0.06 ± 0.14 and 0.06 ± 0.09 , respectively). Fecal consistency scores peaked on d 14 (1.79 ± 0.20 and 1.78 ± 0.25 , respectively) and decreased to near starting levels by d 45 in CFC- and WC-fed calves (0.21 ± 0.26 and 0.09 ± 0.13 , respectively).

No differences were seen between treatments in fecal consistency scores (Supplemental Figure S2; <http://dx.doi.org/10.3168/jds.2014-8422>). Respiratory scores increased for CFC- and WC-fed calves on d 12 (0.41 ± 0.26 and 0.12 ± 0.10 , respectively). Although we detected no significant treatment $\times$ time interaction ($P = 0.9995$), an analysis of simple effects using the SLICE function indicated greater total respiratory scores in CFC-fed calves on d 38 compared with WC-fed calves (0.33 ± 0.37 and 0.14 ± 0.11 , respectively, $P = 0.030$). The percentages of calves in each group with a total respiratory score >0 are shown in Figure 2.

PBMC

The number of PBMC (LSM $\pm$ SEM; Figure 3) per milliliter of blood peaked on d 21 for CFC-fed calves ($4.74 \times 10^6 \pm 4.68 \times 10^5$ cells) and on d 28 for WC-fed calves ($4.39 \times 10^6 \pm 4.97 \times 10^5$ cells). Day relative to colostrum ingestion affected the number of PBMC per milliliter of blood in all calves ($P < 0.0001$). We detected no effect of treatment, breed of calf, or temperature at birth on the number of PBMC during the first month of life.

CD4⁺ T Cells

The number of CD4⁺ T cells out of the total lymphocyte gate was determined by flow cytometry. The number of CD4⁺ T cells (LSM $\pm$ SEM; Figure 4) peaked in CFC-fed calves by d 21 ($3.96 \times 10^5 \pm 8.38 \times 10^4$ cells) and in WC-fed calves by d 14 ($2.95 \times 10^5 \pm$

Figure 2. Percentage of calves fed whole ($n = 17$) or cell-free colostrum ($n = 19$) and having a total respiratory score >0 . Treatment $\times$ time interaction: $P = 0.9995$. Asterisk indicates a difference between treatment groups within a time point in an analysis of simple effects of dichotomized data; $*P \leq 0.05$.

Figure 3. Number (/mL) of mononuclear cells in peripheral blood from calves fed whole ($n = 14$) or cell-free ($n = 17$) colostrum. Data are expressed as LSM $\pm$ SEM.

6.11×10^4 cells). The interaction of treatment $\times$ time was significant for both the number ($P = 0.0016$) and percentage ($P = 0.0002$) of $CD4^+$ T cells. On d 1, the number of $CD4^+$ T cells in CFC-fed calves was lower than that of WC-fed calves (natural log-transformed; 10.12 ± 0.23 cells and 11.40 ± 0.26 cells, respectively; $P = 0.0003$). We also detected a decreased percentage of $CD4^+$ T cells in CFC-fed calves compared with WC-fed calves at h 6 (natural log-transformed; $1.14 \pm 0.21\%$ and $1.87 \pm 0.24\%$ cells, respectively; $P = 0.024$) and on d 1 (natural log-transformed; $0.93 \pm 0.16\%$ and $2.15 \pm 0.19\%$, respectively; $P < 0.0001$). Breed of animal had

a significant effect on the percentage of $CD4^+$ T cells ($P = 0.0032$): Jersey calves had a higher percentage of $CD4^+$ T cells compared with Holstein calves in both CFC-fed (natural log-transformed; $1.84 \pm 0.22\%$ and $1.25 \pm 0.11\%$, respectively; $P = 0.012$) and WC-fed animals (natural log-transformed; $2.25 \pm 0.23\%$ and $1.55 \pm 0.12\%$, respectively; $P = 0.031$).

$CD4^+CD62L^+CD45RO^-$ T Cells

The number of $CD4^+CD62L^+CD45RO^-$ T cells was determined out of the $CD4^+$ T-cell gate by flow cytometry. The number of $CD4^+CD62L^+CD45RO^-$ T cells (LSM $\pm$ SEM; Figure 5) peaked by d 21 in CFC-fed calves ($1.75 \times 10^6 \pm 4.59 \times 10^4$ cells) and by d 7 in WC-fed calves ($1.48 \times 10^5 \pm 3.89 \times 10^4$ cells). Although we observed a trend in the treatment $\times$ time interaction ($P = 0.0712$), an analysis of simple effects using the SLICE function indicated fewer $CD4^+CD62L^+CD45RO^-$ T cells in CFC-fed calves than in WC-fed calves on d 1 (natural log-transformed; 8.90 ± 0.32 cells and 10.53 ± 0.36 cells, respectively; $P = 0.0008$). Additionally, although we found no significant treatment $\times$ time interaction ($P = 0.5609$), an analysis of simple effects indicated a decreased percentage of $CD4^+CD62L^+CD45RO^-$ T cells in CFC-fed calves compared with WC-fed calves on d 1 ($35.32 \pm 4.87\%$ and $51.96 \pm 5.45\%$, respectively; $P = 0.025$) and on d 7 ($33.63 \pm 4.39\%$ and $47.17 \pm 4.78\%$, respectively; $P = 0.040$). Breed and temperature at birth did not affect

Figure 4. The number (per milliliter of blood; left) and percentage (right) of $CD4^+$ T cells expressed as LSM $\pm$ SEM in calves fed whole ($n = 14$) or cell-free ($n = 17$) colostrum. Treatment $\times$ time interaction for number: $P = 0.0016$ and percentage: $P = 0.0002$. Asterisks indicate a difference between treatment groups within a time point in an analysis of simple effects. Test of significance determined using natural log-transformed data for both number (d 1: cell-free: 10.12 ± 0.23 cells; whole: 11.40 ± 0.26 cells) and percentage (h 6: cell-free: $1.14 \pm 0.21\%$; whole: $1.87 \pm 0.24\%$; d 1: cell-free: $0.92 \pm 0.16\%$; whole: $2.15 \pm 0.19\%$) of $CD4^+$ T cells; * $P \leq 0.05$, ** $P \leq 0.01$, *** $P \leq 0.001$.

Figure 5. The number (per milliliter of blood; left) or percentage (right) of $CD4^+CD62L^+CD45RO^-$ T cells from calves fed whole ($n = 14$) or cell-free ($n = 17$) colostrum. Data are expressed as LSM $\pm$ SEM. Treatment $\times$ time interaction for number: $P = 0.0712$ and percentage: $P = 0.5609$. Asterisks indicate a difference between treatment groups within a time point in an analysis of simple effects. Significances were determined using natural log-transformed data for number (d 1: cell-free: 8.90 ± 0.32 cells; whole: 10.53 ± 0.36 cells) of $CD4^+CD62L^+CD45RO^-$ T cells; * $P \leq 0.05$, *** $P \leq 0.001$.

the number or percentage of $CD4^+CD62L^+CD45RO^-$ T cells in WC- or CFC-fed calves.

$CD4^+CD62L^-CD45RO^+$ T Cells

The number of $CD4^+CD62L^-CD45RO^+$ T cells was determined out of the $CD4^+$ T-cell gate by flow cytometry. The number (LSM $\pm$ SEM; Figure 6) of $CD4^+CD62L^-CD45RO^+$ T cells peaked on d 28 in both WC- and CFC-fed animals ($7.31 \times 10^4 \pm 2.43 \times 10^4$ cells and $8.41 \times 10^4 \pm 2.35 \times 10^4$ cells, respectively). Day relative to colostrum ingestion affected the number of $CD4^+CD62L^-CD45RO^+$ T cells in all calves ($P < 0.0001$). The interaction of treatment $\times$ time was significant for both the number ($P = 0.0283$) and percentage ($P = 0.0276$) of $CD4^+CD62L^-CD45RO^+$ T cells. On d 7, the number of $CD4^+CD62L^-CD45RO^+$ T cells was higher in CFC-fed calves than in WC-fed calves (natural log-transformed 10.21 ± 0.21 cells and 9.34 ± 0.22 cells, respectively; $P = 0.005$). There were greater percentages of $CD4^+CD62L^-CD45RO^+$ T cells in CFC-fed calves than in WC-fed calves on d 0.25 ($12.50 \pm 1.63\%$ and $7.07 \pm 1.8358\%$, respectively; $P = 0.0284$), d 1 ($11.11 \pm 1.72\%$ and $5.65 \pm 1.93\%$, respectively; $P = 0.0358$), d 3 ($9.89 \pm 1.33\%$ and $5.59 \pm 1.45\%$, respectively; $P = 0.0305$), and d 7 ($21.74 \pm 2.82\%$ and $13.50 \pm 2.99\%$, respectively; $P = 0.0467$). Breed and temperature at birth did not affect the number or percentage of $CD4^+CD62L^-CD45RO^+$ T cells in WC- or CFC-fed calves.

$CD4^+CD62L^+CD45RO^+$ T Cells

The number of $CD4^+CD62L^+CD45RO^+$ T cells was determined out of the $CD4^+$ T-cell gate by flow cytometry. The number (LSM $\pm$ SEM; Figure 7) of $CD4^+CD62L^+CD45RO^+$ T cells peaked in CFC-fed calves on d 21 ($1.20 \times 10^5 \pm 2.70 \times 10^4$ cells) and in WC-fed calves on d 3 ($8.25 \times 10^4 \pm 1.92 \times 10^4$ cells). The interaction of treatment $\times$ time was significant for the number of $CD4^+CD62L^+CD45RO^+$ T cells ($P = 0.0189$): CFC-fed calves had fewer $CD4^+CD62L^+CD45RO^+$ T cells in peripheral blood compared with WC-fed calves on d 1 (natural log-transformed; 9.14 ± 0.22 cells and 10.08 ± 0.24 cells, respectively; $P = 0.0044$) and on d 3 (natural log-transformed; 10.29 ± 0.22 cells and 10.94 ± 0.24 cells, respectively; $P = 0.047$). Although we detected no significant treatment $\times$ time interaction ($P = 0.479$), an analysis of simple effects using the SLICE function indicated a higher percentage of $CD4^+CD62L^+CD45RO^+$ T cells in CFC-fed calves on d 1 compared with WC-fed calves ($42.44 \pm 3.0\%$ and $33.23 \pm 3.43\%$; $P = 0.050$). Temperature at birth was significant ($P = 0.0502$), such that the percentage of $CD4^+CD62L^+CD45RO^+$ T cells in C-born calves was higher ($39.27 \pm 2.26\%$) than that in H-born calves ($31.47 \pm 1.97\%$).

Monocytes ($CD14^+$ Cells)

The number and percentage of monocytes (LSM $\pm$ SEM; Figure 8) in calves during the first month of life

Figure 6. The number (per milliliter of blood; left) or percentage (right) of $CD4^+CD62L^-CD45RO^+$ T cells from calves fed whole ($n = 14$) or cell-free ($n = 17$) colostrum. Data are expressed as $LSM \pm SEM$. Treatment $\times$ time interaction for number: $P = 0.0283$ and percentage: $P = 0.0276$. Asterisks indicate a difference between treatment groups within a time point in an analysis of simple effects. Significances were determined using natural log-transformed data for number (d 7: cell-free: 10.21 ± 0.21 cells; whole: 9.34 ± 0.22 cells) of $CD4^+CD62L^-CD45RO^+$ T cells; $*P \leq 0.05$.

were determined out of the total lymphocyte gate. We detected no significant treatment $\times$ time interaction for number ($P = 0.5824$) or percentage ($P = 0.1226$) of monocytes. An analysis of simple effects using the SLICE function indicated a higher percentage of monocytes in CFC-fed calves compared with WC-fed calves on d 3 ($41.02 \pm 2.62\%$ and $33.01 \pm 2.89\%$, respectively; $P = 0.041$). Day relative to colostrum ingestion af-

fected both the number and percentage of monocytes in all calves during the first month of life ($P < 0.0001$). Although there was no significant treatment $\times$ temperature at birth interaction ($P = 0.5296$), an analysis of simple effects using the SLICE function indicated a decreased percentage of monocytes in H-born calves compared with C-born calves ($20.65 \pm 2.10\%$ and $31.54 \pm 2.42\%$, respectively; $P = 0.0052$).

Figure 7. The number (per milliliter of blood; left) or percentage (right) of $CD4^+CD62L^+CD45RO^+$ T cells from calves fed whole ($n = 14$) or cell-free ($n = 17$) colostrum. Data are expressed as $LSM \pm SEM$. Treatment $\times$ time interaction for number: $P = 0.0189$ and percentage: $P = 0.479$. Asterisks indicate a difference between treatment groups within a time point in an analysis of simple effects. Significances were determined using natural log-transformed data for number (d 1: cell-free: 9.14 ± 0.22 cells; whole: 10.08 ± 0.24 cells, d 3: cell-free: 10.29 ± 0.22 cells, whole: 10.94 ± 0.24 cells) of $CD4^+CD62L^+CD45RO^+$ T cells; $*P \leq 0.05$, $**P \leq 0.01$.

Figure 8. The number (per milliliter of blood; left) or percentage (right) of monocytes (CD14⁺) from calves fed whole (n = 14) or cell-free (n = 15) colostrum. Data are expressed as LSM $\pm$ SEM. Treatment $\times$ time interaction for number: $P = 0.5824$ and percentage: $P = 0.1226$. Asterisks indicate a difference between treatment groups within a time point in an analysis of simple effects; * $P \leq 0.05$.

B (CD21⁺) Cells

The number (LSM $\pm$ SEM, Figure 9) of B cells, determined out of the total lymphocyte gate, peaked on d 28 for both CFC- and WC-fed calves ($1.45 \times 10^6 \pm 1.75 \times 10^5$ cells and $1.46 \times 10^6 \pm 1.76 \times 10^5$ cells, respectively). Day relative to colostrum ingestion affected the number and percentage of B cells in all calves during the first month of life ($P < 0.0001$ and $P = 0.0002$, respectively). Treatment, breed of calf, and temperature at birth did not affect B-cell numbers in calves during the first month of life. Breed of animal had a significant effect on the percentage of B cells, such that Jersey calves had fewer B cells than Holstein

calves ($22.95 \pm 1.99\%$ and $27.56 \pm 1.22\%$, respectively; $P = 0.046$).

$\gamma\delta$ T Cells

The number of $\gamma\delta$ T cells was determined out of the total lymphocyte gate by flow cytometry. The number (LSM $\pm$ SEM; Figure 10) of $\gamma\delta$ T cells in all animals peaked on d 21 for CFC- and WC-fed calves ($4.11 \times 10^5 \pm 6.05 \times 10^4$ and $3.63 \times 10^5 \pm 6.44 \times 10^4$ cells, respectively). Day of colostrum ingestion affected the number and percentage of $\gamma\delta$ T cells in calves during the first month of life ($P < 0.0001$). We found no dif-

Figure 9. The number (per milliliter of blood; left) or percentage (right) of B cells (CD21⁺) from calves fed whole (n = 14) or cell-free (n = 15) colostrum. Data are presented as LSM $\pm$ SEM. Asterisks indicate a difference between treatment groups within a time point.

Figure 10. The number (per milliliter of blood; left) or percentage (right) of $\gamma\delta$ T cells from calves fed whole ($n = 17$) or cell-free ($n = 18$) colostrum. Data are presented as LSM $\pm$ SEM.

ferences between treatments, breeds, or temperature at birth for numbers of $\gamma\delta$ T cells at any time point.

DISCUSSION

Although the role of colostral antibodies has been defined, the effect of colostral immune cells on neonatal immune development has yet to be elucidated. Previous reports have demonstrated the ability of maternal colostral cells to affect immune cell function within the first weeks of life. In this study, we fed calves whole colostrum containing intact immune cells or flash-frozen colostrum containing lysed cells. It is possible that the flash-freezing process altered levels of cytokines, hormones, or nutrients in colostrum that in turn affected the PBMC composition in the calf. Although the lack of differences in antibody titers and success of passive transfer do not support this interpretation, further research is needed in this area. Rather, the data presented in this study suggest that intact maternal colostral cells adoptively transfer into circulation and affect immune status in the calf. This may have an influence on immune development over time in dairy calves.

In the current study, we found that CFC-fed calves had decreased numbers and percentages of $CD4^+$ T cells within the first 24 h of life compared with WC-fed calves. To our knowledge, this is the first study to measure $CD4^+$ T-cell levels in calves fed either CFC or WC colostrum. Colostrum from first-parity cows contains approximately 25% $CD4^+$ T cells (Ohtsuka et al., 2010). Bovine colostral leukocytes adoptively transfer through the intestine and into circulation (Liebler-Tenorio et al., 2002). Therefore, the adoptive transfer of $CD4^+$ T lymphocytes could account for the

increase in $CD4^+$ T cells in WC-fed calves on d 1 of life. The $CD4^+$ T cells are responsible for increasing phagocytic capacity of macrophages, recruiting neutrophils to the site of infection, and enhancing antibody production by B cells (Zhu and Paul, 2008). In neonatal $CD4^{-/-}$ knockout mice, animals were more susceptible to an enteric pathogen compared with B-cell-deficient or wild-type control mice. These studies suggest that enhancing $CD4^+$ T cells in the neonate may result in increased pathogen clearance and survival (Echeverry et al., 2010).

The number of $CD4^+CD62L^+CD45RO^-$ T cells on d 1 was less in CFC-fed calves than in WC-fed calves. Additionally, the percentage of $CD4^+CD62L^+CD45RO^-$ T cells was less in CFC-fed calves than in WC-fed calves on d 1 and 7. Trafficking of leukocytes from the periphery to lymph nodes is regulated by adhesion molecules such as CD62L expressed on the cell surface (Sallusto et al., 1999). In healthy calves, CD62L expression on $CD4^+$ and $CD8^+$ T cells is highest during the first week of life and gradually decreases as the animal ages (Howard et al., 1992; Hogg et al., 2011). Evidence suggests that colostral cellular components are more likely to come from peripheral circulation than from the mammary gland itself (Harp et al., 1988, 2004; Sheldrake et al., 1988). The difference in number and percentage of $CD4^+CD62L^+CD45RO^-$ T cells on d 1 in WC-fed calves could be explained by adoptively transferred colostral $CD62L^+$ cells that migrated through the intestine and were detected in blood. However, it is also possible that, in CFC-fed calves, $CD4^+$ T cells are shedding CD62L from the cellular surface in response to an antigen or cytokine stimulus. Cattle have decreased expression of CD62L when infected with *Mycobacterium avium* ssp.

paratuberculosis (MAP; Waters et al., 2003), bovine respiratory syncytial virus (McInnes et al., 1999), bovine leukemia virus (Dusinsky et al., 2000), or foot and mouth disease virus (Toka et al., 2011). Therefore, CFC-fed calves could have a decreased ability to clear pathogens, resulting in shedding of CD62L from lymphocytes. Further research is needed to determine if the decrease of CD62L in CFC-fed calves results in a loss of migratory function.

Glycoprotein marker CD45 exists in 2 isoforms: CD45RA, associated with resting, naïve cells, and CD45RO, associated with an activated phenotype (Cossarizza et al., 1996). In calves, the percentage of MAP-specific CD4⁺ T cells expressing CD45RO⁺ increases in vitro upon stimulation with MAP antigens (Waters et al., 2003). Cattle infected with bovine respiratory syncytial virus had greater proportion of CD4⁺CD62L⁺CD45RO⁺ T cells by 10 d postinfection in bronchial lymph nodes (McInnes et al., 1999). These in vitro and in vivo studies suggest that the increase in the number and percentage of CD4⁺CD62L⁺CD45RO⁺ T cells 6 h after birth and on d 1, 3, and 7 in CFC-fed calves in this study may be explained by increased antigenic stimulation.

In this study, we found fewer CD4⁺CD62L⁺CD45RO⁺ T cells on d 1 and 3 in CFC-fed calves compared with WC-fed calves. Typically, CD4⁺CD62L⁺CD45RO⁺ T cells are defined as central memory cells. Antigen-specific colostral memory cells may have an effect in the calf. Donovan et al. (2007) vaccinated dams with inactivated bovine viral diarrhoea virus (BVDV) antigen in late gestation. After parturition, calves were fed fresh, frozen, or leukocyte-free colostrum. Calves fed fresh colostrum had increased proliferative response to BVDV antigens in vitro compared with calves fed frozen or cell-free colostrum. Comparatively, none of the calves' PBMC proliferated in response to an antigen with which the dams were not vaccinated (mycobacterium purified protein derivative; Donovan et al., 2007). An increase in the number of CD4⁺CD62L⁺CD45RO⁺ T cells in WC-fed calves could be attributed to the adoptive transfer of memory cells from colostrum. However, in our study, the percentage of CD4⁺CD62L⁺CD45RO⁺ T cells was higher in CFC-fed calves on d 1 than in WC-fed calves. It is likely that the higher percentage of CD4⁺CD62L⁺CD45RO⁺ T cells in CFC-fed calves can be attributed to lower percentages of other cell types during that time. The percentage of each cell type is determined from the total lymphocyte gate. Alternatively, the increase in CD4⁺CD62L⁺CD45RO⁺ T cell numbers on d 1 of life could be explained by an increase in total number of cells per milliliter of blood. Future studies are needed to determine the function of CD4⁺CD62L⁺CD45RO⁺ T cells in the bovine.

CONCLUSIONS

We showed that CFC-fed calves have fewer CD4⁺, CD4⁺CD62L⁺CD45RO⁺, and CD4⁺CD62L⁺CD45RO⁺ T cells than WC-fed calves within the first month of life. However, the percentages of CD4⁺CD62L⁺CD45RO⁺ T cells and monocytes were higher in CFC-fed calves than in WC-fed calves. We conclude that adoptive transfer of whole immune cells at birth may play a role in the immune status of neonatal dairy calves. Enhancing immune function in calves could lead to increased disease resistance, resulting in healthier animals entering the dairy herd.

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